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PREWORDS

Welcome to the International Symposium on Physiology for Health (INSPIRE), which revolves around the central theme of " The Role of Physiology for Better Health and Quality of Life." We humbly express our appreciation for the opportunity bestowed upon us to organize the symposium. In the course of this event, we have chosen the theme "The Role of Physiology for Better Health and Quality of Life," reflecting the significance of physiology in safeguarding and enhancing human life's quality. The themes addressed in this symposium include Neurophysiology, Exercise Physiology and Nutrition, Stem Cell and Regenerative Medicine, Teaching Physiology, Plant and Animal Physiology, Cardiovascular Physiology, Physiology in Elderly, Ergonomics, Functional Medicine, Miscellaneous (Others).

Physiology, as a rapidly evolving branch of biomedical science, stands as a paramount pillar in the advancement of scientific research and practical application worldwide. In this era of strengthening globalization, physiology serves not only as a foundation for scientific inquiry but also as a vital key in discovering medical breakthroughs that can uplift human life comprehensively.

We fully understand the vital role of physiology in bridging the gap between research and clinical practice across various fields, from medicine to sports. We feel compelled to organize this symposium as part of our commitment to enhancing understanding and knowledge of physiology, both among medical practitioners and students of medical and related sciences. By bringing experienced speakers from various parts of the world, we hope this symposium will serve as a platform for inspiration and education for all participants, contributing to the introduction and application of global medical advancements within our society.

We extend sincere appreciation to all parties who have contributed to the organization of this event in efforts to advance research and internationally standardized publications. May this symposium bring significant benefits for the development of scientific knowledge and public health on a broad scale.

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Literature review

The role of weight training in improving cardiovascular function and prevention of metabolic syndrome

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Abstract: Weight training is a resistance training method using weights as a tool to improve physical condition, including physical fitness and general health. So far, weight training has been believed to only be beneficial in increasing muscle mass. Weight training is also beneficial for cardiovascular function and the prevention of metabolic syndrome diseases such as dyslipidemia and diabetes mellitus. The purpose of this literature review is to determine the effect of weight training on cardiovascular function and metabolic syndrome. Weight training is not only beneficial for increasing muscle mass but also preventing loss of muscle mass when there is a decrease in body mass. In the cardiovascular system, weight training can increase endothelial-dependent flow-mediated dilation which affects blood vessel diameter. Weight training combined with aerobic exercise has been shown to reduce systolic, diastolic, and mean arterial pressure. A decrease in HbA1C levels and body fat percentage by weight training also shows that weight training plays a role in the prevention of diabetes mellitus. Other studies have also shown weight training to have to affect on decreasing abdominal fat levels, increasing insulin sensitivity, and increasing glucose tolerance. Weight training has also been shown to reduce inflammatory biomarkers such as C-reactive protein and Tumor Necrosis factor alpha. In conclusion, weight training plays a role in improving cardiovascular function and the prevention metabolic syndrome which can be seen from its effect on blood pressure, fat content, insulin sensitivity, glucose tolerance, and reduction of inflammatory biomarkers.

Keywords: cardiovascular function; metabolic syndrome; weight training;

1. Introduction

Weight training is a resistance training method using weights as a tool to improve physical condition, including physical fitness and general health. So far, weight training has been believed to only be beneficial in increasing muscle mass. Aerobic training is more frequently studied and found to have an influence on cardiovascular function and prevent the occurrence of metabolic syndrome. Weight training is also beneficial for cardiovascular function and prevention of metabolic syndrome diseases such as dyslipidemia and diabetes mellitus. The purpose of this review is to determine the effect of weight training on cardiovascular function and metabolic syndrome.

2. Methods

This is a review article, we will highlight the effect of weight training or resistance training in increasing muscle, enhancing cardiovascular function, and preventing metabolic syndrome.

3. Effect of weight training/ resistance training

3.1. Increase in muscle mass

A study by Houbet, et al found that resistance exercise training increases muscle mass and strength in Prostate cancer patients. The result is shown in Table 1.

Table 1. Changes in physical performance tests and cardiopulmonary exercise tests over time

	EX + PLA (n = 28a)	EX + PRO (n = 30a) CON (n = 34a)	EX + PLA (n = 28a)	EX + PRO (n = 30a) CON (n = 34a)	EX + PLA (n = 28a)	EX + PRO (n = 30a)
	Baseline	**20 wk**	**Baseline**	**20 wk**	**Baseline**	**20 wk**
Habitual dietary intake						
Energy intake (MJ·d^{-1})	9.2 ± 2.0	9.3 ± 2.2	9.0 ± 1.9	8.6 ± 1.8	9.4 ± 1.8	9.1 ± 1.7
Protein intake (g·kg body weight^{-1}·d^{-1})	1.1 ± 0.3	1.1 ± 0.2	1.0 ± 0.3	1.0 ± 0.3	1.1 ± 0.3	1.1 ± 0.3
Protein intake (% of energy)	16 ± 2	16 ± 3	16 ± 3	16 ± 3	16 ± 3	17 ± 3
Carbohydrate intake (% of energy)	38 ± 9	39 ± 8	42 ± 6	40 ± 7	40 ± 8	39 ± 6
Fat intake (% of energy)	38 ± 7	38 ± 7	36 ± 5	38 ± 6	36 ± 7	36 ± 6
Habitual physical activity						
Average steps per day (steps per day)[b]	6212 ± 2901	5708 ± 2451	5586 ± 2774	5246 ± 2914	7008 ± 2216	5807 ± 1709
% Time sedentary per day (%)[c]	77 ± 7	77 ± 6	79 ± 7[d]	78 ± 9	73 ± 7	74 ± 7
% Time in light activity per day (%)[c]	19 ± 6	19 ± 5	18 ± 6	18 ± 6	21 ± 6	21 ± 6
% Time in moderate activity per day (%)[b,c]	5 ± 3	4 ± 2	4 ± 3[d]	4 ± 3	6 ± 3	5 ± 2
% Time in vigorous and very vigorous activity per day (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 1 shows that weight training or resistance training with or without supplementation with 31 g whey protein can increase the cross-sectional area of the thigh in 20 prostate cancer patients who received androgen deprivation therapy (ADT) compared to patients who did not receive resistance training treatment. This research also shows that there is no effect of using whey protein after weight training on increasing muscle mass, but there is an effect of using whey protein after weight training on increasing muscle mass. This shows that resistance training can increase muscle mass. Resistance training increases muscle mass by causing damage to the muscle being trained or worked. Muscle damage then limits the ability of the muscles to work. However, after a few days, there will be repairs and an increase in damaged muscle mass. This process of repairing and increasing muscle mass requires protein in the process. Several factors affect the increase in muscle mass in resistance training. The first is Practice Habits. Research shows that there is a smaller increase in muscle mass in people who are trained to do resistance training than people who rarely do resistance training. It is suspected that people who have often done resistance training need

more protein supplements to increase muscle mass. The second is age. Research shows an increase in metabolic protein synthesis at an older age requires more protein intake to be able to increase the Cross-sectional Area after doing resistance training.[1]

3.2. Decrease in blood pressure

The effect of resistance training on blood pressure is believed to be through its effect on Nitric Oxide (NO). A previous study found that resistance exercise training (2 days per week) for 18 weeks did not effect on endothelial function in healthy post-menopausal women, while another study has found improvements in endothelial function after 16 weeks of resistance exercise when combined with other lifestyle interventions of aerobic exercise and calorie reduction. Acute resistance exercise increases blood flow intermittently, yields increased shear stress, and improves NO-mediated vasodilation. NO is the main vasodilator produced by vascular endothelial cells. NO functions are also in modulating smooth muscle tone and promoting inflammatory processes. In addition, NO also has an anti-proliferative effect on the walls of blood vessels. NO also stimulates the production of reactive oxygen species (ROS) but this ROS is usually reduced by vascular antioxidant enzymes. Exercise including resistance training can increase blood flow and it is proven to increase NO-mediated vasodilation so that it can lower blood pressure. However, exercise that is too strenuous can increase vascular ROS production which ultimately reduces NO levels and reduces Flow Mediated Dilation (FMD). The effect of exercise on increasing FMD differs between obese and non-obese. From the results of the study, the response of FMD to increased NO due to exercise is impaired in obese people compared to normal people.[2]

3.3. Decrease in body fat

Research shows that when compared to aerobic training, resistance training only slightly reduces body fat percentage. However, other studies have shown that aerobic training combined with resistance training can increase the reduction of fat mass in people with type II diabetes. It's just that resistance training does not provide more benefits when combined with aerobic training in people who do not have diabetes, are overweight, and are middle-aged.[3] Other research shows that resistance training significantly lowers Subcutaneous Abdominal Adipose Tissue (SAT) if combined with aerobic training than Resistance training alone or Aerobic Training alone. Abdominal fat which consists of subcutaneous and visceral fat is one of the risk factors for chronic disease and metabolic syndrome. Compared to peripheral subcutaneous fat, abdominal fat has a more role in triggering metabolic syndrome but whether abdominal visceral fat or abdominal subcutaneous fat has a more role in metabolic syndrome is still being debated. This is because

abdominal subcutaneous fat has a greater mass so it should contribute more. Several studies have explained the mechanism of exercise in reducing body fat. Exercise is believed to trigger the secretion of growth hormone which then triggers hormone-sensitive lipase to break down fat in adipose tissue.[4]

3.4. Control inflammatory cytokines

There are only a few studies that investigate the effect of weight training or resistance training on immune function. Most of the study shows resistance training in 8-12 weeks had minimal effect on resting inflammatory, innate, or acquired immune parameters. Studies by Rall et al. tell us that 3 months of progressive resistance training did not induce changes in leukocyte subsets, cytokine production, and lymphocyte proliferation. But E-Kader and Al-Shreefs research demonstrates that both aerobic and resistance training cause a decrease in TNF-α, IL-6, and CRP levels. On other hand, both of them increase IL-10 levels. However aerobic training is far more effective than resistance training in controlling inflammatory factors. Training or exercise possibly affect inflammatory factors by three mechanisms. They are a reduction in visceral fat mass, a reduction in the circulating numbers of pro-inflammatory monocytes, and an increase in the circulating numbers of regulatory T cells.[5]

3.5. Preventing diabetes melitus

Hemoglobin is the iron-containing oxygen-transport protein present in erythrocytes. Normal adult haemoglobin (HbA) comprises a haem moiety and two globin chains, the α and β chains ($\alpha 2\beta 2$), making up approximately 97% of adult hemoglobin.4,5 Within HbA, approximately 6% is glycated, of which the main component is HbA1c (5%), with minor components of HbA1a and HbA1b (1%).4 HbA1c results from the covalent attachment of glucose to the N-terminal valine of the hemoglobin β-chain in a nonenzymatic process known as glycation. HbA1c is dependent on the interaction between the concentration of blood glucose and the lifespan of the erythrocyte. HbA1c ≥48 mmol/mol (≥6.5%) in a laboratory using a validated method is diagnostic of diabetes. Resistance training plays a role in preventing diabetes. The results showed that resistance training was proven to reduce HbA1C levels in people with Type 2 Diabetes Mellistus. The research also stated that the higher the increase in muscle strength resulting from resistance training, the greater the decrease in HbA1C levels. The research results also show an inverse relationship between increased muscle strength after resistance training and mortality rates in several chronic diseases, especially those related to diabetes such as kidney disease and metabolic syndrome. The length of intervention or training did not affect HbA1C levels. When compared to aerobic training, the effect

of resistance training in lowering HbA1C levels is lower, although several studies have shown no difference between aerobic training and resistance training.[6]

3.6. Preventing dyslipidemia

Research shows that giving resistance training with an intensity of 85% maximum repetitions for 14 weeks in premenopausal women has a significant effect on reducing total cholesterol (from 4.6 to 4.26 mmol/L) and LDL cholesterol (from 2.99 to 2.57 mmol/L). Another study in untrained men who were given resistance training also reduced triglyceride levels and increased HDL cholesterol levels at low to moderate intensity.[7] The mechanism of reducing total cholesterol and raising HDL-Cholesterol can be explained in Figure 1.

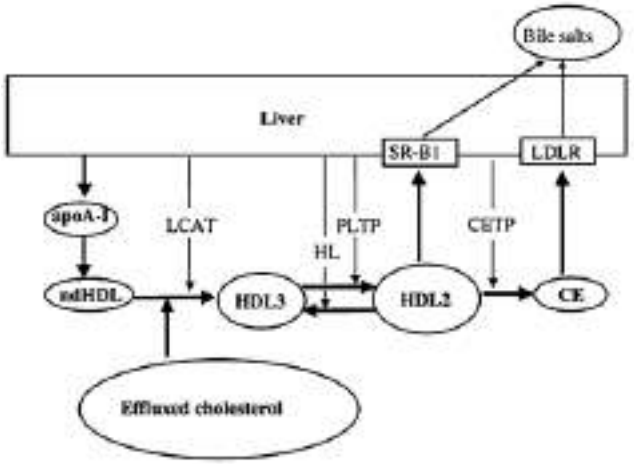

Figure 1. The reverse cholesterol transport pathway delivers free cholesterol from macrophages or other cells to the liver or intestine for excretion

Figure 1 explains that the decrease in cholesterol is thought to be due to the exchange of cholesterol esters between tissue and lipoproteins via HDL cholesterol. On the other hand, although resistance training reduces lipid profiles, it turns out that resistance training does not effect on lipoprotein lipase activity which is believed to be one of the factors affecting lipoprotein levels.[7]

4. Conclusion

Weight Training or resistance training can increase muscle mass and reduce blood pressure by increasing NO in non-obese people. Resistance training can also reduce body fat mass when combined with aerobic training. Besides that resistance training can also reduce HbA1C levels which are an indicator of diabetes mellitus, reducing LDL-cholesterol, reducing total cholesterol and raising HDL which are proof that weight training can prevent dyslipidemia.

5. Acknowledgments

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Literature review

Histopathological changes in animal studies of laryngopharyngeal reflux

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Abstract: Laryngopharyngeal reflux occurred when gastric or duodenal refluxate came intocontact with mucosa of laryngopharyngeal region. Activated pepsin, bile salts, and gastric acid may come into contact with laryngopharyngeal mucosa and may cause chronic inflammation. Unfortunately, majority of animal studies are related to gastrIn regards of lackof studies in animal models, we aimed to review histopathological changes of laryngopharyngeal mucosa in animal models. We found no standardized animal models for laryngopharyngeal models. Animal models can be created with direct application of artificial gastric juice containing activated pepsin and hydrochloric acid or natural gastric juice to laryngopharyngeal mucosa. Expression of genes related to inflammation, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interferon-γ (IFN-γ), were found to beincreased after chronic exposure of laryngopharyngeal mucosa to artificial or natural gastric juice. In all animal studies reviewed, microscopic epithelial damage on laryngopharyngeal epithelial tissue can be found. Studies also showed dysplasia and metaplastic changes to mucosa. Lack of standardized animal models hindered comparison of different studies; in this regard, suitable standardized animal models should be developed in the future.

Keywords: laryngopharyngeal reflux; epithelial damage; animal model

1. Introduction

Laryngopharyngeal reflux is a duodenogastric content reflux resulting in upper aerodigestive tract mucosal damage, mucus hypersecretion, and inflammatory reaction.[1–3] Unlike gastroesophageal reflux, laryngopharyngeal reflux lacks characteristic regurgitation and heartburn due to the fact that laryngopharyngeal reflux commonly occurs as gaseous reflux during daytime.[2] Laryngopharyngeal reflux risk factors include consumption of certain food and drinks, lifestyle, and anatomical anomalies.[4]

Laryngopharyngeal reflux may present as globus sensation, hoarseness, mucus hypersecretion, postnasal drip, chronic laryngitis and pharyngitis, laryngeal erythema, posterior commissure hypertrophy, and diffuse laryngeal edema, with various degree of damage in subglottic and vocal cord mucosal layer.[1,2,4] In some patients, asthma exacerbation may occur due to pepsin in refluxate.[2] Granuloma, polyps, and cysts can be observed in vocal cord, in addition to chronic dry cough caused

by laryngeal irritation. Further down, esophageal stricture and dysplasia can be found. Although inconclusive, sinusitis and chronic otitis media may be related with laryngopharyngeal reflux.[4] Pepsin infiltration in vocal cord can be observed in some studies, suggesting DNA damage observed may be related with pepsin in refluxate.[5] In human gastric juice, hydrochloric acid, pepsin, bile acid, and bilirubin has been observed.[6] With regards to significant comorbidities observed in human laryngopharyngeal reflux, compounding with relative lack of animal studies, we reviewed relevant animal studies related to laryngopharyngeal reflux and discuss relevant findings.

2. Gastric juice and upper GI tract protection

In a study, Wistar rat secretes gastric juice at 12 ± 6 mL each 24 hours and containing 0.72 ± 0.3 mEq HCl in addition to 280 ± 155 mg pepsin.[7] Gastric juice secretion in Wistar rat in 45 minutes after feeding amounted to 1.3 mL, with pH of 3.0; it was estimated that acid secreted in postprandial period equaled to 50 μEq with 60 U pepsin.[8] Fasting period increased gastric pH of rat (3.90 ± 1.0 vs. 3.20 ± 1.0).[9] Some gastric segments had higher pH than other segments: in Lister hooded rats, fasting forestomach pH was 4.3 ± 0.5 (vs. 5.1 ± 0.2 after feeding); while glandular stomach showed lower pH (4.0 ± 0.4 in fasting vs. 3.1 ± 0.3 after feeding).[10] In four months-old rats, gastric pepsin secretion was approximately 1.3 mg per 3 hours, while 14 months-old rats had higher pepsin secretion(1.8 mg per 3 hours), and decreased again in 21 months-old rats (0.9 mg per 3 hours).[11] Corroborating previous research, decreased gastric secretion was observed in elder rats (6.3 ± 2.3 μEq/15 minutes/kg in 3 months-old vs. 1.3 ± 0.5 μEq/15 minutes/kg in 32 months-old rats; $p < 0.05$).[12] On the opposite side of spectrum, gastric pH decreased when rats grew older (1 day, 14 days, 21 days, 28 days, and adults: 5.8, 5.8, 5.7, 5.7, and 4.6 respectively). In rats 28 days-old and older, peak protease activity was observed in pH 2.2.[13]

First-line of defense in esophageal tissues including mucus layer to prevent direct contact between refluxate and epithelial tissue. Second layer of defense including stratified non-keratinized squamous epithelial layer consisting of granulosum, spinosum, and germinativum. Third-line of defense exist as ion transporter in cellular membrane to prevent hydrogen ion damage, acting as chloride-bicarbonate ion exchanger.[14] Epithelial apical junctions consisted from tight and adherent junctions, with tight junctions acting as physical barrier limiting paracellular permeability and apical junction limits epithelial growth and prevents leukocyte and solutes permeability to deeper layers. Claudins, occludins, junctional adhesion molecules, and scaffold proteins can be found in tight junctions, while cadherin, catenin, and nectin can be observed in adherent junctions.[2]

3. Laryngopharyngeal reflux in animals

Induction of laryngopharyngeal reflux in rats can be done through gastric nerve vagotomy. As an alternative, laryngopharyngeal reflux induction may be inducted through laryngeal nerve stimulation in recurrent laryngeal nerve (500 μA AC, with 100 μs pulse width and repetition after each 500 μs).[15] Further alternative for laryngeal damage including direct application of artificial gastric juice in laryngeal and hypopharyngeal tissue (200 μL repeated for 15-45 days). Artificial gastric juice used should contain pepsin (pH 2) or pepsin (at pH 2) with 2-deoxyglucose (10 mg/L).[16]

Mice model of laryngopharyngeal reflux damage can be conducted with similar method as above, with direct application of bile (10 mmol/L, pH 3.0) through feeding tube thrice a day for ten days.[17] Similar model has been employed in another studies, but the application was limited to seven days.[18] Similar variation was conducted in another study, using mixture of bile salts with HCl at pH 3.0 twice a day for 45 days (150-200 μL) directly applied to laryngeal epithelia.[19] In larger animal, indwelling catheter has been used to model laryngopharyngeal reflux. Newborn lamb model has been created, with supraglottic catheter insertion used to directly apply pepsin 300 U/mL (at pH 7 or pH 2) to posterior larynx, thrice a day for six days.[20] In a Bama minipig model aged 4-6 months (average weight: 10 kg), laryngopharyngeal reflux model was created by stenting distal esophagus. After three days, removal of stent was conducted and the minipig model was sacrificed after 14 days.[21] Another study utilized direct, endoscopic acidic pepsin application (1.5 mL pepsin, 1.5 mg/mL, pH 3-4) thrice a day for 4 weeks was studied as laryngopharyngeal reflux model. [22]

4. Outcome of laryngopharyngeal reflux in animals

Laryngeal damage in laryngopharyngeal reflux is thought to occur from pepsin. A study in mice found that pepsin-mediated hyperplasia occurred by increased Glut-1 and H^+/K^+-ATPase expression. Increased Glut-1 expression after pepsin-mediated tissue damage and inflammation caused increased H^+/K^+-ATPase expression, reducing mice laryngeal pH. Pepsin-mediated inflammation itself upregulated Glut-1 through proinflammatory cytokines, including HIF-2α.[16]

Sudden decrease of laryngeal pH after reflux episode may cause laryngeal spasm and apnea. Seizures allowed for relaxation of upper and lower esophageal sphincters, creating reflux and laryngeal spasm followed by apnea and death in animal model.[23] Chronic exposure to acidic pepsin may also increase apnea risk in animal model. A study in rat found that increased response to direct ammonia application to larynx was observed (apnea index: 787 ± 107% vs. 1,227 ± 142%; $p < 0.05$). laryngeal epithelial damage was also observed after application of acidic pepsin (pH 2.0) when compared to denatured pepsin (5.8 ± 0.5 vs. 3.5 ± 0.3; $p < 0.05$).[24] In lamb model, esophageal stimulation may inhibit cardiorespiratory reflexes, including airway protection, laryngeal closure, swallowing, coughing, arterial pressure, and awareness reflexes. The most observed reflex changes were observed in preterm

lamb, further providing evidence of laryngeal reflux-laryngeal spasm relationship.[25] Laryngeal hypersensitivity was also observed through the role of reactive oxygen species causing inflammation after acidic pepsin exposure.[26]

In newborn lambs, reflux laryngitis cause hoarseness, chronic cough, and impaired weight gain (190 ± 18 g/day vs. 99 ± 67 g/day; p = 0.05). macroscopically, epiglottic and vocal cord ulceration was observed in laryngeal reflux. Moderate inflammatory changes were also observed (4 ± 2/15 vs. 0 ± 1/15). Further, longer apnea duration (9 ± 9 s vs. 5 ± 4 s; p = 0.01) and significant SpO_2 reduction was observed (6 ± 8% vs. 3 ± 3%; p = 0.04) after laryngeal reflux.[20] Reflux aspiration by itself may also damage respiratory tract in rat model exposed to either gastric juice, HCl (pH 1.5-2.0), pepsin, or bile salts (0.5 mL/kg) after eight weeks of exposure.[27]

In minipig model, intercellular junction was widened (0.429 ± 0.261 μm vs. 0.960 ± 0.183 μm; p = 0.001) while desmosomes decreased (17.8 ± 1.7 vs 9.5 ± 2.08; p = 0.001) after esophageal stenting.[21] Mice model also showed preneoplastic and dysplastic changes in laryngeal mucosa after chronic reflux. Both pH 7.0 and pH 3.0 bile increased thickness of laryngeal mucosa by 2.1-3.8 times, while exposure to acid (pH 1.5) increased mucosal thickness by factor of 2.9-5.1 times ($p < 0.05$). immunohistochemistry also showed increased NF-kB and ΔNp63 expression after exposure to bile in comparison to acid exposure ($p < 0.005$). Suprabasal layer and basal layer of neoplastic mucosa showed increased Ki67 expression ($p < 0.005$) and reduction of E-cadherin expression ($p < 0.05$).[19]

Metaplastic changes (GMEM, glandulo-metaplastic esophageal mucosa) were also observed in baboon, and thought to be related with reflux exposure. The GMEM changes included glandular-metaplastic epithelial substitution and with no serous-secreting cells. Chronic regurgitation and gastroesophageal refluxes were thought as underlying mechanism of metaplastic changes observed in baboon.[28]

5. Conclusion

In conclusion, no standardized animal model of laryngopharyngeal reflux exists. Rat, mice, lamb, and pig has been utilized so far to model laryngeal damage after exposure to gastric juice, pepsin, and bile. Variable methods, including direct application of substances in question, hindered comparison between different studies. Epithelial damage could be found and thought to occur due to exposure to acid and pepsin, causing inflammation.

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Literature Review

Lower the intensity of low back pain (LBP) by stretching and weight training for the heavy worker

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Abstract: Low Back Pain (LBP) is a musculoskeletal problem that is experienced by almost everyone, especially for heavy workers who often do not pay attention to ergonomic positions while working. Muscles that receive static loads repeatedly and for a long time can cause complaints in the form of muscle injuries, cramps, damage of ligaments, and tendons. Exercise therapy is recommended as first-line treatment and an effective non-pharmacological therapy to lower intensity of low back pain caused by musculoskeletal disorders. Knowing exercises that can have an effect on preventing and reducing LBP, such as stretching and weight training is very important to improve work quality, prevent disability, long treatment and high costs which are one of country's most important burdens. This review aims to explore the types of stretching and weight training that affect the reduction of LBP due to musculoskeletal problems. Stretching can help reduce lactic acid levels in the muscles and increase blood circulation, so that it can help reduce fatigue and pain in the muscles, increase flexibility and range of motion. Meanwhile, weights training can increase bone density and the quality of connective tissue around the spine. This can help maintain the integrity of the spinal structure and prevent injuries or damage that can cause low back pain.

Keywords: exercise; low back pain; physical therapy

1. Introduction

Low back pain (LBP) is currently the leading cause of disability worldwide. It reduces work performance, limits daily activities and increases health care costs.[1–6] Largest increase in disability due to low back pain in decades has occurred in low- and middle-income countries, particularly in Asia, Africa and the Middle East, where health and social systems are ill-equipped to cope with the growing burden of this increase as well as a lack of education regarding health issues and the application of ergonomic work and mismanagement in pain relief, this especially for heavy workers who use a lot of extra physical energy, repetitive movements and a lot of bending and lifting but do not pay attention to ergonomic postures while working.[1, 7, 8] Low back pain has a significant impact on functional capacity because the pain can restrict occupational activities.[9] Exercise therapy is recommended as first-line treatment and an effective non-pharmacological therapy to lower intensity of low back pain caused by musculoskeletal disorders.[3, 6–8, 10–12] Exercise therapy aims to increase muscle and joint strength, and improve

muscle function and range of motion. This should recovery and also bring emotional and psychological benefits leading to decreased pain and disability.[3, 4] Stretching and weight training are exercises that have been shown to reduce low back pain caused by musculoskeletal disorders and can be performed regularly at home and at work so as not to limit the unavailability of exercise center thus it can help improving circulation, improving work posture, improving coordination, relieving stress, increasing flexibility, wiggle room, and preventing injury.[3, 12, 13] The aim of this study is to explore the types of stretching and weight training that affect the reduction of LBP due to musculoskeletal problems and its limitations to reduce pain intensity.

2. Stretching exercises

Stretching can relieve musculoskeletal problems by reducing muscle tension. When a muscle is tense, the muscle fibers shorten because the muscle fibers overlap. By stretching while holding the tensed muscle position for a few seconds, the muscle fiber structure, especially the sarcomers, will stretch due to the reduced overlap of muscle fibers and will automatically cause the muscle fiber structure to be stretched. With the expansion of the muscle fiber structure, the fatigue that occurs can be reduced.[14, 15] According to Da Costa and Vieira, stretching can help reduce the risk of injury and muscle pain by increasing the body's flexibility and range of motion. In addition, stretching can also help reduce lactic acid levels in the muscles and increase blood circulation, helping to reduce fatigue and muscle soreness.[13, 14] Stretching can overcome musculoskeletal problems through several mechanisms. First, stretching can increase the body's flexibility and range of motion, helping to reduce the risk of injury and pain in muscles. Second, stretching can help relieve muscle tension and increase blood circulation. Third, stretching can help improve posture and coordination, so it can help reduce the risk of muscle injury.[6, 13, 14, 16]

Stretching exercises that can be done for people with low back pain include flexibility exercises, stability exercises, and advanced stability exercises. Some of the actions that can be taken are:[17]

Table 1. Stretching exercise: flexibility exercises, stability exercises, and advanced stability exercises[17]

	Flexibility exercises	
1	Single knee to chest Pull one knee up to chest until a comfortable stretch is felt in the lower back and buttocks. Repeat it with opposite knee.	
2	Double knees to chest Pull both knees up to chest until a comfortable stretch is felt in the lower back and buttocks.	

3 Lower trunk rotation stretch
Keeping back flat and feet and knees together, rotate your knees to one side. Repeat with opposite side.

4 Hamstring stretch
Support the back of body high behind the knee. Starting with knee bent, straighten the knee until a comfortable stretch is felt in back of your thigh. Keep your opposite knee bent with foot flat on the floor.

5 Piriformis stretch
Cross the legs with the involved leg on top. Gently pull the opposite knee toward chest until a comfortable stretch is felt in the buttock/hip area

6 Calf stretch
Keeping your back leg straight, with your heel on the floor pointed towards the wall, lean into the wall until a stretch is felt in your calf.

7 Hip flexor stretch
Lying on your back near the edge of the bed, bend one leg with your foot flat on the bed. Hang your other leg over the edge, relaxed. Bend your knee back until a stretch is felt in the front of your thigh.

8 Half kneel hip stretch
Start in a half kneel position. Tighten your stomach muscles as you lean out over your bent knee.

Stability exercises

1 Pelvic tilt
Flatten lower back onto the floor by tightening stomach muscles.

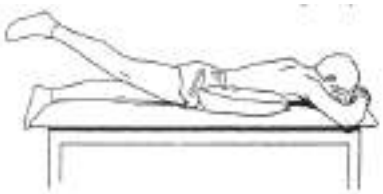
2 Pelvic tilt with arms
While maintaining pelvic tilt, slowly lower one arm over the head. Only go as far as you can while maintaining your back flat on the floor. Slowly return to starting position.

3 Pelvic tilt with legs
While maintaining pelvic tilt, slowly raise one leg a few inches from the floor. Slowly return to starting position while maintaining your back flat on the floor. Only go as far as you can while maintaining your back flat on the floor.

4 Pelvic with bridging
While maintaining pelvic tilt, slowly raise your buttocks off the floor. Slowly return to starting position while maintaining pelvic tilt.

5 Partial curl up
With arms at body side, tilt pelvis to flatten back. Raise the shoulders and head from the floor.

6 Prone with leg raise
Tighten abdominals to keep trunk rigid while you slowly raise straight leg 6 to 8 inches from the floor. Slowly return to starting position



7 Standing pelvic tilt
Stand with your back against the wall. Tighten abdominal muscles while flattening your back against the wall. Progress by holding pelvic tilt and walking away from the wall.

Advanced stability exercises.

1 Pelvic tilt with arms/legs
While maintaining pelvic tilt, slowly raise one leg and lower the opposite arm over your head. Return to starting position while maintaining your back flat on the floor.

2 Pelvic tilt with SLR
While maintaining pelvic tilt slowly raise straight leg 8 to 12 inches from the floor. Slowly lower leg while maintaining your back against the floor.

3 Bridging with straight leg raise
Start with one knee bent and the other leg straight. Maintaining pelvic tilt, lift your buttocks off the floor. Keeping your trunk rigid, slowly raise and lower leg. Slowly return to starting position while maintaining pelvic tilt.

4 Prone with arms/legs
Tighten abdominals to keep your trunk rigid while you slowly raise straight leg and opposite arm simultaneously 6 to 8 inches from the floor. Slowly return to starting position

5 All–fours with arms
While maintaining pelvic tilt, slowly raise your arm until it is parallel with the floor. Slowly return to starting position. Alternate your arms while maintaining pelvic tilt.

6 All–fours with arms/legs
While maintaining pelvic tilt, simultaneously raise your leg and opposite arm. Slowly return to starting position. Alternate sides while maintaining pelvic tilt.

7 Wall push–ups
Place your hands and feet shoulder width apart. Lean into the wall, then push away from the wall while maintaining pelvic tilt.

8 Half kneel to stand
Perform pelvic tilt in half kneel position. While maintaining pelvic tilt move to a standing position, then return to a half kneeling position while maintaining pelvic tilt

The most beneficial program is to include at least 2 sessions per week for 30-45 min each session.[6, 17] and holding for 15-30 seconds and repeating the movement 2-3 times.[17] Stretching exercises do not take much time, but the benefits and positive values of the practices do have a perfect impact on the perpetrator.[2, 18]

3. Limitation of stretching exercises

While stretching has many benefits for low back pain sufferers, it turns out that stretching can also have less of an impact and can even make low back pain worse if the musculoskeletal problem is ligament instability. Yoga is a form of stretching exercise. The effectiveness of yoga in reducing

pain remains controversial. Indeed, yoga can be effective when ligaments are strong, but it will not help when ligament instability, it prevents the muscles from receiving the necessary resistance. Stretching is closely related to ligament strength. Ligaments providing resistance to the spine and core muscles needed to strengthen the body's core, maintain stabilizes and makes physiotherapy or stretching exercises more effective in overcoming pain problems.[1, 2]

The lumbar interspinous muscles are found on both sides of the vertebrae. They attach to the vertebrae at the spinous processes (behind the vertebrae) and extend the length of the spinal column. These muscles are important for lumbar and cervical spine stability. Although the intervertebral muscles play in many roles, their most important role is to stabilize the spine during normal back movements and to help maintain good posture. The left and right intertransversarii muscles are considered as stabilizing muscles during body and trunk movement. These muscles cannot stabilize the spine if their supporting ligamentous attachment, the mamillo-accessory ligament, is damaged. It can cause vertebrae to pop out of their natural position and cause blistering and hernia. This is what causes yoga ineffective to be performed. In other words, stretching will not achieve maximum effect or any benefit in terms of ligament damage.[3, 18]

4. Weight training

Weight training or also known as strength training is a popular sport that helps develop musculoskeletal strength and size.[7, 19] Strengthening the lumbopelvic muscles helps maintain spinal stability during upper and lower extremity movements and improving the neuromuscular mobilization pattern.[6] When a person has long-term lower back pain, the back muscles may have less mass, higher fat content, and stiffer, which makes them more likely to feel fatigued and leads to more intense pain.[20, 21] Over time, this pain and fatigue can lead to a fear of movement, leading to back weakness and instability. Studies have shown that weight training can be safe and can actually reduce pain when done correctly and under the right conditions.[20–23]

The physiological mechanism of weight training which has an effect on low back pain involves several factors, it is increasing the strength of the spinal stabilizer muscles, including the back and abdominal muscles also decreasing body fat. Strengthening these muscles can help maintain good posture and increase spinal stability, thereby reducing pressure on the lumbar area and reducing the risk of low back pain. In addition, weights training can also increase bone density, lean muscle mass and the quality of the connective tissue around the spine. This can help maintain the structural integrity of the spine and prevent injury or damage to the spine which can cause low back pain. Weight training can also increase blood flow to the spinal area, including the intervertebral discs which function as shock absorbers between the vertebrae. By increasing blood flow, nutrients and oxygen needed by the

intervertebral discs, it can reduce the risk of damage to the discs which can also trigger pain.[3, 7, 10, 11, 20, 21]

Apart from that, weight training can also stimulate the release of endorphins, which are natural hormones that can reduce pain and increase feelings of comfort. Weight training can also improve sleep quality and mental well-being in individuals with low back pain.[3, 11] This really helps reduce the intensity and frequency of pain in the lumbar region associated with musculoskeletal problems.[3]

Several studies show that weight training can have a positive influence on LBP. A study by Ibrahim et al. found that a motor control training program and patient education involving weight-bearing exercise can reduce pain intensity and improve physical function in adults with chronic LBP. Additionally, research by Kendall et al. showed that the addition of hip strengthening exercises to a lumbopelvic exercise program can reduce pain and improve function in patients with non-specific LBP.[12] according to Owen et al. (2019) also found that weight training is a type of exercise that is effective in reducing low back pain.[3] The most commonly performed weight training is deadlifts and squats. People living with LBP who have lower reported pain levels and higher baseline lumbar extension strength may be most appropriate to participate in an exercise program that includes deadlifts.[24, 25] However, it is important to note that the effects of weight training on LBP can vary between individuals. Some studies suggest that weight exercise may be ineffective or may even worsen LBP symptoms in some individuals. During the deadlift, the bar from the floor is lifted until the legs get locked and the lifter's posture is erect, if the body position is tilted forward and the back flexion occurs incorrectly when lifting the weight from the floor, it can increase pressure on the lumbar spine. In squats, lowering the body to a lower hip joint than the knee can cause increased stress on the lumbar spine.[19] Therefore, it is important to consult a healthcare professional before starting a weight training program for LBP. Moreohealthcare professional should raise awareness on the biomechanical properties of the lumbar spine and correct spine-protective posture during training to help prevent injuries in the future.[11, 13, 19, 26]

5. Conclusion

From the literature, it is concluded that stretching and weight training are part of exercise that proven to reduce pain in non-specific LBP. This exercise does not require special equipment so that it can minimize the limitations of the tool. In addition, stretching and weight training can increase feelings of pleasure and sleep quality in patients with LBP. Research that combines stretching and weight training is needed to determine the effectiveness of more optimal pain reduction. Apart from that, there is still little research to prove which movements are the safest for non-specific LBP sufferers who have just started exercising. Monitoring by a practitioner is still necessary during training to help prevent

injuries in the future.

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Research Article

Lung function comparison in young adults: The impact of obesity

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Abstract: The impact of obesity on respiratory function in young adults remains a pertinent concern, yet the study on this particular population is still limited. We aimed to compare lung function parameters (FEV1, FVC, and FEV1/FVC) between young adults with obesity and normal body mass index (BMI). This is a cross-sectional study with 39 participants (19 in the obese group (21.42 ± 3.34 years), and 20 in the normal group (23.68 ± 4.07 years)) measured lung function using The NDD EasyOne Spirometry. Data normality was assessed using the Shapiro-Wilk test, followed by t-testsor Mann-Whitney tests. The normality of the data was evaluated using the Shapiro-Wilk test. Normally distributed data were expressed as Mean±SD(95%CI), while skewed data were presented as Median(Range). Statistical comparisons were conducted using t-tests or Mann-Whitney tests, and a $p < 0.05$ indicated a significant difference. We included 39 participants (15(39.47%) females and 23(60.53%) males). The participants were divided into two groups, particularly 19 in the obese group (21.42±3.34 years), and 20 in the normal group (23.68±4.07 years). Compared to the normal group, the obese group exhibited significantly lower FEV1 (3.43(2.69–4.22) vs 2.84(2.54–3.51), p-value <0.001) and FVC (4.05±0.47(3.82–4.28) vs 3.14±0.40(2.95–3.33), $p < 0.001$) values, alongside a higher FEV1/FVC ratio (0.84(0.62–0.88) vs 0.93(0.89–1.00), $p < 0.001$). Young adults with obesity demonstrated altered lung function, reflected by reduced FEV1 and FVC, and an elevated FEV1/FVC ratio. Thus, obese young adults should regularly check their respiratory health and address any issues found.

Keywords: adults; lung function; obesity; respiratory physiology; spirometry

1. Introduction

Obesity is a global health epidemic, with its prevalence steadily increasing over the past few decades.[1] Alongside its well-established associations with cardiovascular disease, diabetes, and musculoskeletal issues, obesity's impact on respiratory health has gained considerable attention in recent years. As the burden of obesity continues to affect individuals across diverse age groups, understanding its specific repercussions on respiratory function has become a matter of paramount importance.

The adverse effects of obesity on respiratory function are well-documented in adults, particularly in older individuals.[2, 3] However, the consequences of obesity on lung function in young adults remain an area of ongoing research interest. The respiratory system's development and maturation continue into early adulthood, making it a crucial phase for investigating the potential consequences of obesity

on lung health.[4] Despite the growing concern surrounding this topic, studies that specifically address the relationship between obesity and lung function in young adults are limited in number.

In light of this research gap, the present study aims to contribute valuable insights by examining and comparing key lung function parameters, such as Forced Expiratory Volume in one second (FEV1), Forced Vital Capacity (FVC), and the FEV1/FVC ratio, in young adults stratified by body mass index (BMI). We intend to shed light on the potential differences in lung function between young adults with obesity and those with a normal BMI, thereby providing a more comprehensive understanding of the impact of obesity on respiratory health within this specific age group.

This research not only addresses an important and relevant issue but also carries implications for public health policies, clinical practice, and future research directions. By better comprehending the consequences of obesity on respiratory function in young adults, healthcare professionals can develop targeted interventions and strategies to mitigate potential health risks associated with obesity during early adulthood.

2. Methods

2.1. Study design and participant

This cross-sectional study aimed to investigate the impact of obesity on lung function in young adults. The study included a total of 39 participants, divided into two groups: an obese group consisting of 19 individuals (mean age: 21.42 ± 3.34 years) and a normal BMI group comprising 20 individuals (mean age: 23.68 ± 4.07 years). Participants were recruited on the Faculty of Medicine, Universtas Andalas.

2.2. Measurement of lung function

Lung function was assessed using The NDD EasyOne Spirometry. This widely recognized spirometry device measures key respiratory parameters, including Forced Expiratory Volume in one second (FEV1), Forced Vital Capacity (FVC), and the FEV1/FVC ratio. Spirometry was conducted in a controlled environment to ensure accuracy and reproducibility of measurements.

2.3. Data normality assessment

The normality of the collected data was assessed using the Shapiro-Wilk test. This test is commonly employed to determine whether data follows a normal distribution. Data that passed the normality test were considered normally distributed, while those that did not were categorized as skewed.

2.4. Data presentation

For normally distributed data, descriptive statistics, including Mean ± Standard Deviation (SD), along with 95% Confidence Intervals (95% CI), were calculated and presented in tabular format. In contrast, skewed data were summarized using the median and range values.

2.5. Statistical analysis

Statistical comparisons between the obese and normal BMI groups were performed to evaluate differences in lung function parameters. Normally distributed data were analyzed using independent t-tests, whereas skewed data were analyzed using Mann-Whitney tests. A significance level of $p < 0.05$ was considered statistically significant, indicating a significant difference between the two groups.

2.6. Ethical considerations

This study was conducted in accordance with ethical guidelines and received approval from the Research Ethics Committee of the Faculty of Medicine, Universitas Andalas (No: 474/UN.16.2/KEP-FK/2021). All participants provided informed consent before participating in the study. Measures were taken to ensure participant confidentiality and data security throughout the study.

3. Results and Discussion

3.1. Participant demographics

A total of 39 participants were included in this study, with 15 (39.47%) females and 23 (60.53%) males. The participants were divided into two distinct groups based on their BMI: an obese group comprising 19 individuals (mean age: 21.42 ± 3.34 years) and a normal BMI group consisting of 20 individuals (mean age: 23.68 ± 4.07 years).

3.2. Lung function parameters

The study examined several key lung function parameters, including Forced Expiratory Volume in one second (FEV1), Forced Vital Capacity (FVC), and the FEV1/FVC ratio in both the obese and normal BMI groups. The results are presented in Table 1.

3.3. Statistical Analysis

Statistical comparisons were conducted to assess differences in lung function parameters between the obese and normal BMI groups. The results revealed statistically significant differences in all measured parameters.

- FEV1: The obese group exhibited significantly lower FEV1 values (3.43 L; 95% CI: 2.69–4.22) compared to the normal BMI group (2.84 L; 95% CI: 2.54–3.51), with a p-value <0.001,

indicating a substantial difference in this parameter between the two groups.

Table 1. Comparison of lung function parameters between covid-19 and control groups. P-value <0.05 means a significant difference

Lung Function Parameter	Normal Group (n=20)	Obese Group (n=19)	p-value
FEV1 (L)	3.43 (2.69–4.22)	2.84 (2.54–3.51)	<0.001
FVC (L)	4.05 ± 0.47 (3.82–4.28)	3.14 ± 0.40 (2.95–3.33)	<0.001
FEV1/FVC	0.84 (0.62–0.88)	0.93 (0.89–1.00)	<0.001

- FVC: Similarly, the obese group had significantly lower FVC values (4.05 ± 0.47 L; 95% CI: 3.82–4.28) compared to the normal group (3.14 ± 0.40 L; 95% CI: 2.95–3.33), with a p-value <0.001, indicating a significant difference in FVC between the two groups.
- FEV1/FVC Ratio: The obese group also exhibited a significantly higher FEV1/FVC ratio (0.84; 95% CI: 0.62–0.88) compared to the normal group (0.93; 95% CI: 0.89–1.00), with a p-value <0.001, suggesting a notable difference in this parameter between the two groups.

These findings highlight significant disparities in lung function between young adults with obesity and those with a normal BMI. The obese group demonstrated lower FEV1 and FVC values, as well as an altered FEV1/FVC ratio, indicating potential respiratory impairments associated with obesity in this population.

3.4. Discussion

The findings of this study provide valuable insights into the association between obesity and lung function in young adults. Our results clearly demonstrate significant differences in key lung function parameters, including Forced Expiratory Volume in one second (FEV1), Forced Vital Capacity (FVC), and the FEV1/FVC ratio, between the obese and normal BMI groups. These findings have important implications for our understanding of the impact of obesity on respiratory health in this specific age group.

The observed reduction in FEV1 and FVC in the obese group compared to the normal BMI group is consistent with previous research.[5, 6] Reduced FEV1 and FVC are indicative of impaired lung function and reduced lung capacity. This phenomenon can be attributed to several factors commonly associated with obesity, including increased fat deposition in the chest and abdominal areas, reduced chest wall compliance, and altered respiratory mechanics.[7] Such structural changes can restrict lung expansion and lead to decreased lung volumes. Additionally, adipose tissue itself can produce inflammatory cytokines, which may contribute to airway inflammation and further compromise lung function.[8]

Of particular note is the significantly higher FEV1/FVC ratio observed in the obese group. This elevated ratio is suggestive of a restrictive pattern of lung disease, where lung volumes are reduced relative to the vital capacity. While restrictive patterns are typically associated with conditions like interstitial lung disease or neuromuscular disorders, our findings suggest that obesity may contribute to such patterns in young adults.[9]

These findings underscore the importance of addressing obesity as a potential risk factor for respiratory impairments in young adults. Early adulthood is a critical period for lung development and function, and the impact of obesity during this phase may have long-term consequences for respiratory health. Recognizing these associations can inform preventive strategies and interventions aimed at mitigating the negative effects of obesity on lung function.

It is worth noting that our study has some limitations. First, the relatively small sample size may limit the generalizability of our findings. A larger and more diverse population would provide a more comprehensive understanding of the relationship between obesity and lung function in young adults. Additionally, while we observed significant differences in lung function, the clinical significance of these differences and their long-term implications require further investigation.

4. Conclusion

In conclusion, our study highlights the significant impact of obesity on lung function in young adults, as evidenced by reduced FEV1 and FVC values and an altered FEV1/FVC ratio. These findings emphasize the need for early interventions to address obesity-related respiratory impairments in this age group. Future research should explore the mechanisms underlying these associations and assess the effectiveness of targeted interventions in improving lung health in young adults with obesity.

5. Acknowledgements

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Research Article

Liver parenchymal cells necrosis in correlation with serum aminotransferase enzymes of oral candidiasis immunosuppressed rats treated with hyperbaric oxygen

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Abstract: Liver is the largest organ in the body which performs many crucial functions. Liver cell necrosis can be caused by the exaggregation of immunosuppressive drugs. An enhancement of the aminotransferase enzymes in blood circulation is often used as marker of liver cell injury. The aim of this study was to investigate the correlation of liver parenchymal cells necrosis and aminotransferase enzymes level in oral candidiasis immunosuppressed rats after hyperbaric oxygen treatment. Methods: A randomly distribution of 18 male Wistar rats was divided into three groups, H (healthy group), OCI (immunosuppressed rats with oral candidiasis) and OCI-HO (immunosuppressed rats with oral candidiasis given hyperbaric oxygen). Dexamethasone 0.5 mg/day and tetracycline 1 %/day are used to create immunosuppressed condition, given orally for 14 days. The number of liver parenchymal cells necrosis was measured using light microscope, while blood samples were collected to count aminotransferase enzymes level. Results: The number of liver parenchymal cells necrosis in OCI group was the highest and declined significantly in OCI-HO group, a statistically significant difference ($p<0,05$) was observed within all groups. Aminotransferase enzymes level showed a significant difference between OCI and OCI-HO groups, while there was no significant difference between H and OCI-HO groups ($p>0,05$). The correlation test showed a positive strong correlation between the number of liver parenchymal cells necrosis with alanine aminotransferase and aspartate aminotransferase levels ($p<0,05$). Conclusion: There was a correlation between the number of liver parenchymal cells necrosis and serum aminotransferase enzymes in immunosuppressed rats with oral candidiasis after hyperbaric oxygen treatment.

Keywords: aminotransferase; hyperbaric oxygen; immunosuppressed rats; liver parenchymal cells

1. Introduction

The liver is the largest organ in the body that performs many important functions. The liver plays a role in the metabolism of foreign chemicals.[1] This function is carried out by the liver parenchymal cells, which make up most of the liver volume.[2] The excessive foreign chemicals and drugs that enter the liver can cause the decrease function of liver parenchymal cells and influence the ability of its regeneration, which then causes liver cell damage.[3]

Continuous use of immunosuppressive drugs with excessive doses can be a predisposing factor for oral candidiasis.[4] The administration of these drugs is also known to have an effect on the occurrence of liver parenchymal cell damage. Previous research showed that giving immunosuppressive drugs for 14 days can affect aminotransferase enzyme levels and cause damage to liver cells.[5, 6] This is indicated due to a decrease in the function of liver parenchymal cells, which play an important role in carrying out liver function. [2, 5]

Parameters of liver damage can be examined from changes in the activity of enzyme levels in the blood formed by liver cells.[7][8] The two aminotransferase enzymes most associated and often used as a marker of liver cell damage are alanine aminotransferase (ALT) and aspartate aminotransferase (AST). If the liver parenchymal cells are injured, the aminotransferase enzyme in the cell will enter the blood circulation due to a change in the permeability of the cell membrane, so that the level of the enzyme in the blood will increase. The increasing of these enzymes can be used to detect the diseases earlier and suggests any inflammation or injury in the liver. Measurement of AST and ALT enzymes can identify the safety of a substance that enters and is metabolized by the liver.[8]

Hyperbaric oxygen treatment is the clinical use of pure oxygen at a higher pressure than atmospheric pressure. This therapy is proven to provide biochemical and cellular benefits to tissues. It can help increase dissolved oxygen in the blood and accelerate the transport of oxygen to tissues.[9] Experimental studies in animal models showed the beneficial effects of hyperbaric oxygen treatment on liver disease.[10] Hyperbaric oxygen treatment promotes the healing of the injured liver.[11] The previous study showed that hyperbaric oxygen treatment provides a protective effect on liver cells from necrosis induced by acetaminophen in a mouse model.[12] This therapy not only can enhance the proliferation of hepatocytes and avert it from necrosis and apoptosis but also enhance microvascular density and sinusoid diameter in animal models.[10] Hence it is the interest of this study to investigate the correlation of liver parenchymal cell necrosis and aminotransferase enzymes level in oral candidiasis immunosuppressed rats treated with hyperbaric oxygen.

2. Methods

This study was a true experiment using a post-test only control group design. Experimental animals used in this study were eighteen male 6 months old of *Rattus Novergicus* strain *Wistar*, weighed 180-200 grams. The rats were divided randomly into three groups after adapted for 7 days. The groups include H: healthy rats group, OCI: immunosuppressed rats with oral candidiasis group, and OCI-HO: immunosuppressed rats with oral candidiasis treated with

hyperbaric oxygen group. The immunosuppressed condition was made by giving dexamethasone 0.5 mg/day and tetracycline 1 %/day orally. On the 4th day, the dose was reduced by 10% and continued for 14 days. *Candida albicans* (ATCC-10231) $6x10^8$ were induced to the rats by applying 0.1 cc of *Candida albicans* on the dorsum tongue of rats using a sterile cotton bud, given once every two days for 12 days.[13, 14] Hyperbaric oxygen treatment 2,4 ATA for 30 minutes at 3 intervals of 5-minute breathing normal air was given to the rats in OCI-HO group, carried out for five days continuously.[6, 12]

At the end of the study, all rats were sacrificed. The liver and blood of all rats were taken for examining the number of liver parenchymal cells necrosis as well ALT and AST levels.[5, 6] The number of liver parenchymal cell necrosis obtained from counting cells that experienced pyknosis, karyorrhexis, and karyolysis, using a light microscope with 400x magnification.[5] This research was conducted in the oral biology laboratory of Dentistry Faculty Universitas Hang Tuah Surabaya, biochemistry laboratory of Medical Faculty Universitas Hang Tuah Surabaya, pathological laboratory of Dr. Ramelan Hospital Surabaya, and Balai Besar Laboratorium Kesehatan Surabaya.

Statistical analyses were done using the Anova test to show the different number of liver parenchymal cells and ALT AST levels among groups, then the Least Significant Difference (LSD) test to show the significant difference among each group. To analyze the correlation between the number of liver parenchymal cell necrosis and ALT AST levels, we used Pearson's correlation test which stated with correlation coefficient. This research was approved by the Ethics Commission of Dentistry Faculty Universitas Hang Tuah Surabaya (Ref. no: EC/010/KEPK-FKGUHT/VII/2019).

3. Results and Discussion

The results showed that the number of normal liver parenchymal cells in OCI group was the lowest compared with H and OCI-HO groups, while the number of liver parenchymal cell necrosis showed the highest (Figure 1). The number of liver parenchymal cell necrosis are significantly different among each group ($p<0,05$) (Table 1).

Table 1. The average number of liver parenchymal cell in each group

Group	Normal (Mean ± SD)	Necrosis (Mean ± SD)
H	$124.7^a \pm 13.7$	$7.1^a \pm 5.9$
OCI	$88.2^b \pm 10.1$	$144.8^b \pm 23.5$
OCI-HO	$114.8^c \pm 12.3$	$77.8^c \pm 15.1$

Mean with different superscript letters are significant at $p<0.05$

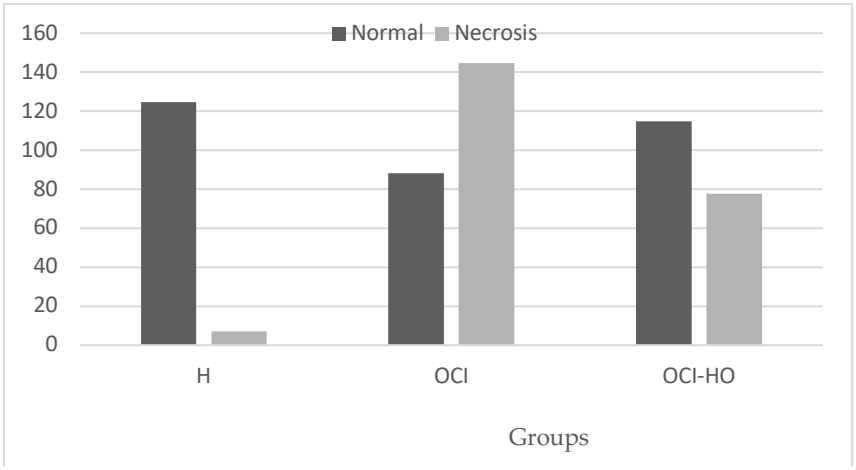

Figure 1. Graphics of average number of liver parenchymal cells in each group

The ALT and AST levels in the OCI group were higher in comparison with other groups, whereas in the OCI-HO group ALT and AST serum showed a decreased (Figure 2). The level of ALT and AST serum in immunosuppressed rats without hyperbaric oxygen treatment (OCI) was significantly different from those with hyperbaric oxygen treatment (OCI-HO), likewise in comparison to the control group (H) ($p<0,05$) (Table 2). However, the levels of ALT and AST serum between H and OCI-HO groups were not significantly different ($p>0,05$) (Table 2).

Table 2. The average ALT and AST levels (U/L) in each group

Group	ALT (Mean ± SD)	AST (Mean ± SD)
H	$110.2^{a} \pm 18.6$	$131.5^{a} \pm 27.6$
OCI	$151.2^{b} \pm 12$	$176.3^{b} \pm 12$
OCI-HO	$119.3^{a} \pm 17.4$	$150.5^{a} \pm 11.3$

Mean with different superscript letters are significant at $p<0.05$

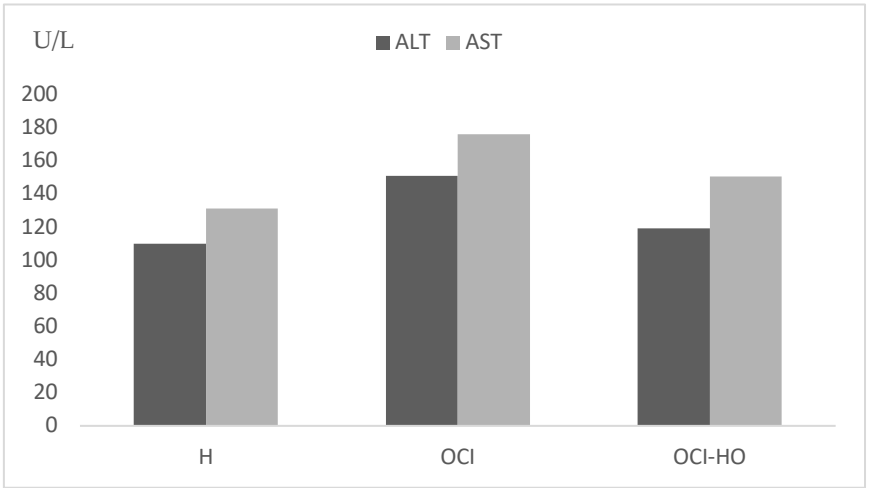

Figure 2. Graphics of average value of ALT and AST levels in each group

To show the correlation between the number of liver parenchymal cell necrosis with ALT and AST levels in immunosuppressed rats with oral candidiasis we used Pearson's correlation test, and showed there was significant correlation ($p<0,05$) between liver parenchymal cell necrosis and ALT AST levels (Table 3).

Table 3. Correlation between the number of liver parenchymal cell necrosis with ALT and AST levels

Parameters	Correlation coefficient (r) value	p value
ALT	0.647	0.004*
AST	0.7	0.001*

Note: *significant at level $p<0,01$

The results of this study showed that the average number of liver parenchymal cell necrosis in OCI group was the highest compared to the H and OCI-HO groups (Figure 1). This could be indicated that the administration of immunosuppressive drugs in this study could trigger damage to liver parenchymal cells and decrease its number.[5] In the OCI-HO group, the average number of liver parenchymal cell necrosis was decrease close to the number of liver parenchymal cells in healthy group. This suggests that hyperbaric oxygen treatment could increase liver parenchymal cell proliferation in injured liver caused by immunosuppressive drugs.[5] Stimulation of liver parenchymal cell proliferation may occur by normalizing the localization of resistance-associated protein 2 (Mrp-2) on the apical membrane of cells and subsequent activation of transporter functions.[10] The study by Sun et al (2018) showed that hyperbaric oxygen treatment can protect liver parenchymal cell against necrosis in a mouse liver transplant model.[10] This therapy is known to increase sinusoidal diameter and microvessel density index in a mouse model of in situ liver transplantation, and also preserve liver parenchymal cells from necrosis and apoptosis.[10] This therapy is known to have hepatoprotective effects in a rat model of hepatocellular necrosis caused by excessive use of acetaminophen.[12] The use of hyperbaric oxygen treatment is very strongly associated with increased function of mitochondria during liver parenchymal cell regeneration, then increasing oxygen delivery to damaged liver tissue. This therapy is also known to decrease malondialdehyde and escalate antioxidant activity, which can then have a positive effect on liver cell regeneration.[10]

This study showed that there was significant difference in aminotransferase enzymes level (ALT and AST) between the immunosuppressed rats without hyperbaric oxygen treatment group (OCI) and the immunosuppressed rats with hyperbaric oxygen treatment group (OCI-HO). The level of ALT and AST showed an increase in group OCI compared to H and OCI-H group. This could be caused by damage to liver parenchymal cells.[6] In other words, all of the rats in OCI group may have shown hepatocellular function disorders. Several previous studies have shown that elevated levels of ALT and

AST enzymes could be used as clues to damage in hepatocellular integrity through persistent necroinflammation.[15] ALT and AST enzymes are located in the liver parenchymal cells, which if these cells are damaged or injured, the enzyme levels in the serum will increase. Necrosis of liver cells or acute liver cell damage will then causes the release of enzymes intracellularly into the blood. The increase in these enzyme levels occurs can be due to damage to liver cells by drugs or toxins. Re-elevation or persistence of elevated ALT enzymes indicates the development of liver disease and necrosis.[2]

This study indicated that the use of hyperbaric oxygen for short periods of time, causing cell oxidative stress, protects the liver against injuries associated with the use of immunosuppressive drugs by a biochemical mechanism that is still unknown.[16] Hyperbaric oxygen exposure can increase the production of reactive oxygen species (ROS), directly related to the amount of oxygen present. ROS overproduction is detrimental for cells because it can damage cell components including proteins, lipids, and nucleic acids leading to significant alteration of health status. However, a moderate increase in ROS can be beneficial because ROS may also act as cellular messengers in many signal transduction pathways.[17] Oxidative stress derived from oxygen-based therapy which play a role as signaling molecule, at relevant levels, can lead antioxidant activity which can then promote the healing process of tissue damage.[18]

The results of the correlation test in this study showed that there was significant correlation between the number of liver parenchymal cell necrosis with serum ALT and AST levels. The correlation coefficient showed a value which means a strong correlation between the number of liver parenchymal cell necrosis and ALT AST levels (Table 3). These correlation results may indicate that the use of immunosuppressive drugs in this study could trigger liver parenchymal cell necrosis, and lead to liver dysfunction. In this study, the number of liver parenchymal cell necrosis was accompanied by changes in the function of liver by examining the level of ALT and AST enzymes. The positive correlation showed that the more higher number of cell necrosis, the serum level of ALT and AST enzymes will increase too. Increased levels of ALT and AST in OCI group was considered to show an indication of liver function damage.[8] The process of oxidation, reduction, hydrolysis, and conjugation of toxic substances was not going on physiologically. Thus, there was indication of impaired liver function. The type of substance contained in an ingredient, the dose given, and the duration of exposure are several factors that can affect liver damage.[2] The limitation of this study is no evaluation of the role of inflammatory cells in the process of liver cell necrosis. Therefore, further research is needed to determine the effect of administering immunosuppressive drugs into the infiltration of inflammation cells, so that further effects and the degree of inflammation on the liver can be studied.

4. Conclusion

There was a correlation between the number of liver parenchymal cell necrosis and serum aminotransferase enzymes (ALT and AST) level in the immunosuppressed rats with oral candidiasis after hyperbaric oxygen treatment 2,4 ATA.

5. Acknowledgments

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Literature Review

Dextrose-prolotherapy: the use of dextrose to enhance physiological healing abilities to relieves pain

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Abstract: Prolotherapy is a complementary and alternative medical (CAM) injection-based therapy used for chronic musculoskeletal pain. The use of prolotherapy has been practiced for a long time and its benefits are well-known. The principle of this therapy involves injecting into tendons, ligaments, or intra-articular and extra-articular spaces. The prolotherapy technique and injection solutions vary based on the condition, clinical severity, and practitioner preferences. Prolotherapy works by inducing temporary mild inflammation in ligaments and tendons, thereby "tricking" the body toinitiate a healing cascade. Inflammation activates fibroblasts, which then form mature collagen precursors and increase growth factors that aid in tissue repair and growth. This process is believed to enhance joint stability, biomechanics, function, and ultimately reduce pain. Several studies have shown that dextrose solution yields better results compared to other control solutions (e.g., NaCl). Prolotherapy has been found beneficial in musculoskeletal injuries such as lateral epicondylitis, lower back pain (LBP), certain tendinopathies, and osteoarthritis. Prolotherapy should not be performed in cases of acute infections such as cellulitis, local abscesses, or septic arthritis. The main risks of prolotherapy include mildpain and minor bleeding caused by needle trauma. In recent times, multidisciplinary groups including family physicians or sports medicine doctors, physiotherapists, orthopedic surgeons, neurologists, or anesthesiologists have incorporated prolotherapy into positive clinical experiences and research reports.

Keywords: dextrose solution; inflammation; injection; pain; prolotherapy

1. Introduction

Prolotherapy is a complementary and alternative medical (CAM) injection-based therapy for chronic musculoskeletal pain. This therapy has been in use for approximately 100 years. However, its modern application can be traced back to the 1950s when prolotherapy injection protocols were formalized by George Hackett, a general surgeon in the United States, based on his more than 30 years of clinical experience.[1]

The prolotherapy technique and injection solutions vary depending on the condition, clinical severity, and practitioner preferences. The main principle involves injecting a small volume of irritating or sclerosing solution directly into the painful ligaments and tendon insertions, as well as into adjacent joint spaces over several treatment sessions. Interest in prolotherapy is high among doctors and

patients, and it is becoming increasingly popular in the United States and internationally, actively used in clinical practice.[1]

The exact number of practitioners currently actively practicing prolotherapy is unknown but may be in the thousands in the United States based on participation in continuing medical education (CME) conferences and relevant doctor listings on websites. Prolotherapy has been assessed as a treatment for various painful chronic musculoskeletal conditions that are refractory to standard therapies. While anecdotal clinical success guides the use of prolotherapy for many conditions, clinical trial literature supports evidence-based decision-making for the use of prolotherapy in conditions such as low back pain (LBP), certain tendinopathies, and osteoarthritis (OA).[1]

2. Methods

Prolotherapy is an injection therapy used for chronic musculoskeletal injuries, including knee osteoarthritis.[2] The goal of prolotherapy injections is to tighten and strengthen weak or loose tendons, ligaments, or joint capsules through the multiplication and activation of fibroblasts.[3] The fundamental principle involves injecting a small volume of irritant solution into specific painful ligaments, tendon insertions, and adjacent joint spaces over several treatment sessions.[2] The foundation of prolotherapy is based on the premise that chronic musculoskeletal pain occurs due to inadequate tissue repair of fibrous connective tissue, resulting in weakness or laxity of tendons and ligaments, also referred to as connective tissue insufficiency.[4]

Historically, prolotherapy was referred to as sclerotherapy because the initial solutions were thought to form scars. However, prolotherapy is now the most commonly used term and is based on the 'proliferation' effect on chronically injured tissues. Additionally, it is also known as regenerative injection therapy (RIT), and some authors refer to the therapy based on the injected solution, even though the exact mechanism of action is not known.[1]

3. Results and Discussion

3.1. Prolotherapy techniques

While there are no formal practice guidelines published, prolotherapy treatment typically consists of multiple injection sessions given every 2 to 6 weeks over several months. During prolotherapy sessions, therapeutic solutions are injected into the painful and sore ligaments, tendon insertions, and adjacent joint spaces. The injected solution (proliferant) has historically been hypothesized to cause local irritation, followed by inflammation and tissue healing, resulting in the enlargement and strengthening of damaged ligaments, tendons, and intra-articular structures. This process is believed to improve joint stability, biomechanics, function, and ultimately reduce pain.[1]

Dextrose is the most commonly used proliferant today. The concentration of dextrose used in prolotherapy varies.[5–7] The higher the concentration of dextrose, the more cells at the injection site experience dehydration, the more cells are activated, and the more tissue repair occurs, thus facilitating the repair of structures that stabilize the joint and surrounding tissues. Commonly used solutions in prolotherapy include lidocaine, dextrose at concentrations of 5%, 10%, 15%, 20%, 30%, Marcaine, or prilocaine.[6]

Dilution can be made by mixing 1 ml of 50% dextrose with 3 ml of 1% lidocaine. A 25% dextrose solution is used for intra-articular injections in the knee. Recent research has shown that a 10% dextrose solution is also effective. Sodium morrhuate 5% is a mixture of sodium salts of saturated and unsaturated fatty acids from cod liver oil and 2% benzyl alcohol. Benzyl alcohol is chemically similar to phenol and acts as a local anesthetic.[7] Dextrose-phenol-glycerin solution consists of 25% dextrose, 2.5% phenol, and 25% glycerin (referred to as DPG or P2G). [4,7] This solution is then diluted with the doctor's choice of local anesthesia before injection. The made dilution (dilution ratio) is 1:1, 1:2, and 2:3. Other solutions that can be used include tetracycline, a mixture of chondroitin sulfate, glucosamine sulfate, and dextrose. [7]

Research states that the majority of participants are not given sedation before prolotherapy and it is believed to reduce the risk of complications. A survey study states that almost all participants are given skin antiseptics before prolotherapy to prevent injection-related infections. [6]

Prolotherapy is used for musculoskeletal pain that persists for more than 8 weeks. Prolotherapy can be used years after the initial issue arises, as long as the patient remains in good health.[4] Indications for regenerative injection therapy, or prolotherapy, include:[7]

1. Chronic pain secondary to ligament or tendon sprains or strains. [4,7]
2. Pain due to overuse or occupational conditions, such as neck or wrist pain in computer operators, tennis elbow, and chronic supraspinatus tendinosis.[7]
3. Chronic postural pain in the cervical, thoracic, lumbar, and lumbosacral regions. [4,7]
4. Recurrent somatic dysfunction accompanied by pain secondary to ligament laxity that may temporarily improve with manipulation. Hypermobility and painful subluxations in mobile joints or structures with extensive joint range of motion.[7]
5. Thoracic and lumbar vertebral compression fractures with associated deformities that exceed the stress on the posterior ligament-tendon complex. [7]
6. Painful and recurrent subluxations of ribs, particularly at the costotransverse, costovertebral, and/or costosternal joints. [7]
7. Osteoarthritis in axial or peripheral joints, spondylosis, spondylolysis, and spondylolisthesis. [4,7]

8. Imbalance in cervical, thoracic, lumbar, lumbosacral, and sacroiliac regions due to secondary ligament laxity.[7]
9. Intolerance to NSAIDs, steroids, or opiates. Prolotherapy may be a treatment option if the patient does not experience improvement after physical therapy, chiropractic or osteopathic manipulation, radiofrequency denervation, steroid injections, surgical interventions for the previously mentioned conditions, or if other modalities are contraindicated.[7]

3.2. Mechanism of action

Ligaments and tendons have poor vascularity, which means they take longer to heal compared to other tissues. Imperfect healing commonly occurs after injuries to ligaments and tendons.[4] Sprains, strains, and overload injuries result in excessive forces that can damage the tissue matrix and cause cell damage or death. Prolotherapy injections tighten and strengthen weak or loose tendons, ligaments, or joint capsules through the multiplication and activation of fibroblasts. Fibroblasts form precursor collagen that strengthens connective tissue.[3]

Prolotherapy works by inducing mild and temporary inflammation in the ligaments and tendons, "tricking" the body into initiating a healing cascade. Inflammation activates fibroblasts, which then form precursor mature collagen. Inflammation also leads to an increase in growth factors.[4] The increased levels or effectiveness of growth factors aid in tissue repair and growth.[4] An increase in the diameter of collagen fibrils, statistically significant ($P < 0.001$) after 6 weeks of proliferant injections in low back pain patients, is observed at month 3.[3] Clinical and experimental studies using electron microscopy have shown that the connective tissue formed after prolotherapy has a structure similar to normal tendons and ligaments.[7] The mechanisms of action of regenerative injection therapy, or prolotherapy, include:

1. Transverse cutting (transection) of the matrix and cells induced by the needle, causing cellular damage, thus stimulating an inflammatory cascade. [7]
2. Compression of cells by the extracellular volume of the injected solution stimulates intracellular growth factors.[7]
3. Chemical modulation (chemomodulation) of collagen through proliferative, regenerative, or repetitive responses induced by the chemical properties of the proliferant substance, mediated by cytokines and various growth factors.[7]
4. Chemical-neuro modulation (chemoneuromodulation) of peripheral nociceptors; antidromic and orthodromic sympathetic transmission; axon reflex.[7]
5. Local hemodynamic modulation through changes in intraosseous pressure, reducing pain. Empirical observations suggest that a combination of dextrose/lidocaine has a longer-lasting

effect compared to lidocaine alone.[7]

6. Temporary repetitive stabilization of hypermobile joints experiencing pain, induced by the inflammatory response to the proliferant, providing a conducive environment for ligament and tendon regeneration and repair.[7]

Research indicates that dextrose has independent effects that can promote local healing of painful extra-articular or intra-articular tissues through the stimulation of inflammatory or non-inflammatory pathways. Recent research also suggests a sensorineural analgesic mechanism. In vitro and animal model data have not fully supported this hypothesis.[8] Studies on the in vitro effects of dextrose on cytokine levels have shown that dextrose transport into human cells uses transport proteins like GLUT 1–4, which interact with cytokines to signal cell growth or repair.[8] In the rat knee ligament model, inflammation responses were reported for each solution, although they were not significantly different from those caused by needle injection alone or saline injection. However, animal model data showed significant biological effects of dextrose and sodium morrhuate compared to controls. Rabbit medial collateral ligaments injected with sodium morrhuate were significantly stronger (31%), larger (47%), thicker (28%), and had larger collagen fiber diameter (56%) compared to controls injected with saline. An increase in cell count, water content, ground substance, and various types of inflammatory cells were hypothesized to account for these changes. Rat patellar tendons injected with sodium morrhuate were able to withstand an average maximal load of 136% (+28%)—significantly more than control tendons that were not injected. In the same study, tendons injected with the saline control were significantly weaker than non-injected controls.[1]

Dextrose has been minimally assessed in animal models. Recent studies have shown that injured rat medial collateral ligaments injected with 15% dextrose had a significantly larger cross-sectional area compared to uninjured controls injected with saline. P2G solution has received the least research attention; although it is actively used in clinical practice, there are no animal or in vitro studies assessing the effects of P2G using injury models. Most doctors report using this solution as a single agent, although its concentration varies. In clinical practice, doctors sometimes mix prolotherapy solutions or use them sequentially in a single injection session, depending on their experience and local practice patterns. Varying concentrations or mixtures have not been assessed in basic science or clinical studies, and there are no clinical trials comparing different solutions to each other.[1]

The potential sensorineural mechanism of injection has been studied in humans. The sensorineural effects of dextrose injections have been proposed based on observations that analgesia arises from subcutaneous perineural dextrose injections along soft peripheral nerves in patients with chronic pain. Transient receptor potential cation channel subfamily V member 1 (TRPV-1), previously known as the capsaicin receptor, is known to produce nociceptive pain with up-regulation. Bertrand and colleagues

stimulated TRPV-1 receptors using a capsaicin cream model to induce pain. The cream contained mannitol or a control cream (vehicle) and was applied to the painful area in a double-blind manner. Chemically, mannitol is related to dextrose. Pain resolution was reported to be faster with mannitol administration. Researchers hypothesized that TRPV-1 receptors might undergo down-regulation, or ion channels or other receptors could be affected. Increased dextrose up to 0.6% around fibroblasts and chondrocytes resulted in increased cytokine levels due to tissue development or breakdown in human tissue in in vitro research.[7]

There are three potential sensorineural mechanisms of dextrose regarding its analgesic effects. The first potential sensorineural mechanism is that dextrose may play a role in key pain modulators, such as ion channels. The ion channel Transient Receptor Potential Vanilloid Receptor-1 (TRPV1) plays a major role in the development of allodynia and hyperalgesia in patients with chronic pain. Chronic neuropathic pain is associated with persistent upregulation of the TRPV1 ion channel. Mannitol, a sugar metabolic molecule with a structure similar to dextrose, has been reported to reduce pain caused by TRPV1 upregulation. While the TRPV1 ion channel does not have a monosugar receptor, monosugars can modulate the effects of TRPV1 expression allosterically.

The second potential mechanism involves dextrose restoring energy reserves in the context of chronic pain. Peripheral nerves tend to be sensitive to glycopenia, leading to histopathological damage when systemic dextrose decreases by as much as 25%. Perineural glycopenia is caused by progressive depolarization and hyperexcitability of nociceptive nerve fibers due to decreased effectiveness of ATP pumps. ATP production depends on dextrose. In one study, nociceptive C fibers exposed to temporary glycopenic conditions showed an increased action potential for 15 minutes and returned to normal when reaching baseline dextrose levels. Dextrose injections can provide analgesia by correcting local glycopenia. However, confirming the occurrence of glycopenia requires techniques like microdialysis and other analytical methods for confirmation. These potential mechanisms shed light on how dextrose may exert its analgesic effects on sensory nerves and pain perception in various contexts.

The third, increasing extracellular dextrose levels with dextrose injections can lead to nerve hyperpolarization through another mechanism. For example, dextrose's activation of potassium channels can increase K+ conductivity, resulting in neuronal hyperpolarization. Increasing extracellular dextrose from normal to 0.5% in intestinal enterocyte cells leads to hyperpolarization through the Na/Dextrose cotransporter (SGLT1). However, SGLT1 has a less significant effect on intermembrane transport in neurons. Although the pain-reducing effect due to nociceptive fiber mechanisms hasn't been confirmed, the hyperpolarization effect is consistent with some reports of using D5 to reduce pain from certain chemotherapy effects.

Epidural injections of D5W can reduce the threshold for firing nociceptive C-fibers through one or

more of the mechanisms described above. However, these potential mechanisms, while informed by medical literature, remain speculative, have not been formally tested, and do not explain the temporal effects of reduced pain with serial injections. Long-term cumulative administration of serial epidural D5W injections may have a beneficial effect on pain management.

In summary, these potential mechanisms suggest that dextrose, when administered in specific contexts, may modulate pain perception through various sensorineural mechanisms, although further research is needed to confirm these effects and understand their full scope.

3.3. Clinical Evidence

Low Back Pain (LBP)

LBP is one of the most common reasons patients seek primary care providers. Approximately 80% of Americans experience LBP during their lifetime. It is estimated that 15% to 20% of patients experience persistent pain, with around 2% to 8% experiencing chronic pain. LBP is the second leading cause of work time lost, following only the common flu. The productivity loss from chronic LBP approaches $28 billion annually in the United States.[1]

Research using randomized control trial methods on LBP has been conducted using P2G and dextrose, involving injections at the ligamentous insertion points of the L4-S1 spinous processes, sacrum, and ilium. Although the outcome measures vary, the average is the percentage of participants reporting more than a 50% improvement in pain/disability scores at 6 months.[1]

Tendinopathy

Degenerative changes in tendinosis and ligamentosis due to sprains, strains, and chronic overload injuries can be addressed by restoring the connective tissue matrix to a normal state through prolotherapy. The strongest data supporting the effectiveness of prolotherapy for each musculoskeletal condition compared to control injections are for chronic, excessive tendon pain previously referred to as tendonitis and now more accurately termed tendinosis or tendinopathy to reflect its underlying pathophysiology. The basis of tendinopathy pathology is a non-inflammatory mechanism resulting from repetitive motion or excessive injury and is associated with painful degenerative tissue.[1]

Histopathological biopsy of tendons in patients undergoing surgery for painful tendinopathy shows thin, frayed, and brittle tendon collagen fibrils, separated from each other lengthwise and cross-sectionally, increased tenocytes with myofibroblastic differentiation (tendon repair cells), proteoglycan ground substance, and neovascularization. Classic inflammatory cells are usually absent. Although these aspects of tendinosis were first described 25 years ago, and experts have advocated for a change in nomenclature (from tendonitis to tendinosis), the misnomer "tendonitis" persists. Prolotherapy has been evaluated as a treatment for four tendon disorders: lateral epicondylitis, hip adductors, Achilles

tendinopathy, and plantar fasciitis.[1]

Animal model research on Achilles tendons showed no transient decrease in tensile strength on days 0, 5, or 10 after receiving 12.5% dextrose injections compared to saline injections or no injections. In another study by Ahn and colleagues, the Achilles tendons of sick mice injected with 20% dextrose showed significantly more fibroblasts on histological examination at week 4 compared to the control group, which had sick tendons but no injections. Research by Kim and colleagues reported that a single injection of 5% or 20% dextrose made with hypertonic saline (1100 mOsm) into the Achilles tendon of sick mice resulted in a significant increase in tendon diameter and the number of fibroblasts in the large field of view compared to equimolar saline (1100-mOsm). This indicates a non-osmolar dextrose-induced proliferation mechanism.[8]

Osteoarthritis

Prolotherapy has been assessed as a treatment for OA in the knee and fingers and is the subject of ongoing research. Treatment recommendations for allopathic and CAM (Complementary and Alternative Medicine) approaches to OA have been published, aiming to correct modifiable risk factors, manage symptoms, and modify the disease. While these modalities may help some patients, none have been proven to provide definitive pain control or disease modification for patients with knee OA.[1]

The Agency for Healthcare Research and Quality (AHRQ) recently evaluated the most common standard treatment options, including glucosamine, chondroitin, viscosupplementation, and arthroscopic debridement. These treatments have not shown effectiveness compared to a placebo. The high burden of knee OA and the lack of a cure continue to stimulate intensive research for new agents to modify the disease and control symptoms.[1]

Reeves and colleagues assessed prolotherapy as a treatment for knee and finger OA. Subjects with finger or knee pain and radiological evidence of OA were randomly assigned to receive 3 sessions of prolotherapy injections with 10% dextrose and lidocaine or lidocaine and bacteriostatic water (control group). In the finger OA trial, the intervention group showed significant improvement in "pain-with-motion" and "range-of-motion" scores compared to the control; pain scores at rest and with grip showed a trend toward improvement but did not reach statistical significance.[1]

In the knee OA trial, subjects in both groups reported significant improvements in pain scores and swelling, the number of bending episodes, and flexion range of motion compared to baseline, but without statistically significant differences between the groups. The twelve-month follow-up in both studies included increased radiological features of OA on plain radiographs: the researchers reported joint space narrowing and osteophyte reduction in the finger study and increased patellofemoral cartilage thickness in the knee study. These radiological findings may suggest disease-modifying properties of prolotherapy.[1]

Jahangiri and colleagues compared dextrose prolotherapy with steroid injections in a blinded, two-group trial for hand osteoarthritis. Participants in both groups with chronic thumb pain and osteoarthritis of the trapeziometacarpal joint (TMJC) were given intra-articular 1 mL and extra-articular 1 mL injections at months 0, 1, and 2. The effects were evaluated at month 6 using the Visual Analog Scale (VAS) 1–10, the Health Assessment Questionnaire Disability Index (HAQ-DI), and lateral pinch strength with a hydraulic pinch gauge. The results of the study showed that the dextrose prolotherapy group had better improvements in motion and range of motion flexion compared to the lidocaine group at month 6.[8]

3.4. Contraindications

Absolute contraindications for prolotherapy are few and include acute infections such as cellulitis, local abscess, or septic arthritis.[1,8] Relative contraindications include acute gouty arthritis, acute fractures, high-dose narcotic use over an extended period as it may lower immune response, and systemic corticosteroid use because it has the opposite effect of inflammation processes. [1,4] Active infections and cancer are contraindications for prolotherapy, as well as other diseases that can interfere with the healing process.[4] Linetsky and colleagues listed the contraindications for prolotherapy as follows:[7]

1. Allergy to anesthesia or proliferant solutions or their components such as dextrose, sodium morrhuate, or phenol.[7]
2. Acutely non-reduced subluxations or dislocations.[7]
3. Acute arthritis (septic or post-traumatic hemarthrosis).[7]
4. Acute bursitis or tendinitis.[7]
5. Capsular pattern of the shoulder and hip indicating acute arthritis with tendinitis.[7]
6. Acute gout or rheumatoid arthritis.[7]
7. Occurrence of neurological deficit.[7]
8. Request for sedation or a large amount of narcotics before or after therapy.[7]
9. Neoplastic lesions involving paraspinal muscle and bone structures.[7]
10. Severe exacerbation of pain or no improvement after local anesthetic block.[7]
11. Relative contraindications to prolotherapy include central spinal canal, lateral recess, and foraminal neural stenosis.[7]

3.5. Side effects

The main risk associated with prolotherapy is mild pain and minor bleeding caused by needle trauma.[1,8] Injection pain can be minimized with local anesthesia and the use of tumescent anesthesia.8

During injection, patients often report aching, fullness, and occasional numbness at the injection site. These side effects usually resolve on their own.[1] Post-injection pain recurrence during the first 72 hours after injection is clinically common, but its incidence has not been well documented. A study on knee osteoarthritis prolotherapy noted that 10% to 20% of subjects experienced such a flare. Burning pain is usually limited and generally responds well to acetaminophen (500-650 mg every 4 hours as needed). Although rare, the onset of severe post-injection pain may require treatment with narcotic medications. NSAIDs are not routinely used after the procedure but may be indicated if pain does not improve with other measures. Most patients with burning pain experience pain reduction within 5 to 7 days after injection; regular activities can be resumed at this time.[1,6]

3.6. Adverse events

Although prolotherapy performed by experienced clinicians appears safe, injections of ligaments, tendons, and joints with irritant solutions pose safety concerns. The theoretical risks of prolotherapy injections include mild headaches, allergic reactions, and infections or neurological damage (nerve). Injections should be performed using universal precautions, and patients should be screened if possible. Dextrose is very safe; it is FDA-approved for intravenous hypoglycemia treatment and calorie supplementation. Sodium morrhuate is a vascular sclerosant used in gastrointestinal procedures and venous sclerosing. Allergic reactions to sodium morrhuate are rare. Although P2G is not FDA-approved for any indications, it has not been reported in clinical trials to cause significant side effects or adverse events.[1]

Historically, a small number of significant complications related to prolotherapy have been reported. Associated with perispinal injections for back or neck pain using highly concentrated solutions, including 5 cases of nerve damage from irritation of the bone marrow and 1 death in 1959 after therapy with zinc sulfate for LBP. Currently, highly concentrated sulfate or prolotherapy solutions are commonly used. In a survey of 95 doctors using prolotherapy, there were 29 reports of pneumothorax after prolotherapy for back and neck pain, two of which required hospitalization for chest tubes, and 14 cases of allergic reactions, although none were classified as serious. Practicing prolotherapy with similar results for spinal prolotherapy: spinal headaches, pneumothorax, nerve damage, and spinal cord and disc injuries have been reported. Researchers concluded that these events are no more common with prolotherapy than with other spinal injection procedures. No serious side effects or adverse events have been reported for prolotherapy when used for peripheral joint indications.[1]

Dagenais and colleagues conducted a survey study of 308 doctors using prolotherapy. The results of the study reported a total of 472 adverse events, including 174 spinal headaches, 123 cases of

pneumothorax, 73 cases of systemic reactions, 54 cases related to nerve damage, 27 cases of bleeding, 29 cases of spinal cord insult, and 2 cases of disc injury.8 Complications such as injection site pain, pneumothorax, allergic reactions, and nerve damage have also been reported in a study by Dorman.[5]

3.7. Practical Aspects of Prolotherapy

Despite limited institutional support, interest in prolotherapy is growing, and it is being performed in increasing numbers. For several decades, prolotherapy has been mainly carried out outside mainstream medicine by independent physicians. More recently, multispecialty groups that include family physicians or sports medicine doctors, physical therapists, orthopedic surgeons, neurologists, or anesthesiologists have incorporated prolotherapy due to positive clinical experiences and research reports. Prolotherapy is considered a valuable procedure, especially for patients who have failed other treatments or those who are not surgical candidates.[1] The Florida Academy of Pain Medicine recommends considering the use of regenerative injection therapy (RIT), or prolotherapy, as a specific therapy for degenerative post-traumatic, overuse, and painful musculoskeletal conditions associated with connective tissue pathology.[7]

4. Conclusion

Prolotherapy is an injection therapy used for chronic musculoskeletal pain. Its use has a long history and known benefits. The principle of this therapy involves injecting into tendons, ligaments, or extra-intra articular areas. The exact mechanism of action of this therapy is not yet known. However, it is hypothesized to have different mechanisms depending on the proliferant or irritant solution used. Hypertonic dextrose solution works through local osmotic cell rupture, phenol-glycerin-glucose (P2G) irritates local cells, sodium morrhuate attracts inflammatory mediator chemotaxis, and sclerosing addresses pathological neovascularization associated with tendinopathy. This process is believed to enhance joint stability, biology, function, and ultimately reduce pain.

The choice of injection solution varies based on the condition, clinical severity, and practitioner preferences. However, some studies suggest that dextrose solution provides better results compared to other control solutions (saline; NaCl). Prolotherapy has also been found effective for musculoskeletal injuries such as lateral epicondylitis, low back pain (LBP), certain tendinopathies, and osteoarthritis. Recent research on osteoarthritis cases indicates that prolotherapy with dextrose solution results in clinically significant improvements in pain scores, function, and stiffness for knee osteoarthritis compared to saline injections and home exercises.

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Research Article

Gut-brain axis modulation by banana peel as the basic ingredient of the vegetarian jerky: A potential anti-depressant functional food

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Abstract: Functional foods can be natural foods or products with one or more particular elements that benefit health and well-being. A plant-based diet can minimize illness risk and stress and help maintain a healthy weight, making it a potential future development. Banana peel (Musa balbisiana Colla) already offers several nutritional properties such as flavonoid and tryptophan is a substrate for synthesizing serotonin (5-HT), making it an ideal basic ingredient for vegetarian jerky. In the present study, the Tail Suspension Test (TST) and Morris Water Maze (MWM) for depressive symptoms, and immunohistochemistry for 5-HT of ileocaecal and brain section were conducted. This post-test-only control group design study was conducted on 50 formalin-fixed and paraffin-embedded samples of ileocaecal and brain that were collected from male Wistar rats that were induced by chronic stress for four weeks and continued with intervention in the form of banana peel jerky (BPJ) for 4 weeks that was supplemented into the diet standard, with the following group divisions.: Groups I-normal and II-chronic stress groups were treated with 15 ml aquadest/kg/d. The test groups III-lower, IV-medium, and V-high doses received a diet supplemented with 15%, 30%, and 60% of BPJ. The results showed that the immobility time in TST and escape latency in the MWM test were significantly lower in the groups supplemented with 15% and 30% BPJ, respectively. We observed a significant association between 5-HT expression in the ileocaecal and brain and between the immobility time duration in TST and 5-HT expression.

Keywords: 5-HT expression; banana peel; brain; chronic stress; ileocaeca

1. Introduction

Happiness and psychological well-being have become two fascinating areas of study in both the social sciences and the health sciences during the last several decades. Anxiety and stress are common occurrences in everyday life that cannot be avoided by any individual. According to WHO, the incidence of stress is relatively high, with about 350 million people worldwide experiencing stress, It has ranked third in the global burden of illness since 2004 and is anticipated to continue to rise in 2030.[1]

Exposure to psychological stresses may result in eustress (mild/moderate) or distress (severe).

Eustress environments motivate people to pursue objectives in life, whereas distress conditions induce bodily and emotional pain Furthermore, there are changes in functional hormonal and neurotransmission, such as increased noradrenergic activity and cortisol levels. Chronic stress can cause structural changes in the brain, such as pyramidal cell atrophy and a decrease in hippocampal volume, as well as an increase in the activity of the hypothalamic-pituitary-adrenal axis (HPA axis), which has a positive correlation with the neurotransmitter serotonin. High levels of cortisol are associated with binding to serotonin receptors. 1A is distributed throughout the brain.[2]

Tryptophan is a precursor to various active metabolites, one of which is serotonin, which regulates mood [3], other studies have found that chronic stress can cause a sustained increase in cortisol, which reduces serotonin release.[4,5] This is due to the activation of the glucocorticoid tryptophan dioxygenase in the liver, which redirects tryptophan from serotonin synthesis to the tryptophan-kynurenine pathway.

Managing stress may be accomplished through a range of activities, such as sports, hobbies, visiting with family, and eating the correct foods. A diet heavy in complex carbohydrates (dietary fiber) can help to boost one's mood.[6] Complex carbohydrate molecules induce the body to generate the hormone insulin, which subsequently attracts numerous neutral amino acids (LAA) into the tissues, allowing the amino acid tryptophan to flow freely via the blood-brain barrier (BBB) to penetrate the BBB and is then utilized in the brain as a precursor to the production of serotonin.[7]

Bananas are high in complex carbohydrates and have been shown to have anti-anxiety properties. Banana peels have a high potential for further processing (the banana peel component accounts for around 40% of the overall weight of the banana) since they contain approximately 170,000 ng/g of phytoserotonin, whereas the flesh has approximately 35,000 ng/g. [8]

Because banana peels deteriorate fast after harvesting, they are further processed into food items that are more durable and have a low water content, one of which is jerky. There haven't been a lot of studies done on the association between food quality and mental health characteristics. Based on this context, this study attempts to appropriately formulate shredded kepok yellow banana peel so that it can be used as a reference for healthy diet choices that can provide protective effects on brain and mental health by increasing the availability of tryptophan in the body for use in serotonin synthesis. It may be worthwhile to investigate whether the consumption of banana peel jerky after chronic stress may affect behavior and serotonin expression in the ileocaecal and brain. Because BPJ contains neurotransmitters, particularly serotonin and its precursor tryptophan, which are known to have neurobehavioral effects, it is possible that behavioral alterations can be linked to them.

This study aimed to assess the relationship between 5-HT expression in the ileocaecal and brain and the duration of immobility in TST, It was a reliable procedure for detecting symptoms of depression in

rats.

2. Methods

2.1. Research design

Following ethical permission from the Islamic University of Indonesia's Faculty of Medicine (approved number 19/Ka.Kom.Et/70/KE/II/2021). This is a pure experimental (true experiment) study using a post-test-only control group design. Male Wistar rats (Rattus norvegicus) were used in this investigation. The male gender was chosen to eliminate hormonal impacts that might affect research outcomes. Mice in a healthy state, as determined by their active movements, 2-3 months old, and weighing 180-200g, are included in the sample, while rats that are weak or dead at the time of the research are excluded. Sebanyak 36 ekor tikus terbagi dalam 5 kelompok, yaitu Groups I-normal and II-chronic stress groups were treated with 15 ml aquadest/kg/d. The test groups III-lower, IV-medium, and V-high doses received a diet supplemented with 15%, 30%, and 60% of BPJ. Groups II-V rats got a 4-week chronic stress induction intervention followed by a 4-week treatment diet.

2.2. Experimental protocol

The independent variable is the composition of BPJ on a standard diet, and the dependent variable is serotonin expression in the ileocaecal and brain of rats that were previously induced with chronic stress for 4 weeks and then given BPJ supplementation to a standard diet at various doses, including 15%, 30%, and 60%. Chronic stress induction includes moist bedding (250 mL water per cage), a water jet (4^0C), cage tilting (45^0), hot water steam, and sawdust removal. Before the termination stage, behavioral testing was performed to determine the efficacy of the 4-week BPJ intervention on overcoming the influence of the 4-week chronic stress induction. Immediately after the last behavioral test, the rats will be anesthetized with ketamine 1 ml/kg body weight (1 ml ketamin-HCl equivalent to ketamine 100 mg; PT Guardian Pharmatama, Bogor, Indonesia).

2.3. Behavioral tests

Tail Suspension Test (TST)

The rats were hung by the tail 60 cm above the floor using cotton yarn immediately after the OFT, or 72 hours following LPS administration. For the latter 4 minutes of a total 6-minute session, the time each rat stayed motionless was recorded (in seconds).

The Morris Water Maze (MWM)

The Morris water maze test was conducted according to the protocols described elsewhere [9–11]. A huge, white-painted circular pool with a diameter of 150 cm and a height of 40 cm served as the test apparatus. Water was poured into the pool to a depth of 18 cm. A white circular platform was put 2 cm below the water's surface. To make the water opaque, fresh milk was poured, which served to conceal the platform. A video camera was installed above the pool's center, and the image of the animal's movements was sent to a laptop computer nearby. The pool was split into four imaginary quadrants of equal size. Four equal-distance starting spots were designated along the pool's circumferential wall. The test began on the last day of the BPJ intervention when the platform in the circular pool was placed.

Preparation of paraffin section

The resected ileocaecal organs and brain are soaked in a buffered formalin solution before IHC (immunohistochemistry) preparations are produced. The IHC process was founded on [12] and was performed to visualize and compare the distributions of serotonin in healthy and diabetic rats. Anti-serotonin (Sigma Chemicals Co, St Louis, MO, USA) antibodies and Fine Test rabbit-DAB (Poly-HRP) were used to detect the target proteins in paraffin sections of the ileocaecal and brain.

Preparation of slides

The paraffin sections were sectioned using a manual rotary microtome (Leica RM 2235, USA) and placed on silane-coated slides (Merck, Darmstadt, Germany) before being kept at ambient temperature. The antigen retrieval and antigen detection slides were then deparaffinized in xylene, followed by tissue rehydration in graded ethanol, 2 minutes of pure water, and 3 minutes of tris buffered saline (TBS). After 20 minutes of antigen collection in citrate buffer pH 6, the slide was put in TBS. The tissue slices were then treated for 1 hour at 37 °C with blocking serum to prevent non-specific immunostaining.

Secondary detection of the primary antibody

Primary antibody (rat serotonin monoclonal antibody (5HT), Sigma Chemicals Co, St Louis, MO, USA, working dilution 1:6000 in 10 mmol sodium phosphate, pH 7.4, salt 0.9% (PBS)) was incubated for 1 hour at 37 °C. The slides were washed three times each with TBS and secondary Trekkie Universal Link Antibody, then incubated for ten minutes. The secondary antibody was washed three times for five minutes with PBS, incubated for 30 minutes at 37 °C with poly-Goat Anti-Rabbit IgG HRP, and then washed three times with PBST buffer. A solution chromogen, 3,3′-diaminobenzidine (DAB); 1 ml DAB working solution = 50 l Reagent A, 50 l Reagent B, and 900 l DAB substrate were created and made accessible in KITS.

Counterstaining

For 1-3 minutes, a hematoxylin Meyer's solution was diluted with distilled water (1:4). For staining, the slides were immersed in a hematoxylin solution. The slides were then cleaned with running water

and dehydrated in ethanol. Finally, the slides were washed with xylol, mounted in Frontier Duo EFH (Canada), and taken with a digital camera using an Olympus CX21/Optilens Optilab Standard light microscope (Carl Zeiss, Oberkochen, Germany).

For immunohistochemical assessment, serotonin expression in each sample was assessed semi-quantitatively according to the modified Kaemmerer method.[13] Immunoreactive Score (IRS) is scored by multiplication percentage of positive cells score with the intensity of staining score produced in that cell (Table 1). The percentage of positive cells score based on: 0 = no positive cells, 1 positive cell less than 30 %, 2 positive cells 30 %- 60%, and 3 positive cells more than 60%. The intensity of staining score produced in that cell indicator is based on: 0 = no color reaction, 1 = light intensity, 2= medium intensity, and 3 = strong intensity. Positive cells with anti-MT-3 indicated a brcoloror.[14]

Table 1. The IRS semiquantitative scale is a product of the percentage of the positive cells score (A) with the intensity of staining score (B), becoming an immunoreactive score (IRS) = AxB, modified kaemmerer method [13]

A	B	AxB
0 = there are no positive cells	0 = no color reaction	0-1 = negative
1 = positive cells <10%	1 = low color intensity	2-3 = low
2 = positive cells 11%-50%	2 = medium color intensity	4-8 = medium overexpression
3 = positive cells between 51%-80%	3 = strong color intensity	9-12 = strong overexpression
4 = positive cells >80%		

Statistical Analysis

Data obtained from uji perilaku menggunakan metode TST akan diperoleh data berupa durasi imobilitas dan MWM escape latency, serta dari analisa IHC akan diperoleh IRS scores of 5-HT was displayed in averages and standard deviation. Data were analyzed using the Statistical Package for the Social Sciences (SPSS-20) program. The Duncan Multiple Range Test post hoc test was used to measure the data of 5-HT comparison between immobility duration and the correlation between groups analyzed using Spearman.

3. Results and Discussion

3.1. Behavioral effects of the post-treatment of BPJ after stress induction

MWM activity is indicated as a time to identify the hidden platform in the control and banana peel floss-supplemented animals in Figure 1. Learning is the capacity to add knowledge to the brain, whereas memory is the ability to recall what was learned. Learning and memory include the entire brain [15] . However, specialized learning necessitates the use of specific areas of the brain. As a result, a chemical that stimulates the central nervous system and raises attentiveness will boost learning and memory. MWM hidden platform is a test of visuo-spatial learning and memory. This learning process

will be disrupted if the hippocampus is disrupted as a result of stressful conditions, where uncontrolled stress can interfere with various memory tasks that rely on the hippocampus, Stress can change synaptic plasticity and the activation properties of hippocampal neurons, which structurally causes changes in neuronal morphology, suppressing proliferation. nerves, and reducing hippocampal volume.[16] The behaviors scored include swim latencies during acquisition, visible platform task, and quadrant duration during the probe trial. Following the consumption of BPJ with varied dosages showed that appropriate dosages of BPJ reduced cognitive impairment caused by the chronic stress regimen. The rats were exposed to chronic stress and fed a control diet supplemented with 30% BPJ had a considerably reduced escape latency, but all BPJ dosage interventions did not differ significantly from Group I. They also spent less time in the target quadrant than the other group.

TST is a behavioral test to examine depressive-like behavior in the animal (Table 3). The depressive-like behavior was measured using the immobility duration, which expresses the degree of depression or tension. In our study, experimental groups without BPJ supplementation exhibited depressive-like behavior, evidenced by higher immobility time when compared to experimental groups with BPJ supplementation. A control diet with 15% BPJ showed an optimal effect on the reduction of immobility time in TST. Lower immobility time showed that the rats actively pursue escape-directed behaviors, suggesting lower depressive-like behavior. Several studies suggested the possibility that banana peel facilitated serotonergic transmission by inhibition of monoamine oxidase enzyme, and it elevated the concentration of norepinephrine and serotonin in the brain.[12,17] A previous study showed that rats administered with pulp and peel extract of *Musa sapientum* had significantly decreased immobility time, as observed in the Forced Swim Test, which indicated an antidepressant-like effect.[18] Formerly, it has been reported that phenolic phytochemicals in bananas reduce neurotoxicity, *Musa paradisiaca* reduced the level of malondialdehyde, which indicated a decrease of free radicals, showing that the antioxidant effect of banana fruit may be protecting the brain from oxidative damage.[17]

Table 2. The mean escape latency time in Morris Water Maze

Groups	Mean escape latency time in MWM (sec.)	Time spent in the target quadrant in MWM (sec.)			
		Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4
I	75.94 ± 21.95^{a}	86.60 ± 34.41^{a}	49.00 ± 43.51^{a}	88.60 ± 42.12^{ab}	65.40 ± 51.48^{a}
II	94.88 ± 21.87^{b}	88.60 ± 36.96^{a}	103.20 ± 23.69^{b}	102.80 ± 38.46^{a}	83.80 ± 33.14^{a}
III	82.14 ± 21.61^{b}	100.83 ± 45.49^{a}	108.60 ± 25.49b	40.60 ± 24.40^{bc}	61.80 ± 30.61^{a}
IV	51.89 ± 18.20^{a}	92.00 ± 36.38^{a}	29.20 ± 11.03^{a}	20.67 ± 14.73^{c}	60.17 ± 54.36^{a}
V	82.58 ± 34.82^{b}	110.40 ±21.47^{a}	54.40 ± 43.71^{a}	78.40 ± 56.96^{ab}	68.83 ± 37.38^{a}

MWM, Morris Water Maze; Groups I-normal and II-chronic stress groups were treated with 15 ml aquadest/kg/d. The test groups III-lower, IV-medium, and V-high doses received a diet supplemented with 15%, 30%, and 60% of BPJ. Each value represents mean±SD. Means with different superscripts in the same column significantly differ ($p \leq 0.05$) according to the Duncan Multiple Range Test. In Time spent in the target quadrant in MWM data.

Table 3. Effect of BPJ on the immobility duration in the TST

Groups	Immobility duration (sec.)
I	60.00 ± 21.70[a]
II	115.40 ± 70.57[b]
III	38.67 ± 24.91[a]
IV	49.67 ± 25.42[c]
V	47.67 ± 33.26[a]

3.2. The 5-HT expression in ileocaecal and brain after 4 weeks of BPF administration after chronic stress induction

In mammals, the neurological system in the brain produces serotonin, which regulates behavior by decreasing hunger, boosting energy expenditure, and increasing sympathetic drive to brown adipose tissue. [19] In addition to this central pathway, several studies have shown that peripheral serotonin is a factor that can increase nutrient absorption and storage, with glucose and fatty acids, in particular, stimulating the release of serotonin from the duodenum, increasing intestinal peristalsis and nutrient absorption. [20]. Peripheral serotonin influences multiple metabolic networks through various serotonin receptors, increasing food absorption and storage while reducing wasteful cycles/thermogenesis. Tph1 in EC cells of the gastrointestinal tract primarily synthesizes circulating serotonin; the action of the cell and surrounding nutrients control expression in EC cells.[20]

This study was conducted to examine the effect of BPJ for 4 weeks after CMS induction of rats on 5-HT expression scores in the ileocaecal and brain of Wistarstar rats. Based on statistical tests, the mean expression of 5-HT in Group I was 7.73, in the treatment Group III had a higher mean expression of 5-HT than IV and V, but statistically significant compared to V and I groups (Figure 3 and Figure 4). This is due to the presence of the amino acid tryptophan in BPJ, which contributes to the synthesis of serotonin in enterochromaffin cells. Dietary tryptophan can be metabolized by host cells in immune cells and epithelia cells (kynurenine pathway) and in enterochromaffin cells (serotonin pathway).[21] The tryptophan content of BPJ in this study was 46.04%. Carbohydrate molecules (glucose, fructose, and sucrose) could stimulate serotonin production in colon and duodenum EC cells. The theory is consistent with the findings of this study, which found that the amounts of carbs and dietary fiber in BPJ were 56.59% and 27.66%, respectively, implying that availability can be directly linked. BPJ nutrition with nutrient availability and serotonin synthesis.[22]

During the four weeks of chronic stress induction in this investigation, the majority of the tryptophan from the regular diet was directed to kynurenine production. Long has researched the alteration in balance between the kynurenine and serotonin pathways in tryptophan metabolism. The major metabolic pathway for tryptophan is the kynurenine pathway, which is performed by the

enzyme indoleamine 2,3-dioxygenase (IDO).[23] Under chronic stress, the production of proinflammatory cytokines stimulates IDO, which increases the kynurenine route while depriving the serotonin pathway of tryptophan and decreasing serotonin synthesis. Reduced serotonin synthesis might be linked to the depression hypothesis.[24] Chronic stress-induced hippocampal shrinkage may be related to an imbalance in neurotoxic/neuroprotective activity. The presence of tryptophan in BPJ may supply the body's need for tryptophan, which can then be utilized for serotonin synthesis rather than kynurenine production.

The results of microscopic images of the ileocecal and brain stained with IHC in Group III, IV, IRS measurements were carried out using 400x magnification in five fields of view (Table 2). The results indicated that all the groups showed individual cells with positive IHC staining for serotonin both in the ileocaecal and the brain (Figure 3 and Figure 4). Serotonin expression in the brain was considerably higher in the usual diet supplemented with BPJ 15% group compared to groups supplemented with BPJ 60% and not statistically different from BPJ 30%. According to the findings, the expression of serotonin in the brain caused by dietary tryptophan is not similar to graded dosages of BPJ. The hypothesis was that food-derived and purified tryptophan preparations have distinct effects on serotonin synthesis in the brain, however, the limitation of this study was that no group received pure tryptophan intervention. Pure tryptophan has been shown to boost serotonin levels in the brain, however, this is not the case with tryptophan derived from other sources.

When viewed from the rate-limiting enzyme for serotonin synthesis, the tryptophan hydroxylase (TPH), with isoforms TPH1 and TPH2. TPH2 synthesizes serotonin in the brain, whereas TPH1 is expressed in the intestine and other peripheral tissues [25], Chronic stress conditions can inhibit the expression of TPH1 and TPH2 in brain tissue and directly reduce serotonin concentrations [26], Therefore, in this investigation, the potential of inducing chronic stress for 4 weeks had a substantial influence on raising the stress of rats, such that the rate of the TPH2 enzyme could not perform efficiently even when an increasing amount of BPJ was administered. The optimal dose of BPJ supplementation on a regular diet was 30%, based on serotonin expression in the ileocaecal. The findings support the theory that serotonin synthesis in the gut is regulated by the activity of the rate-limiting enzyme TPH1.

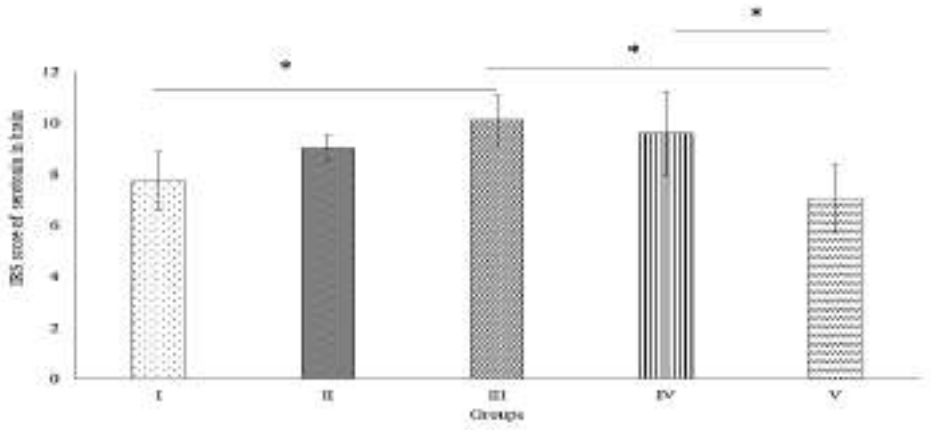

Figure 3. Differences in serotonin expression scores between groups by IRS in the brain. Groups I-normal and II-chronic stress groups were treated with 15 ml aquadest/kg/d. The test groups III-lower, IV-medium, and V-high doses received a diet supplemented with 15%, 30%, and 60% of BPJ

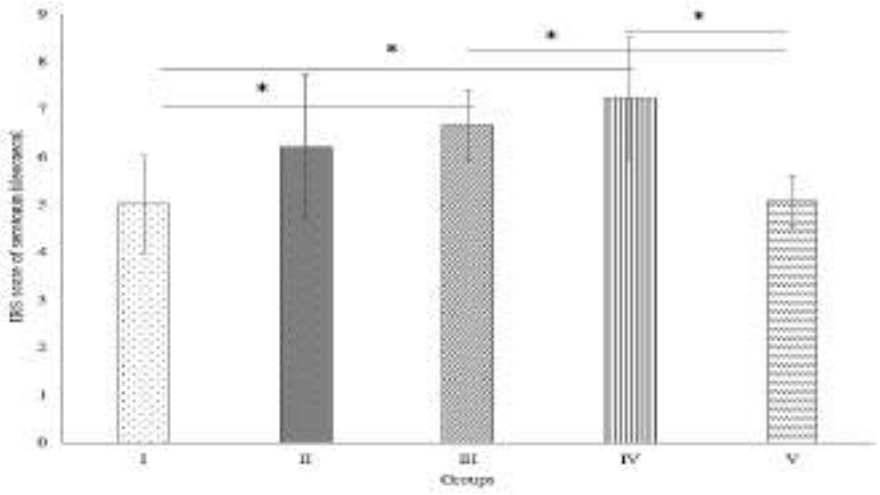

Figure 4. Differences of serotonin expression scores between groups by IRS in ileocaecal. Groups I-normal and II-chronic stress groups were treated with 15 ml aquadest/kg/d. The test groups III-lower, IV-medium, and V-high doses received a diet supplemented with 15%, 30%, and 60% of BPJ

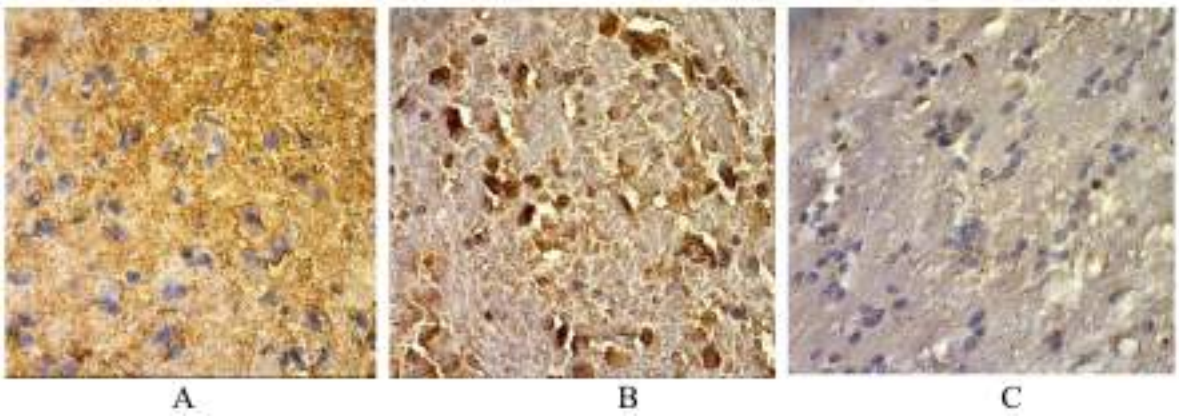

Figure 5. Microscopic photographs of the brain stained with IHC, show weak (A), intermediate (B), and strong positive (C) serotonin expression

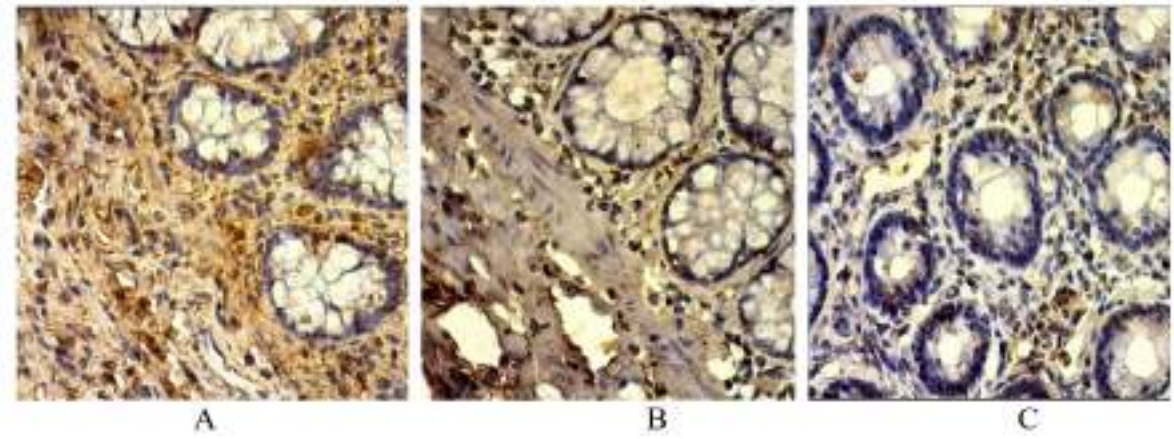

Figure 6. Microscopic photographs of the brain stained with IHC, show weak (A), intermediate (B), and strong positive (C) serotonin expression

3.3. Correlation between behavioral parameters and expression of serotonin in rat's ileocaecal and brain

Table 4 presents the correlation between serotonin expression score (IRS) in the ileocaecal and brain and the results of the immobility duration test in the examined group (without Group I). No statistically significant correlation was observed between immobility duration in TST in both serotonin expression scores in the ileocaecal and brain. The expression of serotonin in ileocaecal significantly positively correlates with the expression of serotonin in the brain. These findings confirm the Gut-Brain Axis concept, according to which the gut microbes play a particular role in behavior, emotion, and cognition. The impact of the digestive tract on brain function has long been the focus of research, where in some conditions such as IBS it has a reciprocal influence on psychiatric conditions such as anxiety. The existence of this connection shows the relevance of the Gut-Brain Axis in pathophysiology and is an

interesting research target for therapy development. [27] Neurotransmitters such as serotonin, adrenaline, and dopamine may play a significant role in controlling the Gut-Brain Axis, which is supported by gut bacteria. The presence of dietary fiber in BPJ also benefits the gut microbiota, which aids in the coordination of the Gut-Brain Axis, which can provide nutritional effects on the brain, particularly fiber, which is acted upon by certain bacteria to produce various metabolites that improve health. These compounds, as well as the vagus nerve, immune system, gut hormones, and the kynurenine pathway, have been hypothesized as mechanisms mediating microbiota-brain crosstalk.[28]

Table 4. Spearman's rank correlation coefficients(R) for the variables tested

		5-HT expression score		
		Ileocaecal	**Brain**	**Immobility duration in TST as a manifest depression-like symptoms**
Serotonin expression score	Ileocaecal		0.693 (*P*= 0.026*)	-0.293 (*P*=0.412)
	Brain			0.152 (*P*=0.675)

*P, statistically significant

4. Conclusion

In chronic stress rat models, the tryptophan level of beef jerky prepared from banana peels increases serotonin expression in the ileocaecal and brain. The findings of the behavioral test that resulted in depression showed that at an optimum dose of 30% BPJ combined with jerky, the effects of prolonged stress induction may be overcome.

5. Acknowledgments

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Research Article

The effect of administration of Longan Leaf Extract (Dimocarpus longan Lour.) on triglyceride, LDL and HDL levels of wistar male rat with high-fat diet

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Abstract: Changes in lifestyle in developing countries have an impact on changes in one's diet and food consumption habits. Poor diet causes health problems, especially cardiovascular disease. Consumption of ready-to-eat foods that are high in cholesterol can cause disruption of fat metabolism in the blood and have an impact on the occurrence of hyperlipidemia, hypercholesterolemia, cardiovascular disease, and diabetes mellitus, thus causing an increase in mortality. Longan leaves are known to contain bioactive compounds such as flavonoids, saponins, and quercetin which have the potential to reduce triglyceride levels. The aim of this study was to prove that longan leaf extract can reduce triglyceride and LDL levels, as well as increase HDL levels in male Wistar rats fed a high-fat diet. This research is a pure laboratory experiment using the Post-Test-Only Control Group Design method. The sample for this study were 24 male Wistar rats (Rattus norvegicus) which were divided into four groups: the negative control group, the group of rats that were given a high-fat diet, the group of rats that were given a high-fat diet and longan leaf extract at a dose of 200 mg/kg BW, and the rat group were given a high-fat diet and longan leaf extract at a dose of 400 mg/kg BW. The results of the One-Way ANOVA test for TG and LDL levels showed a significance of $p = 0.001$ ($p < \alpha$) with $\alpha = 0.05$. The results of the Mann-Whitney U test for HDL levels showed a significant difference with $p = 0.004$ between groups of animals that were given a standard diet and groups of animals that were given a high-fat diet, $p = 0.005$ in groups of animals that were given a high-fat diet and groups of animals that were given a high-fat diet plus longan leaf extract at a dose of 200 mg/kg BW, and the animal group was given a high-fat diet while the experimental animal group was given a high-fat diet plus longan leaf extract at a dose of 400 mg/kg BW. The conclusion of the study showed that administration of longan leaf extract reduced triglyceride and LDL levels, and increased HDL cholesterol levels in white rats fed a high-fat diet.

Keywords: HDL; high-fat diet**;** LDL; Longan leaves (Dimocarpus longan Lour); TG

1. Introduction

The prevalence of cardiovascular disease is increasing over time. One in three people in the world, in 2001 died from cardiovascular disease. This means that 1/3 of the world's population is at high risk of developing cardiovascular disease. Data from the World Health Organization (WHO) in 2012 shows that 17.5 million people in the world died from cardiovascular disease or 31% of the 56.5 million deaths worldwide. Of this figure, it is estimated that 7.3 million were caused by Coronary Heart Disease (CHD), while in Indonesia the Sample Registration System (SRS) Survey in 2014 showed that Coronary Heart Disease (CHD) was the highest cause of death at all ages after stroke, namely 12.9%.[1]

Coronary heart disease (CHD) is a condition that occurs when plaque builds up in the coronary arteries which supply oxygen-rich blood to the heart muscle. Plaque consists of fat, cholesterol, calcium and other substances found in the blood. Cholesterol is a type of lipid that can be found in blood plasma and is considered normal if it is at 200-240 mg/dl (1dl = 100ml) of blood serum. Cholesterol and triglycerides in the blood are wrapped in fat-transporting proteins called lipoproteins. LDL and very low-density lipoprotein (VLDL) carry fat to body cells, including arterial endothelial cells, the oxidation of cholesterol and triglycerides causes the formation of free radicals which are known to damage endothelial cells.[2] The main risk factors for CHD include dyslipidemia. Dyslipidemia is a condition characterized by abnormalities in lipid metabolism in the blood, where there is an increase in cholesterol levels, LDL (Low-Density Lipoprotein), triglyceride levels, and a decrease in HDL (High-Density Lipoprotein) levels. According to the latest molecular medicine research, it was found that the most dangerous type of dyslipidemia is atherogenic dyslipidemia. Atherogenic dyslipidemia LDL cholesterol deposits on the arterial walls are one of the causes of endothelial dysfunction as an initial process for the formation of atherosclerotic plaque.[3]

A meta-analysis of research on hundreds of patients over 10 years shows that an increase in triglyceride levels of 1 mmol/L can increase the risk of cardiovascular disease by 32% in men and 76% in women. According to research results[4], it is also stated that patients at high risk of CHD with reduced LDL cholesterol levels still have a risk of CHD if HDL cholesterol levels are still very low. HDL cholesterol plays an important role in hyperlipidemia. Increasing HDL cholesterol levels is more critical than reducing LDL cholesterol levels in hyperlipidemia. A high-fat diet has a big influence on the concentration of HDL cholesterol and LDL cholesterol in blood plasma.[5]

Increased levels of triglycerides, LDL cholesterol and decreased levels of HDL cholesterol are strong risk factors for coronary heart disease. Therefore, efforts need to be made to control triglyceride and LDL cholesterol levels. One way is by modifying the diet, such as limiting cholesterol and fat consumption and consuming foods that contain cholesterol-lowering substances such as flavonoids, saponins and quercetin.[6]

One of the natural ingredients that can reduce triglyceride levels. LDL and increased HDL are

longan leaves (Dimocarpus longan Lour). Longan is a plant that is widely known and widely cultivated in Indonesia. Longan plants have bioactive compounds that can be utilized, especially in the leaves. Research by Nina (2015) shows the presence of bioactive compounds such as flavonoids, polyphenols, tannins and saponins.[7] Flavonoids are useful for improving blood circulation, especially for preventing blockage of blood vessels, reducing cholesterol levels and fat accumulation in blood vessels, as well as antioxidants which are useful for getting rid of free radicals, while saponins are useful for preventing fat reduction, helping to improve the immune system, improving blood levels. blood sugar and slows the blood clotting process which may help prevent or treat atherosclerosis.[8]

2. Methods

2.1. Time and place of research

The research was conducted from March 2019 to December 2019, taking place at the Biochemistry Laboratory, Faculty of Medicine, Hang Tuah University, Surabaya. Examination of triglyceride levels was carried out in the Biochemistry laboratory at the Faculty of Medicine, Hang Tuah University, Surabaya.

2.2. Longan leaf extract

Longan leaves (Dimocarpus longan Lour) were picked from the village of Longan Gunung Anyar, Surabaya City, East Java, in the amount of 750 gr. Longan leaves (Dimocarpus longan Lour) were then taken to the Biochemistry Laboratory, Faculty of Medicine, Hang Tuah University, Surabaya. Longan leaves (Dimocarpus longan Lour) are then washed with running water, chopped and drained. Longan leaves (Dimocarpus longan Lour) are then dried with the help of a drying cabinet. The dried longan leaves (Dimocarpus longan Lour) were then weighed for dry weight.

Longan leaf simplicia (Dimocarpus longan Lour) was crushed into powder using a blender and sifted through 40 mesh sieve and then the powder weight was weighed at 315 gr. Longan leaf simplicia (Dimocarpus longan Lour) is stored in a tightly closed container. Extraction of longan leaf Simplicia (Dimocarpus longan Lour) was carried out by reflux method using 96% ethanol with a ratio of 1:10. The extraction results (macerate) are collected and after that, the macerate is concentrated using a rotary evaporator to obtain a thick extract and then evaporated using a water bath to remove the remaining ethanol solvent. The yield of ethanol extract from longan leaves (Dimocarpus longan Lour) was 74.5 gr.

2.3. Research implementation stage

A total of 32 Rattus novergicus rats that had been randomly selected were grouped into 4 groups, each group containing 8 rats (Rattus norvergicus), namely the negative control group, rats that received

standard feed and mineral water for 28 days then triglyceride levels were measured. The positive control group, mice that received a high-fat diet for 28 days, then had their triglyceride levels measured. Treatment group 1, rats that received a high-fat diet for 14 days, then on day 15 received a high-fat diet accompanied by administration of longan leaf extract at a dose of 200 mg/kg BW through a sonde until day 28, then triglyceride levels were measured and the group treatment 2, rats that received a high-fat diet for 14 days, then on day 15 received a high-fat diet accompanied by administration of longan leaf extract at a dose of 400 mg/kg BW through a sonde until day 28, then triglyceride levels were measured.

Sampling of rat blood was taken from the heart of rats that had previously been anesthetized by intramuscular injection of ketamine at a dose of 60-75 m/kg BW. The blood was centrifuged with a serum separator for 5 minutes at a speed of 4000 rpm to obtain the serum. Serum triglyceride levels were analyzed using colorimetric enzymatic methods.

3. Results and Discussion

3.1. Triglyceride levels

Table 1. Triglyceride levels of the experimental animal group

No.	Groups			
	K(-) (mg/dL)	K(+) (mg/dL)	P1 (mg/dL)	P2 (mg/dL)
1	77	58	82	45
2	21	57	38	34
3	66	82	101	37
4	63	189	75	40
5	68	110	18	62
6	45	53	23	88
Total	340	649	319	261
Mean	39,17	91,50	56.17	51,00
Std. Deviation	22,702	52,409	34,406	20,649

Note:
K(-): Group of Experimental Animals Given Standard Feed
K(+): Animal Experiment Group Given High Fat Diet
P1: Group of experimental animals given a high fat diet and longan leaf extract 200 mg/kg BW
P2: Group of experimental animals given a high fat diet and longan leaf extract 400 mg/kg BW

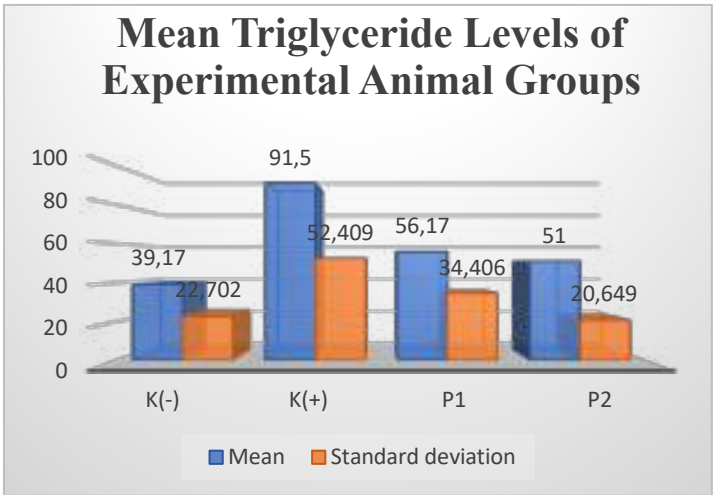

Figure 1. Mean triglyceride levels of experimental animal groups

According to Table 1 and Figure 1, the average triglyceride level of the experimental group that was given a standard diet was 39.17 mg/dL, which was lower than the group of experimental animals that were given a high-fat diet. The latter group had an average triglyceride level of 91.50 mg/dl. Meanwhile, the experimental animal group that was given a high-fat diet and 200 mg/kg BW of longan leaf extract had an average triglyceride level of 56.17 mg/dL, which was lower than the experimental animal group that was given a high-fat diet but slightly higher than the experimental animal group that was given a high-fat diet with longan leaf extract 400 mg/kg BW. This group had an average triglyceride level of 51.0 mg/dL.

Table 2. One-way anova test results for blood triglyceride levels in the experimental animal group

	Sum Of Sguares	Df	Mean Square	F	Sig
Between Groups	9124,792	3	3041,597	2,497	0,001

According to Table 2, the One-Way ANOVA test results demonstrated a significance of p = 0.001 ($p < \alpha$). This indicates that there was a significant impact of longan leaf extract on the triglyceride levels of the experimental groups. The groups that were studied included the one that was given a standard diet, the one that was given a high-fat diet, and the experimental group that received a high-fat diet along with longan leaf extract.

Table 3. Post hoc analysis of triglyceride levels

(I)	(II)	Mean Difference	Sig
K(-)	K+	52.3333	0.003
	P1	17.0000	0.409
	P2	11.8333	0.564
K(+)	P1	35.3333	0.001
	P2	40.5000	0.000
P1	P2	5.16667	0.800

According to Table 3, the results indicate that there was a significant increase in blood triglyceride levels in both the group of experimental animals that were given a standard K(-) diet and the group that were given a high-fat K1 (+) diet. However, in the group K (+) which was given a high-fat diet supplemented with longan leaf extract at a dose of 200 mg/Kg BW (P1) and in the group that was given the same high-fat diet but with a dose of 400 mg/Kg BW (P2), there was a significant decrease in triglyceride levels.

3.2. Blood LDL cholesterol levels

Table 4. Blood LDL cholesterol levels of experimental animal groups

No.	Groups			
	K(-) (mg/dL)	K(+) (mg/dL)	P1 (mg/dL)	P2 (mg/dL)
1	7	18	11	9
2	13	15	12	11
3	13	13	11	12
4	11	20	13	10
5	12	25	16	13
6	10	20	11	10
Total	66	111	74	65
Means	11,00	18,50	12,33	10,83
Std. Deviation	2.28035	4.23084	1.96638	1.47196

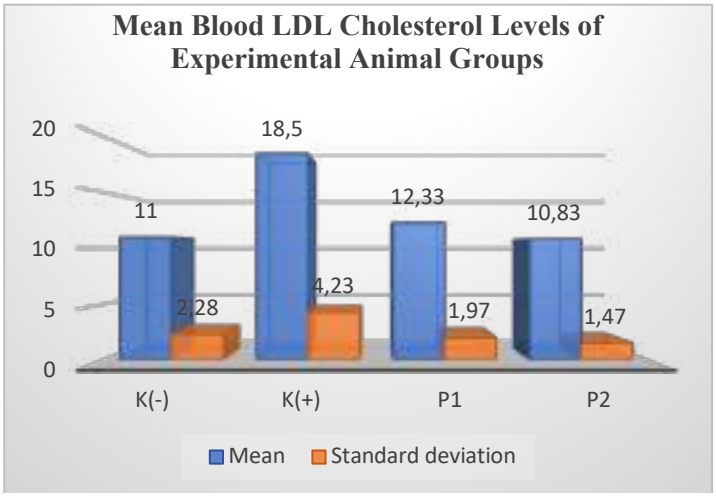

Figure 2. Mean blood LDL cholesterol levels of experimental animal groups

Note:
K(-): Group of Experimental Animals Given Standard Feed
K(+): Animal Experiment Group Given High Fat Diet
P1: Group of experimental animals given a high fat diet and longan leaf extract 200 mg/kg BW
P2: Group of experimental animals given a high fat diet and longan leaf extract 400 mg/kg BW

Based on Table 4 and Figure 2, the average blood LDL cholesterol level of the experimental group of animals that received a standard diet was 11 mg/dL, which was lower than the experimental group that received a high-fat diet, with an average level of 18.5 mg/dL. The experimental group that received

a high-fat diet along with 200 mg/kg BW of longan leaf extract had an average blood LDL cholesterol level of 12.33 mg/dL, which was lower than the group that only received a high-fat diet but slightly higher than the group that received 400 mg/kgBW of longan leaf extract along with a high-fat diet, with an average blood LDL cholesterol level of 10.83 mg/dL.

One-Way Anova Test Results for Blood LDL Cholesterol Levels

Table 5. One-way ANOVA test results for LDL cholesterol in the blood of the experimental animal group

ANOVA	Sum of Square	df	Mean Sqare	F	Sig.
Between groups	235.667	3	78.556	10.786	0,001

Based on table 5, it can be seen that the results of the One-Way ANOVA test show a significance of $p = 0.001$ ($p < \alpha$), meaning that there is a significant effect of giving longan leaf extract on blood LDL cholesterol levels between the group of experimental animals given a standard diet and the group of experimental animals given a high-fat diet, and a group of experimental animals were given a high-fat diet and longan leaf extract at a dose of 200 mg/KgBW and 400mgKgBW.

Post Hoc LDL Cholesterol Levels in Experimental Animal Group

Table 6. Post hoc test results for blood LDL cholesterol levels

(I)	(II)	Mean Difference	Sig
K(-)	K+	7,500	0,001
	P1	1,333	0,402
	P2	0,1667	0,916
K(+)	P1	6,167	0,001
	P2	7,667	0,001
P1	P2	1,500	0,347

The results from table 6 showed that when experimental animals were given a standard K(-) diet or a high-fat K1(+) diet, their LDL cholesterol levels in blood significantly increased. However, in the K(+) group, both P1 and P2 subgroups, which were given a high-fat diet supplemented with longan leaf extract at doses of 200 mg/Kg BW and 400 mg/Kg BW respectively, showed a significant decrease in blood LDL cholesterol levels.

3.3. Blood HDL cholesterol levels

Based on Table 7 and Figure 3, the average HDL cholesterol level in the group of experimental animals that were given a standard diet was 30.16 mg/dL, which was higher than the group of animals that were given a high-fat diet (19.50 mg/dL). When an experimental animal group was given a high-fat diet and 200 mg/kg BW longan leaf extract, their average blood HDL cholesterol level was 28.33 mg/dL. This was higher than the experimental animal group that was only given a high-fat diet, but

slightly lower than the group that received a high-fat diet and longan leaf extract 400 mg/kgBW, which had an average blood HDL cholesterol level of 29.00 mg/dL.

Table 7. Blood HDL cholesterol levels of experimental animal groups

No	Groups			
	K(-) (mg/dL)	K(+) (mg/dL)	P(1) (mg/dL)	P(2) (mg/dL)
1	30	16	30	32
2	27	22	26	29
3	26	26	29	27
4	32	16	27	30
5	31	14	28	29
6	31	23	30	27
Rata-rata	30,16	19,50	28,33	29,00
Std. Deviation	1.72240	4.80625	1.63299	1.89737

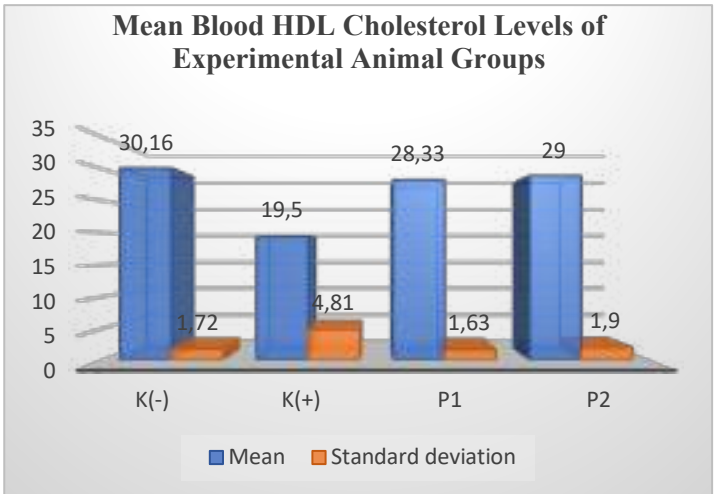

Figure 3. Mean blood LDL cholesterol levels of experimental animal groups

Kruskal Wallis Testing

Table 8. Kruskal Wallis test results for blood HDL cholesterol levels

Kruskal-Wallis H	Df	Sig.
14,989	3	0,002

Based on Table 8, the significance value is 0.002. Therefore, $p < \alpha$ or p is significant, indicating a significant effect of longan leaf extract on increasing blood HDL cholesterol levels.

Mann-Whitney U test

According to Table 9, the experimental group that received a standard K(-) diet, the experimental group that received a high-fat K1(+) diet, and the experimental group that received a high-fat diet with longan leaf extract at a dose of 200 mg/kg BW, all showed an increase in blood HDL cholesterol levels. Group K(+) with group P1, which was given a high-fat diet supplemented with longan leaf extract at a dose of 200 mg/Kg BW, and group P2, which was given a high-fat diet supplemented with longan leaf

extract at a dose of 400 mg/Kg BW, also showed a significant increase in HDL levels and blood cholesterol.

Table 9. Mann-Whitney U test result for blood HDL cholesterol levels

(I)	(II)	Sig
K(-)	K+	0,004
	P1	0,060
	P2	0,221
K(+)	P1	0,005
	P2	0,004
P1	P2	0,624

3.4. Discussion

In the group of experimental animals that received a high-fat diet and in the group of experimental animals that were given treatment one and two, it was found that there was a significant reduction in triglyceride levels, a decrease in blood LDL cholesterol levels and an increase in blood HDL cholesterol levels. The decrease in triglyceride levels and LDL cholesterol levels as well as the increase in HDL cholesterol levels is caused by longan leaf extract which contains saponins and flavonoids that can reduce triglyceride levels and LDL cholesterol levels as well as increase blood HDL cholesterol levels through several mechanisms.[9,10]

Research by Salamah and Widyasari 2015 shows the presence of bioactive compounds in longan plants such as flavonoids, tannins and saponins, especially in the leaves.[7] Flavonoids are useful for improving blood circulation, especially for preventing blockage of blood vessels, reducing cholesterol levels and fat accumulation in blood vessels, as well as antioxidants which are useful for getting rid of free radicals.[11] Flavonoids themselves are antioxidants because they can capture free radicals by freeing hydrogen atoms from their hydroxyl groups. The released H atom will bind to 1 free radical so that the peroxyradical will be stabilized and cause a decrease in the activation energy. The decrease in the activation energy will then prevent oxidation of the cholesterol so that it will lower cholesterol.[12] Flavonoids can also reduce triglyceride levels by increasing the action of the lipoprotein lipase (LPL) enzyme in breaking down triglycerides. Where LPL enzyme activity increases due to reduced lipid peroxidation. Increased activity of this enzyme can convert VLDL into intermediate-density lipoprotein (IDL) so that VLDL secretion in the liver decreases. Decreasing VLDL levels will also lower triglyceride levels.

Saponins are effective in lowering cholesterol, namely by inhibiting the absorption of cholesterol in the intestine by forming insoluble complex bonds with cholesterol, binding with bile acids to form micelles and increasing the binding of cholesterol by fibre to be secreted.[13]

4. Conclusion

Based on data analysis and interpretation of research results, it can be concluded that

1. A high-fat diet can significantly increase blood triglyceride levels in male white rats (Rattus norvegicus) Wistar strain
2. Provision of longan leaf extract (Dimocarpus longan lour) can significantly reduce triglyceride levels and LDL cholesterol levels in the blood of male white rats (Rattus norvegicus) Wistar strain fed a high-fat diet.
3. Provision of longan leaf extract (Dimocarpus longan lour) can significantly increase HDL cholesterol levels in the blood of male white rats (Rattus norvegicus) Wistar strain fed a high-fat diet.

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Literature Review

Lifestyle medicine for healthy ageing and longevity

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Abstract: The global population's rapid aging, attributed to declining fertility rates and extended lifespans due to technology, raises concerns about well-being in older age. Personal choices significantly impact premature deaths, overshadowing genetic, social, and healthcare factors. Six critical lifestyle elements—smoking, alcohol, inactivity, finances, social adversity, and negative traits—significantly influence longevity. Research demonstrates that a healthy lifestyle improves quality of life and reduces disability periods. The importance of maintaining functional capacity, emphasized by the United Nations for healthy aging, stands out, particularly in countering muscle-related issues prevalent in older age. Lifestyle Medicine, integrating modern practices into evidence-based medicine, encompasses nutrition, exercise, stress management, and more to prevent and treat chronic illnesses. It addresses chronic inflammation, linked to diseases like atherosclerosis and diabetes, through tailored lifestyle interventions. A lifestyle vital sign assessment questionnaire streamlines personalized healthcare by focusing on key habits. Lifestyle medicine intersects with palliative care, aiming for holistic management of chronic illnesses and improved quality of life. In conclusion, lifestyle medicine advocates for exercise in health promotion, disease prevention, and treatment in older adults. Its integration into clinical practice necessitates personalized approaches considering individual factors and limitations. These interventions align with palliative care, emphasizing tailored strategies to support well-being across various life stages.

Keywords: healthy ageing; lifestyle medicine; longevity

1. Introduction

Recently, the global population is aging, and this aging is happening at the fastest pace ever before. Some countries such as France had about 150 years to adjust their population from 10 to 20% of people over 16 years old. While other countries like China and Brazil may only need about 20 years to adopt their societies for this movement.[1] There are two drivers for this population aging, first is a failing fertility rate which is a global phenomenon, and the second seems to be that overall people live longer because of a better health due to better technology solutions.

However, the technology solutions that we live for longer is no guarantee for well-being. The question is how the 60-year-old human today is expected to live for advancing years. There are many reasons for aging. The first factor of course is genetic inheritance, but the big factories where we live are who we are and what our health behaviours are. In several advanced countries, for example in the

United States and Canada, CDC Data show 8 out 10 elderly (> 65 years old) have one chronic disease and most of them have 2 more comorbidities. The prevalence increases with advanced age.[2]

Even if the entire population had access to excellent medical care, only a small fraction of premature death would have been prevented. The biggest challenge lies with personal behaviour. Personal behaviour contributes to around 40% to premature death, advancing genetic predisposition (30%), social circumstances (15%), health care (10%), and environmental exposure (5%).[3]

An analysis on the effects of social and behavioural elements that contribute to increase in early mortality has been published. From 57 elements were analysed, and were then pinpointed six factors that have the most impact on longevity. They are smoking, alcohol abuse, lack of physical activity, financial difficulties, social adversity, and negative psychological characteristics. All of these are lifestyle factors.[4]

2. Healthy lifestyle and wellbeing

At the beginning of the 90s, 20% of women and 10% of men of all older people were sedentary. These sedentary lifestyles were then increased around 2-3-fold after 20 years. As it has been known that physical activity has an important role for health quality and influences the prevalence of chronic disease.[5] It is so important that while we may live longer one of the key factors of this behavioural pattern can actually compress disabled periods of life. An interesting longitudinal study has presented that a good lifestyle, with a good social support and social network diet and physical activity improve the quality of life at any race. This study compares healthy lifestyle and unhealthy lifestyle. (Table 1)

Table 1. Healthy and unhealthy lifestyle

Lifestyle component	Healthy	Unhealthy
Smoking	Never	Current
Alcohol	≤ 1 drink per day	≥ 2 drinks per day
BMI	18-24.9	30+
Exercise	1 hour per day	< 1 hour per day
Walking	48 blocks per week	6 blocks per week
Social network	Highest quartile	Lowest quartile
Social support	Top quartile	Bottom quartile

The results of study showed that greater distance walk and better diet quality were associated with a relative compression of the disabled period. Obesity was associated with a relative expansion of disable period Smoking was associated with a loss of both able and total years. A healthy lifestyle is predicted life was better and disabled years much lower than if they had an unhealthy lifestyle, whether it's women or men of any race. These results are necessary to keep in mind that a healthy lifestyle is needed not only for longevity but also for good years without any daily living difficulty.[5]

Functional Capacity

In the concept of healthy aging, it is needed to emphasize that the United Nations declared in the decade of healthy aging, that there are key Concepts what is functional capacity. Functional capacity is to maintain a good level of physical and psycho-cognitive capacity. While, fitness in terms of physical options meaning that the ability to do things on par with demands for a life. Besides, well-being is experiencing high quality of life. Healthy aging is talking about not only longevity, but also about how to develop and maintain a functional capacity that facilitates well-being, not just the absence of disease for every elderly person.[6]

For example, if we have the strength in our leg muscles to stand and move being mobile in the safe way. When we start our young life at 18-20 years old, we are very strong, we can climb, we can work for a long distance, and we don't fall easily or we find our balance really fast. If we maintain a good physical activity level, we can reach the elder year in mobile independent and far away from metabolic disease. However, if we adopt a sedentary lifestyle, then we leave earlier to reach the functional lower threshold. When we fall due to weak muscles or loss of balance, it will increase the risk of accidents, for example, head injury and risk to our life. A statistic has shown that 1 of 3 sixties years old got fall or slip at least once per year. And also, it will cause premature inability even premature death. For this reason, the functional capacity has a strong effect on increasing the life span.[7]

We lose muscle in the condition of atrophy. A reduction in muscle volume due to loss of contractile tissue. It usually happens in menopause women or after sixties years old men. Muscle atrophy could happen due to iatrogenic muscle loss or muscle loss syndrome. Muscle loss syndrome including sarcopenia due to aging, frailty cachexia, and malnutrition. Since 2018, European work Group on Sarcopenia in older People (EWGSOP) places muscle strength in this case as handgrip strength or sit to stand test as the primary sign to define sarcopenia additional with low muscle quantity and low physical performance. The new definition was stated to help the general practitioner to define sarcopenia easier compared to muscle quantity and physical performance.[8]

The disused muscle will increase the risk of sarcopenia. If we maintain a good muscle mass and function so the sarcopenia will be less pronounced in older age. Active older person has several advantages. it will increase muscle mass by 50%, it will also increase cardiovascular endurance by 80%, reduction in osteoporosis rate by 70%, lower triglyceride and cholesterol, increase the sensitivity of insulin, and better brain function. In overall, the person who exercises and stays active through their lifespan has a biological age that is younger (up to 20 years) than their chronological age.[8]

3. Lifestyle medicine

Evidence based in lifestyle medicine is needed. It is the integration of modern lifestyle practices into evidence based medicine while in cooperation with health promotion, non communicable disease (NCD) prevention, and chronic disease management. Lifestyle medicine is composed of 8 pillars: nutrition, physical activity, sleep hygiene, stress management, substance use (cigarettes, alcohol), sexual health and fertility, social connection, and environmental exposure (especially toxic substances). The European lifestyle medicine organization has defined lifestyle medicine. Lifestyle medicine is a branch of medicine which has a goal to maintain optimal health and to prevent, treat and reverse chronic illness across all life stages. The health intervention used in lifestyle medicine include evidence-based behavioural strategies, while considering equity and sustainability, to help patients or clients in enhancing self management skills for optimizing all components of lifestyles.[9]

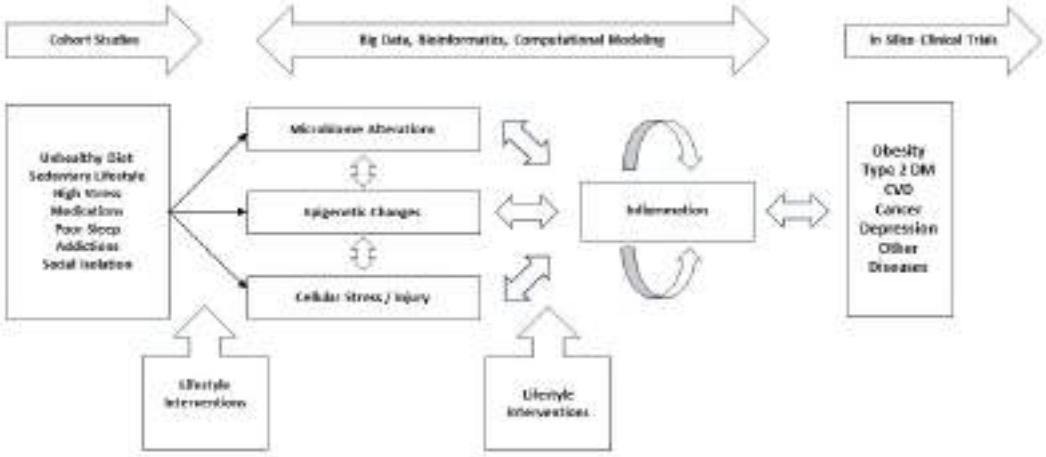

Figure 1. Lifestyle associated pathogenesis

A clear schematic figure has been presented. From the evidence, unhealthy diets, sedentary lifestyle, high stress, extreme use of medication, poor sleep, and social isolation have an impact negatively on health. All these impact negatively on health via cellular stress at the genetic changes and microbiome alteration, and cellular stress or injury. Which all these in turn cause inflammation. The inflammation then puts the plates into a circle which creates that much on from bio-epigenetic changes and stress. A lifestyle intervention can help prevent these by downward processing their health. Lifestyle intervention can help rescue a health problem by addressing all these factors. And also if the disease has happened, lifestyle intervention can help break the inflammation cycle and prevent all the diseases. There are links between chronic inflammation to the state of atherosclerosis, metabolic syndrome, type-2 diabetes and other chronic diseases. These efforts are added to big data, bioinformatics, and in silico

clinical trials results. It is now already providing strong evidence and a call for further education of entities and health professionals in lifestyle medicine.[10]

An important approach has been arranged to provide A lifestyle vital sign assessment questionnaire in the health care setting. The questionnaire composite 10-item lifestyle questions that are derived from scientific review.[11]

- Physical activity:
    - Average days per week engage in moderate or strenuous physical exercise
    - Average minutes engage in moderate or strenuous exercise
    - Days a week perform muscle strengthening exercise
    - Hours usually spend sitting or reclining at work or at home
    - Difficulty of walking ½ mile or climbing one flight of stairs
- Diet
    - Consume highly processed meat
    - Consume sugary food/drinks
    - Consume 5 or more fruits and vegetables
- Sleep
    - Sleep quality for most nights during the past month
- Smoke
    - Currently smoke cigarettes

Lifestyle medicine is also important in palliative care. Palliative care is a holistic approach. It is not only to manage individuals in the terminal stage, but also caring for the quality of life of the patient in chronic disease, and support the family on how to handle the end stage condition. The approach of palliative care is needed to address key symptoms such as: pain, anxiety, insomnia, edema, constipation, fatigue, and low level of functional capacity. The use of lifestyle intervention receiving palliative care or hospice care is a complex undertaking, requiring tailoring recommendation to individual patients.

4. Conclusion

Lifestyle medicine developments can meaningfully inform clinical practice and health policy. There is evidence based rationale for using exercise and physical activity for health promotion and disease prevention and treatment in older adults. Balance and gait training together with strengthening should guide priorities. Decision making should consider realistic sequencing and transfer ability as well as clients/patients preference. Lifestyle medicine aligns also with palliative care aims. Lifestyle medicine

prescription should be individualized, considering risk factors, medical history, musculoskeletal limitations, functional ability, tolerance, and personal preferences.

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Literature Review

Various aspects of *Anopheles vagus* as a vector of parasitic and virus diseases in Indonesia

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Abstract: Indonesia is a country in Southeast Asia that is abundant in mosquito species. *Anopheles vagus* is one of the dominant populations of mosquitoes in the region. It has been found throughout Sumatra, Sulawesi, Java, and Kalimantan. This review collects the latest data from various studies in Indonesia on the *An. vagus* in terms of morphology, habitat, bionomy, and its role as a vector in Indonesia. This narrative review based on a literature search, focuses on studies conducted in Indonesia. The database is obtained from the complete articles studied in Indonesia and English, reviews, and books with the keyword "*Anopheles vagus*". A literature search yielded 16 articles. The scope of research from the existing literature was broad, consisting of morphology, bite patterns, ecology, detection of *Plasmodium* parasites, insecticide resistance, and analysis of the developmental status of *An. vagus* preadult, and its vector capacity. The literature study described that *An. vagus* is the most common mosquito found by the cattle bait method although it is sometimes also caught by the human bait method. These mosquitoes have been foraging for blood feed at night, indoors and outdoors, and prefer to rest outdoors. From previous research, *Plasmodium*, microfilaria, and japanese encephalitis virus were detected in their body. From all available data, it can be concluded that this mosquito has the opportunity to become the main vector for malaria, filariasis, and viruses in Indonesia. There is a need for in-depth surveillance and gene-level research on these mosquitoes to understand mosquito behavior and the extent of its role in disease transmission in the environment.

Keywords: *Anopheles*; *Anopheles vagus*; morphology; behavior; bionomic

1. Introduction

Data from Disease Vectors and Reservoirs Riset (Rikhus Vektora) indicates that more than 70% of new infectious diseases are caused by vector-borne illness.[1] *Anopheles* mosquitoes are one of the Diptera that play a role as vector-borne diseases. As many as 80 species of this mosquito have been identified in Indonesia, one of which is the *Anopheles vagus* mosquito.[1] *Anopheles vagus* is very abundant; it is spread throughout Mainland Asia and Southeast Asia, including Indonesia.[2–7]

Although *An. vagus* is not the main vector of malaria, in several countries in Asia, this mosquito has been confirmed as a secondary vector of malaria especially when outbreak occur.[8–11] This literature study describes various findings from various studies in Indonesia regarding *An. vagus*: morphology, bionomics, molecular biology, and evidence of its role as a vector in Indonesia.

2. Methods

This narrative review was conducted from July–August 2023. We used the keyword '*Anopheles vagus*' to search all article, books, and other review from Indonesia related to this topic. For this study, complete articles in both Indonesian and English are included.

3. Results and Discussion

The keyword "*Anopheles vagus*" yielded 28 study articles, both in Indonesian and English. However, only sixteen research were concerning *An. vagus* for their study, namely morphology, vectorial capacity, and the role of *An. vagus* as a vector, and insecticide resistance status. From sixteen articles, three articles were *An. vagus* review studies, and thirteen articles were research studies defined concerning the diversity of species, bionomic features, biting behavior, density, ecology, and habitat of *Anopheles* including *An. vagus*.[12–27]

Morphology Pattern *Anopheles vagus*

Larvae of *An. vagus* generally have the same characteristics as other *Anopheles* mosquitoes. The body part consists of a head, neck and abdomen, as well as an anal segment without a siphon. The spiracles are located in the eighth abdominal segment. On each abdominal segment there is a pair of palmate hairs and a dorsal layer.[28] *Anopheles vagus* has a distinctive morphological characteristic in the form of a pale ring at the tip of the palpi, the length of which is at least three times the length of the dark ring below. The proboscis has a pale part at the tip.[12,13]

Dark and pale prehumeral is a distinctive pattern of *Anopheles vagus*. The difference between A*n. vagus* and other members of *Cellia* group is also in the femur and tibia, where the legs of *An. vagus* do not have spots like those of *An. sundaicus* and *An. subpictus*.[29]

According to a previous study, *An. vagus* salivary glands contain an immunogenic protein that's important as a biomarker of *Anopheles* exposure to humans. This protein can initiate the formation of specific antibodies of the host and develop natural specific antibodies against *Plasmodium.*[30]

Table 1. The summary spesific study for *Anopheles vagus* in Indonesia

No	Titles/theme of study	Type of study	Authors/years	Population size and sample site	Focus	Methods	Key finding	Ref
1	Morphological Identification of Sibling Species *Anopheles vagus vagus* and *Anopheles vagus limosus* Originating from Bangsring Village, Banyuwangi	Research	Wahyuni *et al* /2018	*An. vagus vagus* and *An. vagus limosus* from Bangsring Village, Banyuwangi Village , East Java	Morphology identification between two sibling species: *An. vagus vagus* and *An. vagus limosus*	Collection method with cattle bait	Two sibling of *An. vagus* are different on the palpi and proboscis	[12]
2	Morphologycal variation of *Anopheles Vagus* Donitz, 1902 (Diptera: Culicidae) From Brackish and Fresh Water	Research	Siti Alfiyah and Mujiono 2014	*Anopheles vagus* from brackish water and fresh water, Tuntang district, Semarang regency	Understanding the differences in morphology and caetotaxy	Mosquitoes collection and larvae survey	*An. vagus* from different habitats vary in size and spot in the wings (costa).	[13]
3	A laboratory Study of the Pre-Adult Filaria and Malaria Vector, *An. vagus* in East Nusa Tenggara Province	Research	Lobo *et al* /2019	*Anopheles* spp mosquitoes from Manggarai Barat and Kupang regencies	Identifying the preadult life cycle of *An. vagus*	Rearing adult female mosquitoes by wild collected	The immature *An. vagus* takes 11-31 days at 21.1°C to develop. Stadium IV larvae was the most vulnerable stage.	[14]
4	Confirmation of *An. sinensis* and *An. vagus* as Malaria Vectors in Muara Enim District, South Sumatra Province	Research	Budiyanto *et al* / 2018	Mosqoitoes and larvae	To confirm the malaria-carrying *Anopheles* species' existence and biting habits	Human Landing Collection and larvae collection	Both *Anopheles sinensis* and *An. vagus* carry *Plasmodium falciparum* sporozoites	[15]
5	Distribution and The Habitat Characteristics of *An. vagus* (Diptera: Culicidae) Larvae at Paddy Fields in The Vicinity of Dramaga IPB University Campus Dramaga Bogor West Java	Research	Novianto et al/ 2021	Larva collection from any breeding habitat	Analyzing the *An. vagus* larval stage distribution in 4 villages in the Dramaga subdistrict of West Java	Larva collecting with a dipper	*Anopheles vagus'*s habitat features in paddy fields	[16]

6	The mortality rate of *anopheles vagus* exposed to the insecticide permethrin is 2% (w/w) in mosquito net fibers	Research	Yahya and Astuti 2013	*Anopheles vagus*	Knowing the effectiveness of insecticides permethrin contained in mosquito net fiber	Bioassay test WHO	Permethrin are effective as insecticides for *Anopheles vagus*	[17]
7	Investigation of resistance of *Anopheles sp.* against pyrethroid insecticides and the possibility of voltage gated sodium channel (VGSC) gene mutations	Research	Didid et al / 2018	Non-blood-feeding wild-caught female mosquitoes around cattle	Identifying mutations in the VGSC gene codon 1014 marker for the dominant population of *Anopheles* mosquitoes in Muara enim, OKU, and Lahat regency in order to assess the state of pyrethroid pesticide resistance.	Cattle bait collection	Three regencies of *Anopheles vagus* showed no signs of pyrethroid pesticide resistance.	[18]
8	Species diversity and biting activity of malaria vectors (*Anopheles spp.*) in Lifuleo Village, West Kupang District, East Nusa Tenggara	Research	Rahmawati et al/2014	*Anopheles spp*	Knowing the diversity of *Anopheles spp.* mosquitoes, and population fluctuations of each species in Lifuleo Village, West Kupang District, Kupang Regency	Human landing collection; resting collection, and light trap collection.	*An. Barbirostris* has peak bloodsucking inside the house at 22:00–04:00 and outside the house at 21:00–04:00; *An. subpictus* inside the house 20:00–01:00 and outside the house 22:00–23:00; *An. vagus* inside and outside the house 22:00–23:00,; *An. Umbrosus* outdoors 20:00–03:00	[19]

9	Isolation of Japanese encephalitis virus from *Anopheles annularis* and *Anopheles vagus* in Lombok, Indonesia	Research	Olson et el /1985	*Culex spp, Aedes spp,* and *Anopheles spp* from lombok.	Isolation of the virus from mosquitoes (serological study)	Mosquitoes collection by miniature light trap	First report of JE virus in *An. annularis, An. vagus*, and *Cx. tritaeniorhynchus*	[20]
10	Mosquito-specific viruses (family Flaviviridae, genus Flavivirus) Isolated from *Anopheles vagus* mosquitoes in Bali	Research	Damayanti et al /2021	Mosquitoes species from Badung, Bulelelng and Jembrana regency, Bali	Molecular with one-step RT PCR	Mosquitoes collection with cattle bait by light trap method	Mosquito-specific viruses from Flavivirus detected in *An. vagus*	[21]
11	Japanese encephalitis in Indonesia: An update on epidemiology and transmission ecology	Review	Garjito et el / 2018	Article, local publications, unpublished JE documents, and presentation by JE researchers were also collected	Mosquitoes as JE Vector	A systematic search of international and national published articles	*An. vagus* and other 2 genus are vector of Japanese encephalitis	[22]
12	Various aspects of malaria in Pesawaran district, Lampung province	Review	Ritawati and Supranelfy	Books, articles, and scientific journals	Entomological data related to species, malaria morbidity, community behavior towards malaria and data on the use of anti-malarial drugs	Literature search	*Plasmodium* was identified in sixteen species *Anopheles,* including *An. vagus*	[23]
13	*Anopheles vagus* as a vector in Indonesia	Review	Permadi et al/ 2014	Article, reviewing books and scientific journals.	Evidence o*f An. vagus* as a malaria vector	Literature search	*An. vagus,* zoofilic mosquitoes, contain *Plasmodium* in several research studies.	[24]

14	Study of the genetic diversity of *An. barbirostris* and *An. vagus* mosquitoes in Two Malaria Endemic Areas in West Java	Research	Sumantri RA and Iskandar DT/ 2005	Female *An. barbirostris* and *An. vagus* from Sukabumi Regency and Tasikmalaya regency, West Java Province	Isoenzym analyzis	Alloenzym variety and alel frequency	Genetic diversity of the *An. barbirostris* mosquito and *An. vagus* is not yet categorized as polymorfism.	[25]
15	Confirmation of the status of *An. vagus* as a secondary vector in the malaria outbreak in Sukabumi district	Research	Munif *et al* /2008	Female *Anopheles spp* from Sukabumi regency West Java Province	Evidence *An. vagus* as vector	Enzyme-Linked Immunosorbent Assay (ELISA)	*Plasmodium falciparum* was detected in *An. vagus.*	[26]
16	Vector capacity and inoculation rate of entomological *An. vagus* from malaria endemic area in banten province	Research	Astuti *et al*/ 2015	*Anopheles spp* mosquitoes from Lebak and Pandeglang Regencies, Banten Province	Determining vector capacity and entomological inoculation rate of *Anopheles vagus*	Human Landing Collection, ELISA and Molecular PCR	A*n. vagus* can be a potential vector to transmit *Plasmodium vivax* malaria based on vectorial capacity value.	[27]

Bio ecology habitat of *Anopheles vagus*

There are three main topographic zones that make up the habitat of *An. vagus*: brackish water zones, coastal plains, and hills and mountains.[13,31] These larvae can almost be found in areas with calm or slightly flowing water such as small pools on beaches, springs, puddles, ditch, paddy fields, swamps in muddy ponds, animal footprints, brackish water, and various artificial containers such as used tires, drums, and boats.[16,32,33]

Bionomic and behavior of *Anopheles vagus*

Anopheles vagus is known as a zoophilic mosquito, although it sometimes likes human blood .[2,32,34] *Anopheles vagus* has varying biting peak times. In a study in South Sumatra, the peak of biting mosquitoes was at 01.00-02.00 am [15] , but in research in eastern Indonesia, this mosquito peaked biting inside and outside the house at 22:00–23:00 pm.[19] They also prefer to bite outdoors rather than indoors and prefer to rest in outdoor areas, but nevertheless, they still have low vectorial capacity and inoculation rates.[27] Factors that make mosquitoes roles as vectors hence : the age of mosquitoes, contact between humans/hosts with mosquitoes, frequency of biting, and susceptibility of mosquitoes to parasites. The capacity to be a vector is influenced by environmental, behavioral, biochemical, and cellular factors that affect the relationship between vectors, pathogens to be transmitted by vectors, and hosts where pathogens will be transmitted. In molecular examination, this mosquito also has a very high level of holomology with its subspecies, namely *An. vagus limosus*.[35] It has susceptibility to synthetic pyrethroid based on no mutation identified in the VGSC gene.[18] Filariasis worms were identified in the body of *An. vagus* mosquitoes in the East Nusa Tenggara region.[36] Japanese encephalitis virus was also detected in this mosquito in the Lombok region and western Java of Indonesia. The presence of *Plasmodium* in *An. vagus* through ELISA and PCR examinations in various studies in Indonesia.[15,24,27,37] All this evidence has raised awareness of these mosquitoes becoming important vectors of malaria, filariasis, and Japanese encephalitis transmission in Indonesia.

4. Conclusion

The abundant population, diverse habitat and breeding place, and parasites and viruses in their body from various studies in Indonesia make *An. vagus* has the possibility in the future being of the important vectors in Indonesia. In the future, further research can be done at the gene level and conduct broader surveillance on these mosquitoes to understand mosquito behavior and the extent of its role in disease transmission in the environment.

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Literature Review

Bioinformatics analysis of interleukin-10 and its mutation

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Abstract. Interleukin-10 (IL-10) has an important role in anti-inflammatory activity. Study of the molecular characteristic is needed before conducting further research, thus can provide insight and understanding regarding the properties and characteristics of IL-10. Therefore, this article aims to provide knowledge regarding the bioinformatics analysis of IL-10 and changes in the protein if there is a mutation at the gene level. Method: Bioinformatic analysis of IL-10 and its mutation was obtained from several websites that can be accessed freely on internet, such as NCBI, PSIPRED, PROTPARAM, TMHMM, PROTSCALE, PEPTIDECUTTER, PROSITE, NETNGLYC, TARGETP, SIGNALP, Swissmodel, and Pymol. Result: The mutation model used is IL-10 C177T gene mutation. Human IL-10 gene is located on chromosome 1q32.1. The IL-10 protein is 178 amino acids (aa) long. The IL-10 C177T gene mutation causes several differences in protein characteristics. Most of the IL-10 protein is located outside the cell membrane. The C177T mutation does not increase the hydrophobicity of the IL-10 protein amino acids. Of the 37 enzymes, only 18 are predicted to be able to cut IL-10 protein. Normal and mutant APP proteins have the same 1 glycosylation site, namely at amino acid position 134. The prediction of the target location of normal and mutant APP proteins on cells is not different. there was no difference in protein structure between normal IL-10 protein and C177T mutant IL-10. Conclusion: bioinformatics analysis is very useful in determining the molecular characteristics of IL-10 and its mutation.

Keywords: bioinformatics; interleukin-10; mutation

1. Introduction

Interleukin-10 (IL-10) is a cytokine that has an important role in anti-inflammatory activity.[1] This cytokine functionally has diverse target cells, resulting in broad anti-inflammatory activity.[2] Although Th2 cells were the first identified cellular source of IL-10, production of this cytokine by CD4+ and CD8+ T cells has also been identified.[3] Cells of the myeloid and lymphoid lineages produce IL-10 in response to different stimuli. These cells include macrophages, monocytes, dendritic cells (DCs), neutrophils, mast cells, eosinophils, and natural killer (NK) cells, in addition to CD4+, CD8+ T cells, and B cells.[4]

The main biological function of IL-10 appears to be in dendritic cells (DCs) and macrophages. Interleukin-10 is a potent inhibitor of antigen presentation.[5] It inhibits the expression of major

histocompatibility complex (MHC) class II as well as upregulation of the costimulatory molecules CD80 and CD86. It also inhibits DC differentiation from monocyte precursors, and also inhibits DC maturation. Thus, many of the characteristics of the immune inhibition of IL-10 can be traced to its effect on APC to prevent the production of Th1-associated cytokines IL-2 and interferon-ɣ (IFN-ɣ), as well as Th2-associated cytokines IL-4 & IL-5.[6,7]

Another important effect of IL-10 is to inhibit the production of proinflammatory cytokines and mediators from macrophages and DCs.[8] The main inflammatory cytokines, IL-1, IL-6, IL-12, and tumor necrosis factor (TNF), are dramatically suppressed after exposure to IL-10. Inflammatory chemokines of both the CC and CXC types are also suppressed by IL-10, as is the production of macrophage matrix metalloproteases. Interleukin-10 can further inhibit inflammation by increasing the release of IL-1 receptor antagonists by macrophages. It can also target naïve CD4+ T cells, possibly through inhibition of the CD28 signaling pathway.[9,10]

Bioinformatics science is growing fast in this digital era. It can also be used for many research in medical and biomedical science.[11] Study of molecular characteristic is needed before conducting further research. It can provide insight, thus can assist researchers in understanding the properties and characteristics of a compound. Therefore, this article aims to provide knowledge regarding the bioinformatics analysis of IL-10 and changes in the protein if there is a mutation at the gene level.

2. Methods

Bioinformatic analysis of IL-10 and its mutation was obtained from several websites that can be accessed freely on internet. The mutation model used is IL-10 C177T gene mutation. To analyze genetic and protein characteristics of IL-10, we use the features present on the National Center for Biotechnology Information's website (www.ncbi.org). To analyze the prediction of protein secondary structure due to mutation, we use PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/). To analyze changes in the physicochemical characteristics of a protein that undergoes a mutation, we use PROTPARAM (https://web.expasy.org/protparam/). To analyze changes to the Topology of Trans-membrane Proteins, we use TMHMM (https://services.healthtech.dtu.dk/services/TMHMM-2.0/). To analyze change in the hydrophobicity, we used PROTSCALE (https://web.expasy.org/protscale/). To predict cleavage by proteases, we used PEPTIDECUTTER (https://www.expasy.org/#proteome). To predict the potential of glycosylation sites, we used NETNGLYC. To analyze protein domain motifs, we used PROSITE. To predict the location of proteins in cells, we use TARGETP. To predict bearer location code, we use SIGNALP. To predict the 3D shape of the mutated IL 10 protein structure, we used

Swissmodel and Pymol.

3. Results and Discussion

3.1. Interleukin-10 gene

The Interleukin-10 (IL-10) gene was obtained from the NCBI site with the code NC_000001.11 (206767602..206772494, complement). The official name of this gene in humans is Interleukin 10 with the symbol "IL 10". This gene is also sometimes written with the following symbols: CSIF; TGIF; GVHDS; IL-10; IL10A.[12]

The location of the IL-10 gene in humans is located on chromosome 1q32.1. This means that the gene that carries the genetic information for IL-10 is on chromosome no. 1, position of the long arm (q), 3rd arm, band 2 and sub band 1. Figure 1 shows a schematic of the location of the IL-10 gene nucleotides. After analyzing the sequence with the identity code NG_012088, the IL-10 gene has a total length of 11,892 base pairs (bp), consisting of 5 exons and 4 introns. The IL-10 gene sequence reference can be seen in Figure 2.[13,14] Interleukin-10 has two isoforms, namely interleukin-10 isoform 1 precursor (NP_000563.1)[15] and interleukin-10 isoform 2 (NP_001369553.1)[16]. The IL-10 gene has 4 transcript variants as seen in Figure 3[17], namely NR_168466.1 and NR_168467.1 (non-coding transcript types) and NM_000572.3 and NM_001382624.1 (protein-coding transcript types).[18]

Figure 1. Gene location of IL-10[12]

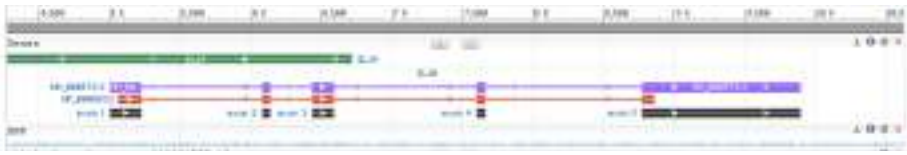

Figure 2. Ref sequence (NG_012088)[13]

Figure 3. Gene annotation of IL-10[17]

3.2. Protein IL-10

The IL-10 protein (P22301.1) has a length of 178 amino acids (aa).[19] The IL-10 is most commonly found in the appendix.[20] Its molecular weight is around 18 kDa (monomer). Human IL-10 contains a non-glycosylated, unpotential N-linked glycosylation site, at position 134. IL-10 also contains one less cysteine residue than in mouse. The IL-10 shows two disulfide bonds in the chain at positions 30 and 126, and forms a nondisulfide linked homodimer. The length of the alpha-helix A --> F in human IL-10 is 21, 8, 19, 20, 12 and 23 aa, respectively. As is well known, the A --> D helices of one monomer noncovalently interact with the E and F helices of the second monomer, forming a noncovalent V-shaped homodimer. Functional areas have been mapped on the IL-10 molecule. At the N-terminus, pre-helix residues A #1-9 are involved in mast cell proliferation, while at the C-terminus, helix residues F #152-160 mediate leukocyte secretion and chemotaxis.[21] The IL-10 protein sequence (P22301.1) can be seen in figure 4.

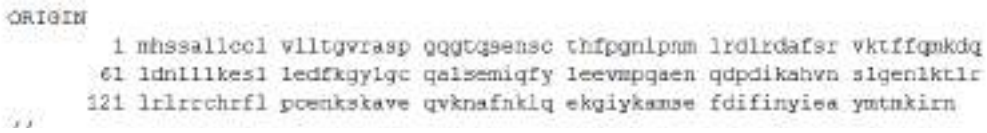

Figure 4. Protein sequence of IL-10 (P22301.1) [19]

3.3. Model of mutation

On the Genetics Home References website, which is now affiliated with MedlinePlus, there is no data on the form of the IL-10 gene mutation. Meanwhile, on the NCBI OMIM site, single nucleotide variants (SNV) were found with the codes rs376415487, rs768418064, rs1572537757, and rs568879359 (OMIM: *124092).[22] For example, in a dSNP with code rs376415487, there is a missense variant of the IL-10 gene mutation, with the change of amino acid arginine to amino acid glutamine at amino acid position 177 (NP_000563.1: p.Arg177Gln).[23]

3.4. Secondary structure prediction (PSIPRED)

At the 177 amino acid position, Protein IL-10 isoform the normal precursor is arginine with a coil-shaped amino acid secondary structure. Meanwhile, at the same position, the mutant IL-10 protein is in the form of glutamate with an identical secondary structure, namely it remains in the form of a coil. Similarly for the other amino acid sequences. (Figure 5).[24,25]

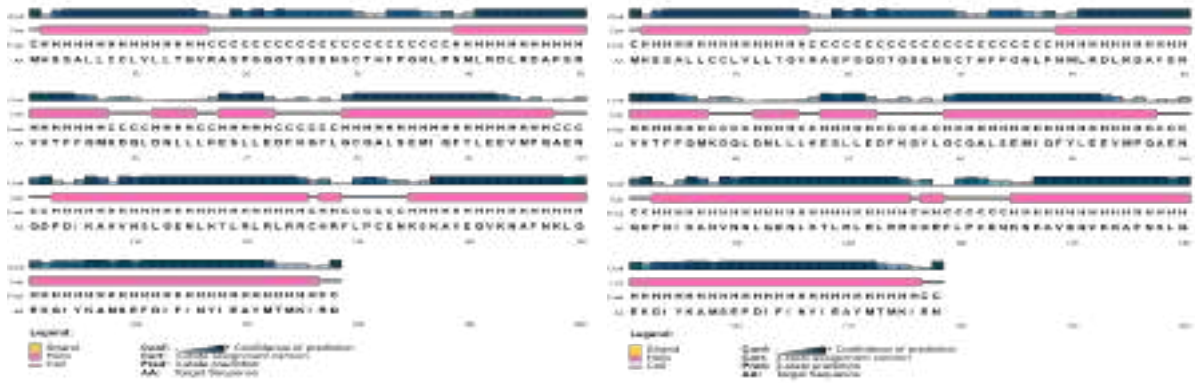

Figure 5. Normal (left) and mutant (right) IL-10 protein secondary structures at amino acid positions 1-178[24]·[25]

3.5. Physical-Chemical characteristics (PROTPARAM)

Mutation of the IL-10 C177T gene causes several differences in protein characteristics. Character differences occur in molecular weight, composition of the amino acids alanine and glycine, atomic composition, aliphatic index and hydroplasticity. Differences also occur in the index of instability; however, normal and mutant proteins are included in the unstable group. See table 1.[26]

Table 1. Physical-chemical characteristics of normal and mutant IL-10 protein

Characteristic	Protein il-10 normal	Protein il 10 mutant
Molecular weight	20516.76	20489.69
Amino acid composition	Total negative residue (Asp + Glu): 21 Total positive residue (Arg + Lys): 23	Total negative residue (Asp + Glu): 22 Total positive residue (Arg + Lys): 22
Atom composition	Carbon C 904 Hydrogen H 1443 Nitrogen N 251 Oxygen O 265 Sulfur S 14 Formula: $C_{904}H_{1443}N_{251}O_{265}S_{14}$ Total number of atoms: 2877	Carbon C 903 Hydrogen H 1438 Nitrogen N 248 Oxygen O 267 Sulfur S 14 Formula:$C_{903}H_{1438}N_{248}O_{267}S_{14}$ Total number of atoms: 2870
Instability Index	56.64 Classification instable	58.41 Classification instable
Aliphatic Index	84.94	84.94
Grand average of hydropathicity (GRAVY):	-0.354	-0.348

3.6. Trans-membrane Protein Topology (TMHMM)

Most of the IL-10 protein is located outside the cell membrane. The transmembrane position is at amino acid positions 701-723, a small portion is inside the cell. The C177T mutation causes less protein to be present in the cell (Figure 6).

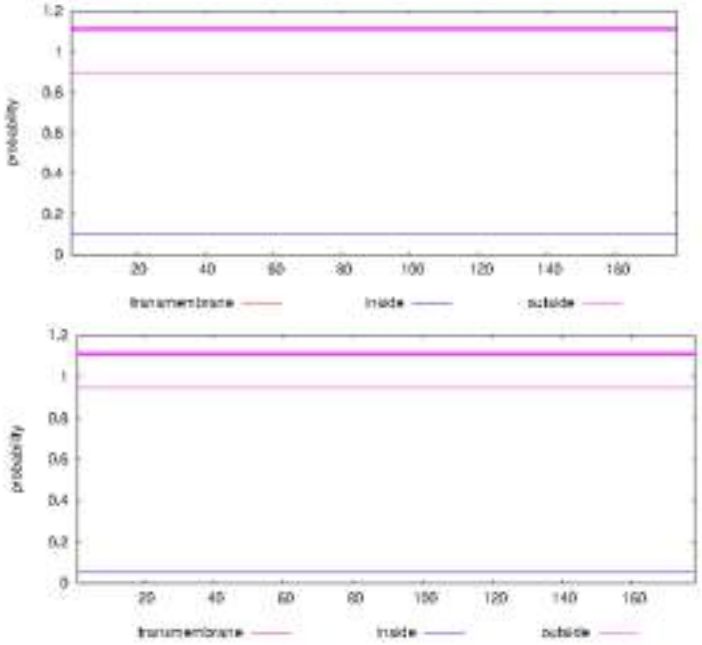

Figure 6. TMHMM protein IL-10 normal (top) dan mutant (bottom)[27]

3.7. Hydrophobicity of IL-10 (PROTSCALE)

Hydrophobicity analysis using the Hphob.IL-10 (PROTSCALE) method, and converted to scatter form with the help of Microsoft Excel, showed that the C177T mutation did not increase the level of hydrophobicity of the IL-10 protein amino acids (Figure 7).[28]

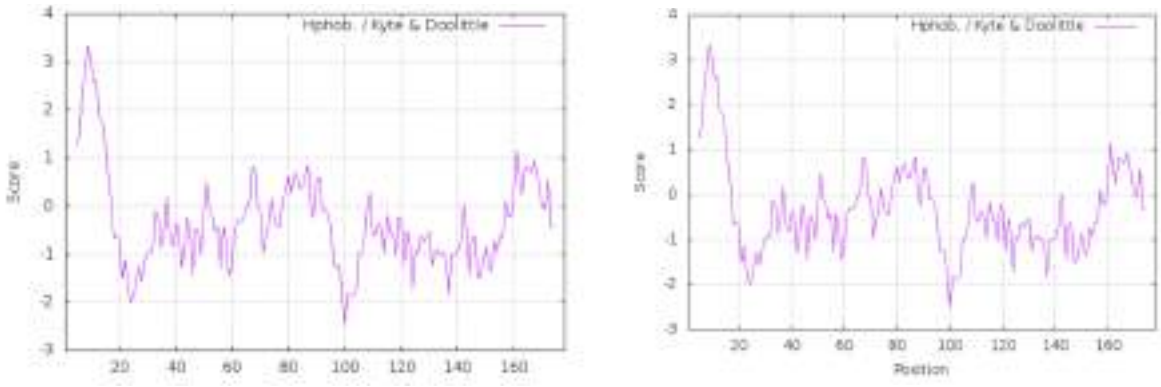

Figure 7. PROTSCALE protein IL-10 normal (left) dan mutant (right)[28]

3.8. Prediction of cleavage by protease (PEPTIDECUTTER)

From the 37 protease enzymes available on the website www.expasy.org/tools/#proteome, only 18 enzymes are predicted to be able to cut the IL 10 protein. The normal 177 amino acid sites can be cut by

the enzymes Arg-C proteinase, Clostripain, and Trypsin. The mutant 177 amino acid site can be cut by the enzymes Glutamyl endopeptidase, Proteinase K, and Staphylococcal peptidase I. Each of the 177 amino acid sites in normal and mutant can only be cut by certain enzymes and no enzyme can cut both simultaneously.[29]

3.9. Prediction of potential glycosylation sites (NETNGLYC)

The normal and mutant IL-10 proteins have the same 1 glycosylation site, namely at the 134 amino acid position (Figure 8).[30]

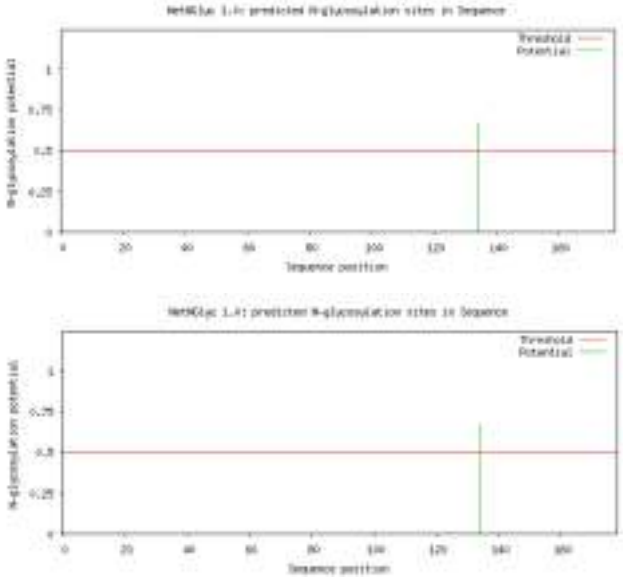

Figure 8. Position of glycosylation sites (top) and mutant (bottom)[30]

3.10. Protein domain motif (PROSITE)

The normal IL-10 protein domain motif is: KgylgCQalseMIqFYLeEVM at positions 85-105. There is no difference in protein domain motifs between normal IL-10 and IL-10 C177T mutant.[31]

3.11. Protein location prediction in cells (TARGETP)

The prediction of the target location of normal and mutant IL-10 proteins in cells was not different, namely mostly in the secretory pathway (0.918) and a little in the mitochondria (0.067) and elsewhere (0.036). This is related to the function of the IL-10 protein as a signal peptide that must be on the cell surface.[32]

3.12. Location code carrier prediction (SIGNALP)

The mutation occurs at the CC 177 position, so there is no difference in the protein end region (AA 1-178) between normal IL-10 and the mutant in the prediction of the carrier of the protein location code

and its cutting site. Peptide signals are at amino acids 1-18 (mean S; 0.915), with the S peak at position 2 (0.937). Predicted cleavage site is between 18 and 19, with max cleavage score: 0.846 (position 19). With a high mean-S and D-score (0.899), it indicates that the IL-10 protein and the mutant are included in the "secretory protein" group.[33]

Table 2. Prediction of IL-10 protein locations

Name	Len	mTP	SP	Other	Loc	RC
Sequence	178	0,067	0.918	0.036	S	1
Cutoff				0.000	0.000	0.000

targetp v1.1 prediction results
Number of query sequences: 1
Cleavage site predictions not included.
Using NON-PLANT networks.

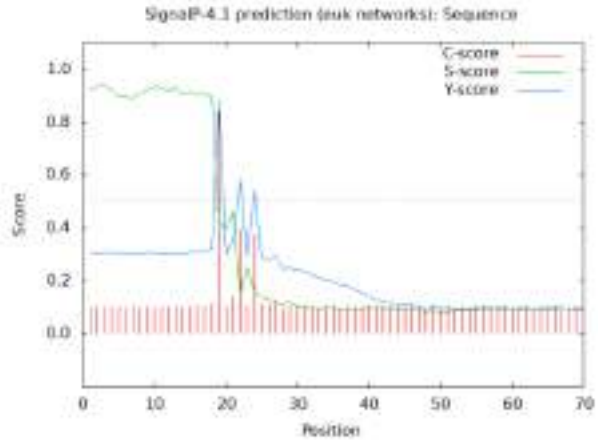

Figure 9. Signal prediction of IL-10[33]

3.13. Image of protein structure

The 3-dimensional shape of the IL-10 protein structure is referenced from the Swiss model. The protein structure refers to the 2ilk.1.A template with a residue range of 24-178.[34] After being analyzed with the Pymol program, there was no difference in protein structure between the normal IL-10 protein and the C177T mutant IL-10.[35]

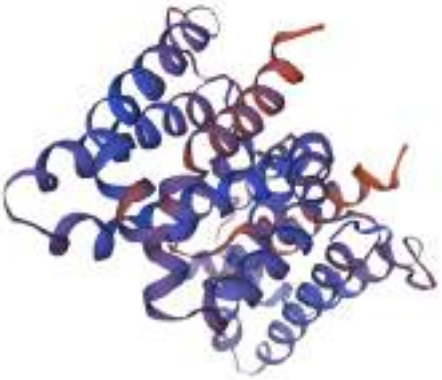

Figure 10. Template 2ilk.1.A[34]

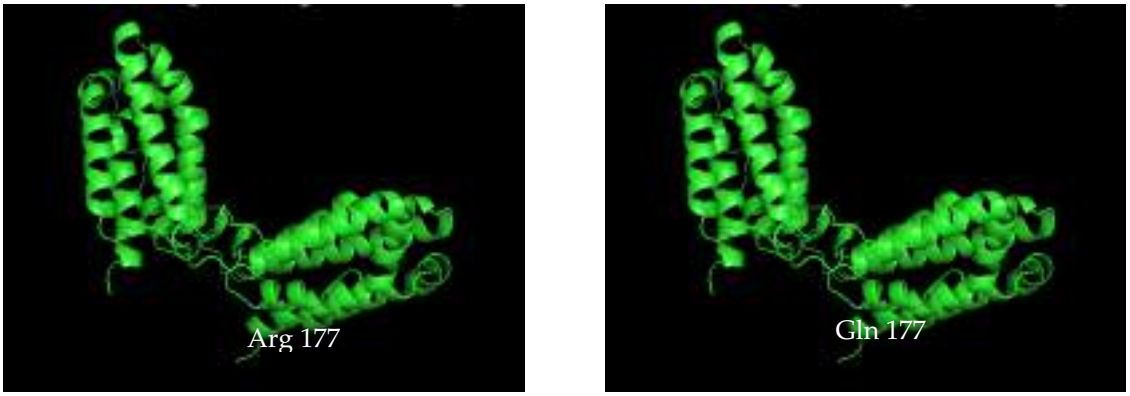

Figure 11. Structure of normal IL-10 (left) and C177T mutant IL 10 (right)[35]

4. Conclusion

Human IL-10 gene is located on chromosome 1q32.1. The IL-10 protein is 178 amino acids (aa) long. The IL-10 C177T gene mutation causes several differences in protein characteristics. Most of the IL-10 protein is located outside the cell membrane. The C177T mutation does not increase the hydrophobicity of the IL-10 protein amino acids. From the 37 enzymes, only 18 are predicted to be able to cut IL-10 protein. Normal and mutant IL-10 proteins have the same 1 glycosylation site, namely at amino acid position 134. The prediction of the target location of normal and mutant IL-10 proteins on cells is not different. there was no difference in protein structure between normal IL-10 protein and C177T mutant IL-10. The bioinformatics analysis is very useful in determining the molecular characteristics of IL-10 and its mutation.

5. Acknowledgments

The author would like to personally thank Prof. Dr. dr. Irfanuddin, Sp.KO, M.Pd.Ked (professor

at medical faculty, Universitas Sriwijaya) who introduced the author to various tools in the field of molecular bioinformatics.

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Research Article

Urine endothelin-1 levels in obesity : Premilinary study

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Absract: The increasing levels of endothelin-1 (ET-1) in the kidneys can be detected through elevated levels of ET-1 in urine. Early and mild kidney damage has been reported to elevate renal ET-1 levels especially in obesity. The aim of this study was to compare the urine ET-1 levels between the obese and non-obese groups. One hundred healthy respondents, consisting of 50 obese (IMT>30 kg.m$^{-2.}$) and 50 normal-weight individuals, were included in this cross-sectional study. Each group of respondents was divided into 52 individuals aged 18-20 years and 48 individuals aged 26-30 years. Urine ET-1 levels and blood pressure were compared between the obese and normal-weight groups within these age groups. Urine ET-1 levels were examined using ELISA from urine samples collected, while blood pressure was measured using a digital device (Omron). The research results indicated that in the 18-20-year-old group, obesity was found in 69% of males and 31% of females, blood pressure was significantly higher (p<0,001) and urine ET-1 levels were also significantly higher (p = 0.012) in the obese group compared to the normal-weight group. In the 26-30-year-old group, obesity was observed in 46% of males and 54% of females. Blood pressure was significantly higher (p<0,001) in the obese group compared to normal-weight group. Urine ET-1 levels were higher in the obese group, but the difference was not statistically significant (p=0,591). Obesity in the 18-20-year-old group led to kidney disturbances, while in the 26-30-year-old group, kidney disturbance was also present but might be due to other systemic factors affecting renal ET-1 levels.

Keywords: Endothelin-1 kidney; Endothelin-1 urine; obesity

1. Introduction

Endothelin-1 (ET-1) in the kidneys is produced by the glomerulus and tubules and functions to inhibit the absorption of sodium and water, as well as regulate blood vessel diameter.[1,2] Increased ET-1 in the kidneys leads to higher permeability of the filtration barrier, damage to podocyte cytoskeleton and endoplasmic reticulum, and stimulation of tubular cell apoptosis, thereby causing kidney damage.[2,3] Elevated renal ET-1 due to kidney damage is excreted in the urine, resulting in increased urinary ET-1 levels.[4,5] Hence, the examination of urinary ET-1 can be used to assess kidney conditions, although this technique is still limited in clinical use.

Our initial data show that obesity in mice results in increased kidney ET-1 levels and early disturbances in the glomerulus.[6,7] Urinary ET-1 examination is not extensively reported, especially in obesity among young adults. This preliminary study aims to compare urinary ET-1 levels between a group of obese young adults and a group of normal-weight individuals.

2. Methods

This cross-sectional study involved 50 obese young adults (BMI ≥ 30 kg.m^{-2}) and 50 individuals with normal weight (BMI < 25 kg.m^{-2}). The respondents' ages were between 18-20 years and 26-30 years. Blood pressure and urinary ET-1 levels were measured. ET-1 levels were measured using ELISA technique with a quantitative sandwich method using an ELISA kit (BioSource). ET-1 in the sample binds to the coated antibodies in the well of the kit, followed by biotinylated ET-1 antibodies binding to the existing ET-1 in the sample. Absorbance was measured at a wavelength of 450 nm. Blood pressure was measured using a digital blood pressure monitor (Omron).

Data were analyzed using independent t-tests to measure the differences in mean urinary ET-1 levels and blood pressure between the obese and normal-weight groups in each age group. Correlations between urinary ET-1 levels, BMI, and blood pressure were analyzed using Pearson correlation. This study obtained ethical approval from the Ethics Commission of the Faculty of Medicine, Hasanuddin University, with No.136/UN4.6.4.5.31/PP36/2022.

3. Results and Discussion

Table 1. Respondent's characteristic

Variables	18-20 years		p*	26-30 years		p*
	Obesity (n=26)	Control (n=26)		Obesity (n=24)	Control (n=24)	
Sex						
Male	18 (69%)	12 (46%)		11 (46%)	14 (58%)	
Female	8 (31%)	14 (54%)		13 (54%)	10 (42%)	
Blood Pressure						
Systolic (mmHg)	127.58 ± 10.26	111.58 ± 9.37	0,000	123.42 ± 6.03	112.08 ± 7.79	0,000
Diastolic (mmHg)	85.27 ± 7.19	74.96 ± 6.39	0,000	83.54 ± 4.21	74.58 ± 5.09	0,000
BMI (kg.m^{-2})	33.69 ± 4.53	20.58 ± 1.44	0,000	34.55 ± 3.90	21.69 ± 0.89	0,000
ET-1 urine (ng/L)	55.55 ± 19.13	40.93 ± 21.38	0,012	107.23 ± 49.43	100.29 ± 38.79	0,591

p* calculated with independent t test

Significantly higher urinary ET-1 levels (p=0.012) were found in the obese group compared to the control (normal-weight) group in the 18-20 age group. However, in the 26-30 age group, while urinary ET-1 levels were higher in obesity compared to the control, the statistical significance was not observed

($p = 0.591$) as seen in Table 1. Systolic and diastolic blood pressures were significantly higher ($p<0.001$) in obesity compared to the control in both age groups.

This study indicates a positive correlation between urinary ET-1 and obesity (BMI ≥ 30 $kg.m^{-2}$) in young adults (18-20 years). This suggests that in obesity with BMI ≥ 30 $kg.m^{-2}$, kidney ET-1 increases, indicating kidney disturbances. Increased plasma ET-1 in obesity, believed to be produced by adipocytes and overproduction from blood vessel walls, could lead to increased ET-1 circulation to various organs, including the kidneys. In the kidneys, ET-1 can cause disruptions such as hyperfiltration and disturbances in the glomerulus and tubules.[8,9] If kidney disturbances persist, blood vessels and smooth muscle cells in the kidneys also produce ET-1, exacerbating kidney damage due to ET-1 accumulation.

In the 26-30 age group, increased urinary ET-1 levels were not significantly different in obesity compared to normal weight. Urinary ET-1 levels were significantly higher in the 26-30 age group compared to the 18-20 age group, and as numerous studies have reported, ET-1 levels tend to increase with age. The reason for the lack of significant difference in urinary ET-1 levels between obese and normal-weight individuals in the 26-30 age group is unclear, but it is assumed that older age might entail various organ disturbances contributing to ET-1 elevation, which was not examined in this study. Conversely, in the younger group (18-20 years), the elevated ET-1 levels were likely attributed solely to obesity.

In individuals with kidney disease, increased urinary ET-1 is not always accompanied by elevated plasma ET-1.[1,4] Local kidney disturbances can elevate kidney ET-1 levels, leading to glomerular and tubular disruptions. Although this study did not assess kidney disturbances, some animal studies have shown a link between increased kidney ET-1 levels and glomerular disturbances.[7] Sing et al also reported a tendency of increased urinary ET-1 in obese adolescents, correlated with urinary TNF-alpha levels indicating inflammation and endothelial dysfunction.[8–10] Furthermore, increased urinary ET-1 levels have been associated with decreased estimated glomerular filtration rate (e-GFR) in patients with polycystic kidney disease.[5] Human studies, to our knowledge, are limited in reporting early kidney disturbances due to obesity in young age.

Systolic blood pressure also appears to correlate with BMI ≥ 30 kg/m2, although few of the obese subjects had systolic pressures exceeding 140 mmHg, likely due to their young age. However, compared to normal-weight subjects (BMI < 25 kg/m2), systolic pressure was significantly higher in obesity in our data (Table 1). Hypertension has been reported to cause kidney disturbances and increased urinary ET-1.[3] Conversely, diastolic pressure in our data did not correlate with BMI ≥ 30

kg/m2, possibly due to the compensatory mechanisms in other body tissues to maintain normal diastolic pressure in the young population.

The presence of hypertension in obesity is linked to increased plasma ET-1, leading to systemic blood vessel vasoconstriction. In normal conditions, ET-1 production is limited to maintain normal blood vessel tone. However, increased production of ET-1 due to a large number of adipocytes can elevate blood pressure. In this study, both in the 18-20 and 26-30 age groups, systolic and diastolic blood pressures were significantly higher in obesity compared to normal weight, although none were categorized as hypertensive.

Table 2. Correlation between BMI, blood pressure and ET-1 urine levels

	ET-1 urine levels	
BMI ($kg.m^{-2}$)	r = 0,342	p = 0,013
Systolic pressure (mmHg)	r = 0,297	p = 0,033
Diastolic pressure (mmHg)	r = 0, 166	p = 0,241

p : Pearson's correlation

Table 2 shows a weak correlation between urinary ET-1 levels, BMI, and systolic blood pressure. Conversely, diastolic pressure did not correlate with urinary ET-1 levels.

The influence of BMI on increased urinary ET-1 levels showed a weak positive correlation (Table 2), indicating that many other factors contribute to elevated urinary ET-1 levels. A weak positive correlation was also observed between urinary ET-1 levels and systolic blood pressure (Table 2), while diastolic pressure did not correlate (Table 2). This suggests that kidney damage characterized by increased urinary ET-1 levels affects blood pressure, particularly systolic pressure, but there are still many other factors influencing the elevation of both systolic and diastolic pressures.

This study have several limitations include the limited number of respondents and the lack of laboratory examinations to assess kidney disturbances. Nevertheless, it provides sufficient preliminary information that obesity in young age requires special attention.

4. Conclusion

In conclusion, urinary ET-1 levels correlate with obesity (BMI ≥ 30 $kg.m^{-2}$), likely contributing to early kidney disturbances. However, future studies should involve comprehensive laboratory examinations to confirm kidney disturbances.

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Research Article

No disruption of blood glucose levels in students of the Hidayatullah Islamic boarding school Surabaya who performDawood's fast

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Abstract: Intermittent fasting is beneficial in reducing mortality from cancer, increasing insulin-sensitivity and reducing oxidative stress. One form of intermittent fasting that is often applied lately is the 'Dawood's Fast'. Research on the benefits of Ramadan fasting and Monday-Thursday fasting has often been carried out, but research on the benefits of Dawood's Fast is still rarely carried out. Blood glucose is the sugar contained in the blood which is formed from carbohydrates in food and stored as glycogen in the liver and skeletal muscles. Blood glucose levels are influenced by factors such as age, insulin hormone, type and amount of food consumed and physical activity. The fasting state will result in a momentary hypoglycemia condition which can affect the body's metabolism. The purpose of this study was to determine the effect of Dawood's Fast on changes in glucose levels in human blood. This study is quasi-experimental using a comparative pre-test and post-test (non-equivalent control group). The subjects of this study were 34 people who were divided into 2 groups, namely the treatment group or the group that underwent the Dawood's Fast for 6 consecutive weeks and the control group or the group that did not undergo the Dawood's Fast for 6 consecutive weeks. The results of statistical analysis using the Wilcoxon Signed Ranks test in the control and treatment groups showed no significant difference ($p>0.05$). Meanwhile, the Mann Whitney U test to compare the control and treatment groups showed that there was a significant change in blood glucose levels of students at the Hidayatullah Islamic Boarding School in Surabaya when compared to the control group and the treatment group which were given the Dawood fast treatment in week 4 ($p<0.05$). While the statistical test to compare the difference between fasting blood sugar and blood sugar 2 hours PP using One-Way Anova showed results ($p> 0.05$) so there was no significant difference. This study conclude of this study is that giving Dawood's fasting treatment will not reduce human blood glucose levels, so it is safe for health.

Keywords: blood glucose levels; dawood's fast; intermittent fasting

1. Introduction

Fasting is a form of worship that is practiced by all religious communities in the world. Many religions incorporate fasting periods into their rituals such as Muslims, who fast from dawn to

dusk during Ramadan and Christians, Jews, Buddhists and Hindus, who observe traditional fasts on certain days or calendar years.[1] Ovivo Corna uses fasting as a treatment for various types of diseases.[2] Dawood's fast is a sunnah fast that the Prophet Dawood used to do. Fasting is done by fasting one day and one day not. According to terms, fasting means holding back, abstaining, or controlling oneself from eating, drinking, intercourse, and other things that cancel one's self from sunrise to sunset.[3]

Diabetes is a chronic disease that affects almost every organ in the human system.[4] Diabetes Mellitus (DM) is a chronic disease characterized by blood glucose levels that exceed normal, namely when blood sugar levels are equal to or more than 200 mg/dl, and fasting blood sugar levels above or equal to 126 mg/dl. DM is known as a silent killer because the sufferer is often not aware of it and when it is known that complications have occurred.[5]

One of the non-communicable diseases that has shown an increase so far is diabetes mellitus (DM). The World Health Organization (WHO) reports in its Global Status Report on NCDs that the population suffering from diabetes mellitus in 2008 numbered around 347 million and 80% of them came from poor and developing countries. Not only that, in 2008 as many as 1.3 million people died from diabetes mellitus.[6] WHO estimates that by 2030, diabetes mellitus will be the seventh-highest cause of death in the world.[7]

The IDF (International Diabetes Federation) states that more than 10 million Diabetes Mellitus patients suffer paralysis and worrying and life-threatening complications such as heart attacks, strokes, kidney failure, blindness, and amputations. By 2035 it is estimated that nearly 600 million people will live with diabetes mellitus and around 470 million people will experience impaired glucose tolerance.[8] The high incidence of DM sufferers has an increasing effect on complications. According to Soewondo et al (2010) in Purwanti (2013) there are 1785 people with diabetes mellitus in Indonesia who experience complications.[9]

The prevalence of diabetes mellitus in Indonesia in 2013 was 2.1%. This figure is higher than in 2007 (1.1%). A total of 31 provinces (93.9%) showed a significant increase in the prevalence of diabetes mellitus[5]. The benefits of fasting are reducing mortality from cancer and cardiovascular disease, increasing insulin sensitivity and reducing oxidative stress and inflammation. In addition to increasing life span and reducing the incidence of disease, fasting has also been shown to have other advantages. Fasting has been shown to protect normal cells and mice, but not cancer cells against oxidants and chemotherapeutic agents in general. [1]

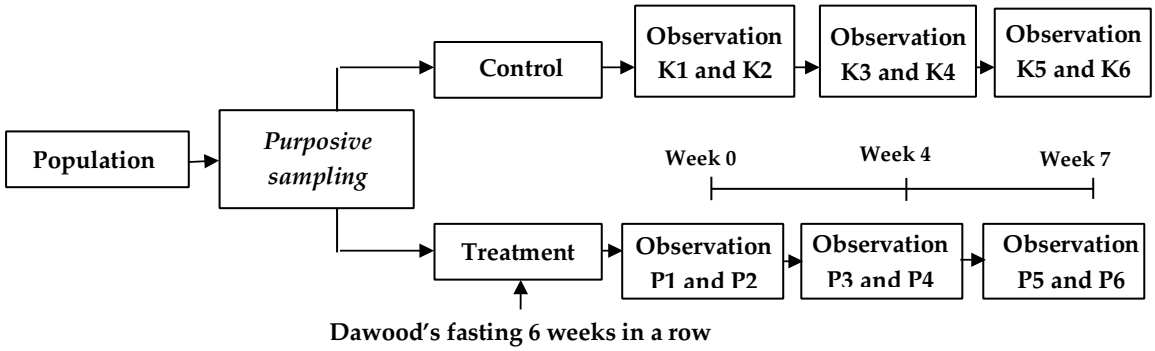
Fasting promotes ketogenesis, promotes potent changes in metabolic pathways and cellular processes such as stress resistance, lipolysis and autophagy and can exert medical effects, in some

cases as effective as drugs approved for use such as seizure relief and brain damage associated with amelioration of rheumatoid arthritis. [1] Blood glucose levels are influenced by several factors such as age, insulin hormone, emotions, stress, type and amount of food consumed and physical activity undertaken. Lifestyle changes such as shifting eating patterns to foods that lack fibre and contain lots of sugar are now very popular, especially among young people. This is one of the risk factors for being overweight and if it continues continuously it will increase the incidence of Diabetes Mellitus. One age group that is at risk of being overweight is the adolescent age group.[10] The benefits of fasting have been carried out by many researchers, but researchers more often research Ramadan fasting and fasting on Mondays and Thursdays. The benefits of Dawood's fast are still very rarely carried out by researchers because Dawood's fasting is not as popular as fasting in Ramadan which every year Muslims are obliged to do. Meanwhile, the Dawood's fast is a sunnah fast that is rarely practiced by Muslims, compared to Monday-Thursday fasting. The general research purpose is to determine the effect of Dawood's Fast on changes in glucose levels in human blood and the special research purpose is to prove the effect of Dawood's Fasting on fasting blood glucose levels and glucose 2 hours after eating for students of Hidayatullah Islamic Boarding School Surabaya.

2. Methods

Research design

This research is a quasi-experimental study using a comparative pre-test and post-test non-equivalent control group design. This study was designed to fulfill the research objective of knowing Dawood's fasting pattern on changes in blood glucose levels in students at the Hidayatullah Islamic Boarding School, Surabaya. The sample in this study was divided into two groups, namely a control group and a treatment group from the sample selected by purposive sampling. The population studied was the students of the Hidayatullah Islamic Boarding School in Surabaya.



Notes :
K1: The control group whose blood was taken during the pre-test and after 8 hours of fasting
K2: The control group whose blood was drawn during the pre-test and 2 hours after drinking 75 gr of sugar water
K3: The control group whose blood was drawn at week 4 and after 8 hours of fasting
K4: The control group whose blood was drawn at week 4 and 2 hours after drinking 75 gr of sugar water
K5: The control group whose blood was drawn at the time of the post-test and after 8 hours of fasting
K6: The control group whose blood was drawn during the post-test and 2 hours after drinking 75 gr of sugar water
P1: The treatment group whose blood was taken during the pre-test and after 8 hours of fasting
P2: The treatment group whose blood was taken during the pre-test and 2 hours after drinking 75 gr of sugar water
P3: The treatment group whose blood was taken at week 4 and after 8 hours of fasting
P4: The treatment group whose blood was drawn at week 4 and 2 hours after drinking 75 gr of sugar water
P5: The treatment group whose blood was taken during the post-test and after 8 hours of fasting
P6: The treatment group whose blood was taken during the post-test and 2 hours after drinking 75 gr of sugar water

The research was conducted at the Islamic boarding school Hidayatullah Surabaya, Jalan Kejawan Putih Tambak VI/1 Keputih Sukolilo Surabaya 60111 and the Clinical Pathology Laboratory of RSUD Dr. Soetomo Surabaya in October 2019 until the beginning of the second week of November 2019. Statistical analysis was carried out to determine whether there was an effect of David's fasting on blood glucose levels. From the experimental results, the data were analyzed using the statistical program which includes the following analysis steps: (1) Analysis of data distribution and concentration of data or descriptive statistics; (2) Test the normality of the data with Shapiro-Wilk to find out whether the data distribution is normal; (3) If the data is normally distributed, proceed with the variance homogeneity test; (4) If the variance is homogeneous, one-way ANOVA will be carried out. If the one-way ANOVA yields $p < 0.05$ (there is a significant difference), then proceed with analyzing the differences; (5) If the data normality test is not normally distributed or the data homogeneity test is not homogeneous, the data cannot be analyzed with one-way ANOVA, but with the Wilcoxon Signed Ranks Test. If the Wilcoxon Signed Ranks Test produces $p < 0.05$ (there is a significant difference), then proceed with analyzing the differences.

3. Results and Discussion

3.1. The average fasting blood glucose levels

Based on Figure 1, it is known that the average fasting blood glucose level of students at Hidayatullah Islamic Boarding School Surabaya which was measured during the pre-test and 8 hours after fasting obtained data at (K1) which was 76,500 and at (P1) namely 75,250, fasting blood glucose level for Islamic boarding school students Hidayatullah Surabaya which was measured

during the 4th week and 8 hours after fasting (K3) was 82.500, and at (P3) was 79.3125, and the Fasting Blood Glucose Level of Santri Islamic Boarding School Hidayatullah Surabaya which was measured during the post-test and 8 hours after fasting (K5) is 80.875 and at (P5) is 73.375.

Based on the results of the study, it can be seen descriptively that the mean value in each control and treatment group is different. In group (K1) the mean was 76,500 mg/dL and in (P1) 75,250mg/dL. Where these results are lower than group (P1) than (K1). The mean in group (K3) was 82.500 mg/dL and (P3) 79.3125mg/dL, which means the results were also lower than in group (K3). Meanwhile, the average in group (K5) was 80.875 mg/dL and in (P5) was 73.375 mg/dL, which means the results were the lowest compared to group (K5).

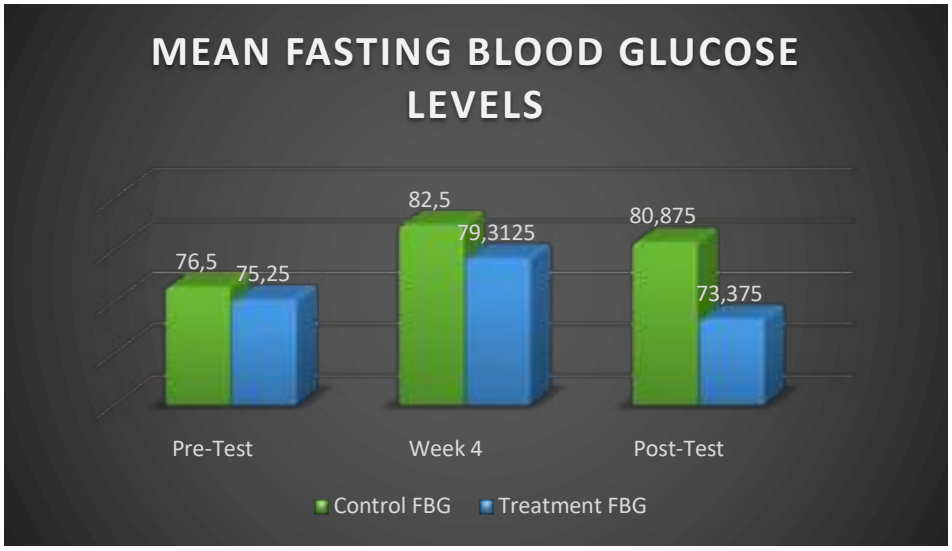


Figure 1. Figure of mean fasting blood glucose levels at the time of pre-test, 4th week, and post-test

So it can be concluded that the fasting blood glucose level of the treatment group was lower when compared to the fasting blood glucose level of the control group after being given Dawood's Fasting treatment for 6 consecutive weeks. Based on Figure 1, there are differences in fasting blood sugar levels pre-test, week 4 and post-test in the control group and the treatment group. In the treatment group doing the sunnah fasting of Dawood's Fast will experience a decrease in blood glucose levels because when fasting will experience the breakdown of glycogen into glucose so blood glucose levels will decrease during fasting.[11]

3.2. The Average Post-Prandial Blood Glucose Levels

Based on Figure 2, it is known that the average 2-hour blood glucose level of PP Santri Pondok Pesantren Hidayatullah Surabaya which was measured during the pre-test and 2 hours after

drinking 75 g of sugar water obtained data at (K2) of 100.750 mg/dL, and at (P2) i.e. 101.188 mg/dL, 2 Hour PP Blood Glucose Level Santri Pondok Pesantren Hidayatullah Surabaya which is measured during the 4th week and 2 hours after eating (K4) is 106.9375mg/dL and at (P4) is 110.0625mg/dL, and 2 Hour PP Blood Glucose Level Santri Pondok Pesantren Hidayatullah Surabaya which was measured during the post-test and 2 hours after eating (K6) was 101.8125 mg/dL and at (P6) was 114.625 mg/dL.

Based on the results of the study, it can be seen descriptively that the mean value in each control and treatment group is different. In group (K2) the mean was 100.750mg/dL and (P2) 101.188mg/dL. Where the results are higher from group (P2) than (K2). The mean in group (K4) was 106.9375 mg/dL and in (P4) 110.0625mg/dL, which means the results were also higher than group (K4). Whereas in group (K6) the average was 101.8125 mg/dL and (P6) 114.625mg/dL, where these results are higher than the group (P6).

So it can be concluded that the 2-hour PP blood glucose level in the treatment group was higher when compared to the 2-hour PP blood glucose level in the control group after being treated with Dawood's fasting for 6 weeks in a row.

Based on Figure 2, glucose levels 2 hours pp in the treatment group are higher than in the control group. These levels will increase to 120-150 mg/100 ml of blood after humans consume carbohydrates or glucose. This is what is called a physiological increase in blood sugar. Then 2-3 hours after consuming food, these high levels will return to normal. When fasting for 12-18 hours, this blood sugar level will decrease to the point of 60-70 mg/100 ml of blood. This decrease is also referred to as a physiological decrease in sugar.[12] During fasting, cortisol functions to increase the process of gluconeogenesis to increase the formation of glucose in the liver. This is what causes physiological adaptations to occur during fasting to maintain a balance in the body's condition to remain in normal conditions.[13]

The results of all the data found that 2 groups were not normally distributed, namely in groups K1 and P1 with a value ($p < 0.05$). While for the other groups, it has a value ($p > 0.05$). then the conclusion: the normality test of Shapiro Wilk all data is normally distributed. Except for groups K1 and P1 which are not normally distributed. Because there is data that is not normally distributed. So testing whether there is influence using nonparametric analysis is by using the Wilcoxon Signed Ranks Test.

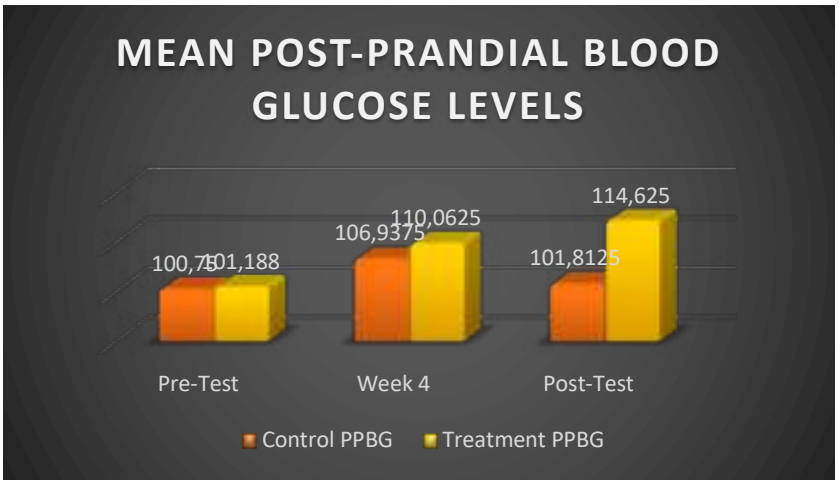


Figure 2. Figure of mean post-prandial blood glucose (PPBG) levels at the time of pre-test, 4th week, and post-test

3.3. The results of the Wilcoxon signed ranks test data variance of glucose levels of Santri Hidayatullah Islamic Boarding School Surabaya with three measurements starting from the pre-test, week 4, and post-test

Table 1. The results of Wilcoxon signed ranks test control group

Wilcoxon Signed Ranks Test Control Group					
Criteria		**FBG**		**PPBG**	
	Groups	**K3**	**K5**	**K4**	**K6**
FBG	K1	0.712	0.605		
	K3	–	0.977		
PPBG	K2			0.438	0.660
	K4			–	0.569

Table 2. The results of Wilcoxon signed ranks test treatment group

Wilcoxon Signed Ranks Test Treatment Group					
Criteria		**FBG**		**PPBG**	
	Groups	**P3**	**P5**	**P4**	**P6**
FBG	P1	0.660	0.851		
	P3	–	0.453		
PPBG	P2			0.201	0.078
	P4			–	0.877

Based on the results of the Wilcoxon Signed Ranks test in Table 1 and Table 2, the p-values were obtained in the control group and before eating when compared with the pre-test with the 4th week, the pre-test with the post-test, and the 4th week with the post-test, yielding p = .712 and .605 and .977.

Whereas at 2 hours after eating when compared with the pre-test with the 4th week, the pre-test with the post-test, and the 4th week with the post-test, the values of p = .438 and .660 and .569 were obtained. and in the treatment group before eating when compared with the pre-test with the 4th week, the pre-test with the post-test, and the 4th week with the post-test, the values of p = .660 and .851 and .453 were obtained. Whereas at 2 hours after eating when compared with the pre-test with the 4th week, the pre-test with the post-test, and the 4th week with the post-test, the values of p = .201 and .078 and .877 were obtained. So that the p-value > 0.05 for all groups. So it can be concluded that there is no significant difference between fasting glucose levels and glucose levels 2 hours PP in the control and treatment groups during the pre-test, week 4 and post-test. To find out which control group and which treatment has a significant difference, a Man Whitney U analysis must be carried out.

Based on Tables 1 and 2, the results of the control group also obtained results that were not much different from the treatment group. According to the researcher, the stability of blood glucose levels in the control group was caused by very good individual habits to control their blood sugar levels. Aside from a healthy diet, this can also be done in other ways, for example by physical activity, and is supported by questionnaire data that the respondents filled out themselves.

After analysis using the Wilcoxon Signed Ranks test, it can be concluded that the test shows a p-value > 0.05, so statistically there is no difference in the mean in all groups both in the control group which is the same as fasting and 2 hours post-prandial blood glucose levels, and in the same treatment group compared fasting blood glucose levels and 2 hours pp both during the pre-test, week 4 and post-test.

3.4. The results of the Mann-Whitney U test data variance of glucose levels of Santri Hidayatullah Islamic Boarding School Surabaya with three measurements starting from the pre-test, week 4, and post-test

Table 3. Mann Whitney U analysis test results

Groups	Significance					
	FBG – 1	PPBG – 1	FBG – 2	PPBG – 2	FBG – 3	PPBG – 3
K1 – P1	0,317					
K2 – P2		0,484				
K3 – P3			0,015			
K4 – P4				0,017		
K5 – P5					0,278	
K6 – P6						0,714

After the Wilcoxon Signed Ranks Test was carried out, it was continued with an analysis test using the Mann Whitney U Technique, where this test was carried out to compare the control group with the treatment group starting from fasting blood glucose levels and 2 hours pp from the pre-test to the post-

test.

The results are:

1. There is no effect on the control group with the treatment group whose blood was drawn during the pre-test and before eating and the control group with the treatment group whose blood was drawn during the pre-test and 2 hours after eating, with p values of 0.317 and 0.484 ($p>0.05$)
2. There is an effect on the control group with the treatment group whose blood was taken at week 4 and before eating and the control group with the treatment group whose blood was drawn at week 4 and 2 hours after eating, with p values of 0.015 and 0.017 ($p <0.05$).
3. There is no effect on the control group with the treatment group whose blood was drawn during the post-test and before eating and the control group with the treatment group whose blood was drawn during the post-test and 2 hours after eating, with p values of 0.278 and 0.714 ($p>0.05$)

Based on Table 3 it can be concluded that there were changes in fasting blood glucose levels and 2 hours pp blood glucose levels during week 4 compared to the control group with the treatment group which showed a p-value <0.05, so there was a significant difference. This is because, at the time of fasting, blood glucose levels will fall, causing a decrease in insulin secretion, which then increases the work of counter-insulin hormones, namely glucagon and catecholamines which result in the breakdown of glycogen. After a few hours of fasting, glycogen reserves will begin to decrease. As a result of reduced insulin in circulation, this will lead to the release of fatty acids from adipocytes whose oxidation will form ketones and will be used as energy by the body's organs.[14]

3.5. The test results analysis of difference in 2 hours PP blood glucose levels with fasting blood glucose levels of Santri Hidayatullah Islamic Boarding School Surabaya

Test results Analysis using 3 times the method. The first is to do a normality test, if the data is normally distributed then it is continued with a homogeneity test then if the data is homogeneous it is continued with the One-Way ANOVA test, and if the data is not normally distributed then it is continued with the Wilcoxon test. It is done through blood glucose levels 2 hours pp minus fasting blood glucose levels in three measurements starting from the pre-test, week 4, and post-test, which can be seen in Table 4. Because the results of the Normality Test show that the data is normally distributed, it can be continued with the Homogeneity Test. The homogeneity test can be seen in Table 5.

Table 4. Shapiro-Wilk normality test results

Groups	Shapiro – Wilk		
	Statistic	Df	Sig.
Control	0.790	3	0.091
Treatment	0.956	3	0.597

Table 5. Homogeneity test results

Levene statistic	df 1	df2	Sig
4.314	1	4	0.106

Based on Table 5, the results of the variance homogeneity test show a significance of 0.106. This shows p> 0.05, thus the data above has a homogeneous variant. To prove the hypothesis, the One-way ANOVA test was carried out. One-way ANOVA test can be seen in Table 6.

Table 6. Homogeneity test results

	Sum of squares	Df	Mean squares	F	Sig
Between Groups	133.604	1	133.604	4.009	0.113
Within Groups	130.374	4	32.539		
Total	263.978	5			

Based on the results of the One-Way ANOVA test in Table 6, the value of p = 0.113 is obtained. So that the p-value > 0.05, it can be concluded that H0 is accepted and H1 is rejected, which means that there is no significant difference between the difference in blood glucose levels 2 hours pp and fasting blood glucose levels, both in the control group and the treatment group.

Based on Table 4 the normality test on the difference in 2 hours pp blood glucose levels, with fasting blood glucose levels shows a p-value > 0.05, so the data is normally distributed and followed by a homogeneity test, based on Table 5 the results show p > 0.05, so the data produced is homogeneous. After the homogeneity test, it is continued with the One-way ANOVA test, based on Table 6 where the results of this test obtained a p-value > 0.05. So the conclusion is that there is no significant difference.

Based on the discussion above, it can be concluded that there is an effect of Dawood's fasting on the blood glucose levels of Santri Pondok Pesantren Hidayatullah Surabaya, starting from the 4th week of fasting. During fasting, the body experiences physiological adaptations and adaptive responses that have a positive impact on health. Dawood's fasting can be accepted as a type of calorie restriction that can be practiced in everyday life. Bener and Yousafzai (2014) also support that fasting helps make blood glucose levels more stable due to more regular eating patterns and relatively the same calorie intake from day to day, as well as helping to regulate increased levels of glucose and insulin in the body, help lower glucose levels, lower blood pressure and triglycerides. The decrease in blood sugar levels when fasting is also caused by a decrease in insulin secretion.[15]

4. Conclusion

From the results of the research that has been done, it can be concluded that there is an effect of Dawood's Fasting on the blood glucose levels of Islamic boarding school students at Hidayatullah Surabaya, during week 4. However, during the post-test, there was no effect of Dawood's fasting on the blood glucose levels of Islamic boarding school students at Hidayatullah Surabaya. So that Dawood's fasting will not make someone fall into a state of continuous hypoglycemia.

The suggestions for the next research were:

1. Conduct further research to determine the best way to lower human blood glucose levels using the intermittent fasting method.
2. Conduct further research on the mechanism of action of "Dawood's Fasting" for each individual and the best type of intermittent fasting that can be applied to see any differences.
3. Similar studies can be carried out by examining other variables such as cholesterol levels to prove other benefits of Dawood's Fast.

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Literature Review

The effects of acute aerobic exercise on salivary cortisol level and serum cortisol level

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Abstract: Cortisol plays an important role in stress response, both physical and mental. Acute aerobic exercise includes trigger cortisol production. It was reported that changes in cortisol level in blood, always associated with salivary cortisol. This study was to assess the effect of an acute aerobic exercise on salivary cortisol level and serum cortisol level. Search data base used by Google Scholar with keywords english. Keywords used were "acute aerobic exercise", "salivary cortisol", "serum cortisol", which were found in 11 journal articles under the following criteria that are suitable for analysis according to the purpose, periodof 5 years, suitability of data topics and the effects analysis. It was found that five articles focusing on effects acute aerobic exercise on salivary cortisol level and six articles focused on effects acute aerobic exercise on serum cortisol level. There were 9 journal articles which had significant effects and 2 others had no effects. We found that acute aerobic exercise can affect the hormonal response on blood or saliva.

Keywords: acute aerobic exercise; salivary cortisol; serum cortisol

1. Introduction

Cortisol is a steroid hormone from the glucocorticoid group which is synthesized from cholesterol. The secretion is largely regulated by the hypothalamus pituitary adrenal (HPA) axis. Secretion cortisol regulated by ACTH secreted by glands anterior pituitary. ACTH release by the anterior pituitary is regulated by corticotropin-releasing factor (CRF). Corticotropin-releasing hormone (CRH) then secreted to in plexus capillary principal part of the pituitary portal system in the hypothalamus going to gland anterior pituitary for stimulate ACTH secretion. Hormone adrenocorticotropic (ACTH) then will work through track cyclic adenosine monophosphate (cAMP) for stimulate adrenal cortex secretes cortisol.[1]

Cortisol has a direct negative feedback effect on the hypothalamus to decrease CRF formation and the anterior pituitary gland to decrease ACTH production. Both of these feedbacks help regulate cortisol concentrations in plasma which are influenced by two factors, namely circadian rhythms and stress.[2]

Cortisol plays an important role in stress response, both physical and mental. Exercise is one of the stress trigger. Exercises in different forms, types, and intensities have been shown to affect cortisol release differently.[3] Acute aerobic exercise includes trigger cortisol production.

The measurements of cortisol level in blood, urine and saliva used to study the cortisol include involvement in exercise.[4] It was reported that changes in cortisol level in blood, always associated with salivary cortisol. However, there is literature that says that the recommended blood specimens for cortisol level but there is other literature that says that salivary specimens have offered advantages over serum cortisol.[4,5]

The purpose of this paper is to discuss the effect of an acute aerobic exercises on cortisol from saliva and serum specimens.

2. Methods

This study is a literature review with a narrative review approach following the PRISMA guidelines. The research database was taken from Google Scholar using the keywords "salivary cortisol; cortisol serum; acute aerobic exercise, endurance exercise". In collecting and searching, 1,510 journals and research articles were found on the search keywords, only 47 journals were found according to the search keywords. Based on the results of the collection procedure, 11 journals were found that met the research inclusion criteria, namely: 1) publications in the 2019-2023 period; 2) in English; 3) studies that include research subjects, namely aerobic acute exercise and endurance sports; 4) type of journal is original research journal (not literature review), full text, and accessible; 5) the theme of the contents of the journal is salivary cortisol levels and serum cortisol levels in acute aerobic exercise and or endurance sports. In the end, 11 studies met the criteria for reassessment. Even so, this study also adds a discussion that is adapted based on other related sources. Screening studies according to PRISMA guidelines is presented in Figure 1.

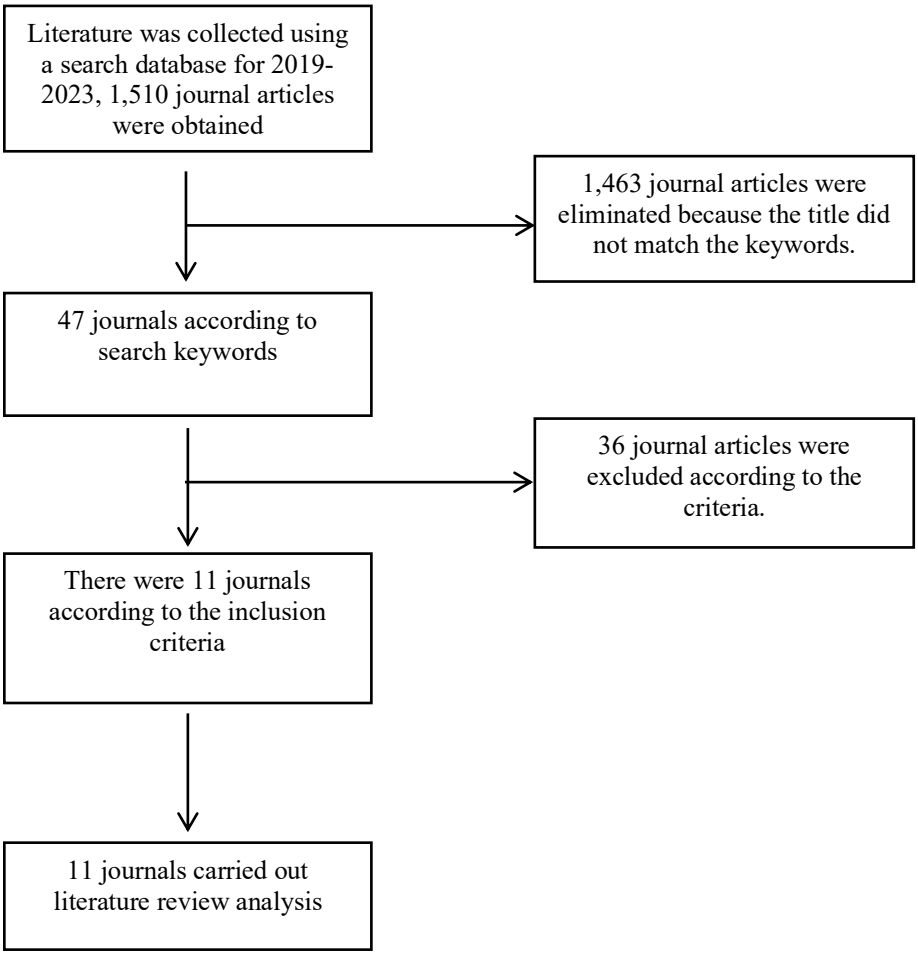

Figure 1. Figure of literature selection criteria

3. Results and Discussion

3.1. Result

Table 1 shows that the review of research of cortisol on exercise.

Table 1. Review of research of cortisol on exercise

No.	Title (Author)	Respondents	Method	Results			
1	A Comparison of Continuous and Interval Exercise on Cognition in Young Adults (Emily C.	Twenty-two college-aged men	Continuous (CONT) and interval (INT) cycling exercise. Began by 10:00 am. One mL	PRE CONT (ug/dL)	Post CONT (ug/dL)	Pre INT (ug/dL)	Post INT (ug/dL)
				0.58 ± 0.35	0.79 ± 0.62	0.63 ± 0.37	0.68 ± 0.42

	Tagesen, et al)		of saliva was collected before and after exercise.	Conclusion: The results showed after continuous exercise, cortisol concentrations were elevated, but after interval exercise, cortisol concentrations were unchanged.				
2	Cardiorespiratory Fitness, Blood Pressure and Ethnicity Are Related to Salivary Cortisol Responses after an Exercise Test in Children: The ExAMIN Youth SA Study (Sabrina Köchli, et al)	324 black and 227 white school children (aged 7.4 ± 1.0 years)	Cardiorespiratory fitness (CRF). Saliva samples were collected in a fasting state in the morning as a baseline measurement before 8 a.m, 30 min after the baseline sampling and a final sample 30 min after the CRF test.	Ethnicity	Base-line (saliva sample in the morning, <08:00 a.m.)	Pre sample (saliva sample before shuttle run) (in[mg/l])	Post sample (saliva sample after shuttle run) (in[mg/l])	Change from pre to post (in[mg/l])
				black	−1.84 (−1.91; −1.77) ***	−2.13 (−2.22; −2.05)	−1.74 (−1.82; −1.66) ***	0.40 (0.29; 0.50) **
				white	−2.26 (−2.34; −2.17)	−2.03 (−2.13; −1.93)	−1.41 (−1.51; −1.31)	0.62 (0.49; 0.75)
				Superscript symbol denotes significance for: * p < 0.05; ** p <0.01; *** p < 0.001. Conclusion: The results showed black ethnicity was related to lower salivary cortisol at baseline and post shuttle run test and a lower cortisol reactivity compared to the white ethnic group (p < 0.01)				
3	Decreased Activity of the Hypothalamic Pituitary-Adrenal Axis after Acute Aerobic Exercise in Obese Women (Sugiharto, et al)	20 obese female teenagers (20-24 age years old).	Subject performed in three groups of CTL (n=7, control group), IAE (n=7, interval time aerobic exercise) and CAE (n=7, continuously aerobic exercise). The blood sampling was carried out from the cubital vein. A 3 ml of blood was taken.	Group	Before (ng/mL)	After (ng/mL)	P -Value	
				CTL	327.36 ± 225.61	336.64 ± 192.47	P ≥ 0.05	
				IAE	334.99 ± 218.06	170.34 ± 10.15	P ≥ 0.05	
				CAE	328.65± 143.09	129.98 ± 16.53	P ≤ 0.05	
				Conclusion: The results showed the significant different serum cortisol between before and after the continuously aerobic exercise (CAE Group) (P ≤ 0.05)				

4	Effects of A High Intensity Interval Session on Mucosal Immune Function and Salivary Hormones in Male and Female Endurance Athletes (Camila Monje, et al)	Twenty subjects (10 males and 10 females) are long distance national level runners (age 18-40 years old)	HIIT season after 2 hour of fasting, between 3:00 pm and 6:00 pm. Saliva samples were collected 5 min before and 20 min post-exercise	Cortisol (nmol/L)	All participants	Males	Females
				PRE	9.7±1.9	10.3±1.7	9.0±1.9
				POST	18.6±7.4	17.3±6.9	19.9±8.1
				p-value	<0.001	0.002	0.005
				Conclusion: The results showed exercise increased the concentration of salivary cortisol (males: p=0.002; females: p=0.005)			
5	Endocrine responses after a single bout of moderate aerobic exercise in healthy adult humans (Maria Dourida, et al)	Twelve healthy (8 male and 4 female with age: 30.6 ± 4.4 years)	30-minute aerobic exercise. Blood samples were collected before (t0), at the end of the exercise bout (t30), and 30 min after the completion of exercise (t60).	Cortisol (nmol/L)	All participants	Males	Females
				T0	13.9 ± 1.2	13.2 ± 4.7	15.1 ± 7.6
				T30	10.3 ± 1.1	10.8 ± 3.8	9.3 ± 4.7
				T60	7.1 ± 1.2*	7.2 ± 2.5*	7.0 ±3.5*
				Conclusion: The results showed CORTISOL decreased after exercise, reaching significance ($p < 0.01$) 30 min after the completion of the exercise bout.			
6	Immune Response (Cortisol, TNFA, HMGB1) in Trained and Untrained Adolescent after 12 Minutes Run Exercise (Ilhamjaya Pattelongi, et al)	15 male basketball-trained and 15 male untrained basketball students at SMAN 1 Banjarbaru (15-18 age years)	Subject asked to performed 12 minutes run exercise (multistate technique) of moderate intensity. Blood sample taken 5 cc from the brachial vein after running.		Trained group	Untrained group	***P*** (post-hoc)
				Cortisol (ng/ml)	1440.1	26.3	0.000
				Conclusion: The results showed the cortisol concentration level was significant differences with the trained group compared to untrained group.			
7	Improvement of serum cortisol levels in obese female college students after	20 female students (20-23 age years) with BMI between 25-28 kg/m^2	Subject performed in two groups: control (n=10) and exercise (n=10). The	Group	Pre	Post	***P*** (post-hoc)
				Control	225.33 ± 55.22	241.87 ± 76.07	0.231
				Exercise	223.28 ± 58.84	131.85 ± 18.52	0.001

	moderate-intensity acute exercise (Wahyuningtyas Puspitorini, et al)		exercise group being exposed to moderate intensity acute exercise (60-70% HR max). Serum samples were taken 30 minutes pre exercise and 6 hours post exercise.	Conclusion: The results showed no change in cortisol levels in the pre-and post-test control groups, but cortisol levels in the exercise group showed a significant decrease		
8	Perceived stress and salivary biomarkers in educators: comparison among three stress reduction activities (Doreen Wagner a and Sharon M. Pearcey)	26 full time teachers (26-64 age years) participated (10 men and 16 women).	Subject asked to performed self-selected activities (11 participant chose yoga, 9 chose meditation and 6 chose aerobic exercise workout). Saliva samples were taken pre activity, immediately after activity and 30 minutes post activity.	***P*** (post-hoc) aerobic exercise workout Conclusion: The results showed the cortisol concentration level was significant differences with the aerobic exercise workout group higher at the 30 minute post activity compared to meditation group and yoga group.	Yoga 0.000	Meditation 0.000
9	Salivary endocrine response following a maximal incremental cycling protocol with local vibration (Monèm Jemni, et al)	12 male (age 18–28 years)	Subject asked to performed two maximal incremental cycling test (vibration and no vibration group) with protocol. Salivary samples were taken pre-exercise and immediately post-exercise during each trial.	Group ***P*** (post-hoc) Conclusion: The results showed the cortisol concentration was significantly higher after the Vib cycling test in comparison to the no Vib.	Vib 0.001	No vib 0.001
10	Testosterone	Male	3 days after	Group	Cortisol levels (nmol/L)	

	and Cortisol Responses to HIIT and Continuous Aerobic Exercise in Active Young Men (Cristian Cofré-Bolados, et al)	undergraduate subjects (n = 13) studying physical education	the test, the subjects showed up at 19:30 h in the laboratory and during three non-consecutive sessions, separated by 72 h, they performed the control sessions (CON), High-intensity interval training (HIIT), and a continuous aerobic exercise (AEE). Blood samples were collected pre-session (rest), immediately after the session (0 h), and 12 h post-session (12 h)		Resting time	0 time	12 h after exercise
			Control	8.16 ± 3.3	8.16 ± 3.3	13.17 ± 3.4
			HITT	8.75 ± 3.3		13.25 ± 2.2
			AEE	10.13 ± 4.5	11.95 ± 5.6	13.99 ± 2.1
			Conclusion: The results showed no differences of the measurements of free cortisol levels between AEE and HITT group.			
11	The impact of aerobic fitness on arterial stiffness and adrenal cortex hormones in middle-aged and older adults (Nobuhiko Akazawa, Koichiro Tanahashi, Keisei Kosaki, Hiroshi Kumagai, Satoshi Oikawa, Ai Hamasaki and Seiji Maeda)	198 middle-aged and older (age 50–79 years) healthy adults participated (81 men and 117 women). All women were postmenopausal.	All subjects performed a symptom-limited cycling exercise test (Lower fitness and Higher fitness). Blood samples were collected from the antecubital vein after overnight fasting.	Serum cortisol for lower fitness (μg/dl) 10 ± 4	Serum cortisol for higher fitness (μg/dl) 9 ± 4		
			Conclusion: There were no significant differences in cortisol concentration levels between lower fitness and higher fitness groups.				

From Table 1 it was found that five articles focusing on effects acute aerobic exercise on salivary cortisol level and six articles focused on effects acute aerobic exercise on serum cortisol level. There

were 9 journal articles which had significant effects and 2 others had no effects.

3.2. Discussion

The present literature review aimed to assess the effect of an acute aerobic exercise on salivary cortisol level and serum cortisol level. It was found in our study that acute aerobic exercise promotes change in salivary cortisol level and serum cortisol level.

Therefore, the characteristics of sample collected, such as: gender, number of participants, level of fitness, sport practiced and intensity of intervention, becoming a limitation in our analysis.

A large number of studies analyze the likely effects of acute aerobic exercise on cortisol level by bringing together individuals of both genders in their samples.[6–8] This fact limits the result found due to the exercise of different sex – related cortisol concentration levels in healthy individuals. It was found in our study that acute aerobic exercise no differences observed between male and female participants in their samples.[9,10]

The measurements of cortisol level in blood, urine and saliva used to study the cortisol include involvement in exercise.[4] It was found that five articles focusing on effects acute aerobic exercise on salivary cortisol level.[6,7,9,11,12] It was found that six articles focused on effects acute aerobic exercise on serum cortisol level.[8,10,13–16] There were two articles which had no significant effects is the measurements of cortisol level in blood serum. [8,16]

Several author report changes in cortisol concentration level in practitioners of aerobic sport such cycling [8,11,12], running [6,9,13,14], treadmill protocol. [10,15,16]

4. Conclusion

We found that acute aerobic exercise can affect the hormonal response on blood or saliva. There were 2 journal articles which had no significant effects is the measurements of cortisol level in blood serum. There were differences between the results of studies with level of fitness, sport practiced and intensity of acute intervention.

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Research article

Leptin gene polymorphism -2548g>a (rs7799039) in the occurrence of type-2 diabetes mellitus in Medan city

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Abstract. Hormone leptin is a hormone secreted by adipose tissue to regulate satiety in the hypothalamus. Previous research has found that polymorphisms in the leptin gene cause disruption of the satiety center and play a role in insulin resistance. This study aims to determine how the polymorphism of the Leptin gene affects patients with Type-2 Diabetes Mellitus in Medan. This study is quantitative descriptive research with cross-sectional design. The study was carried out in November 2022-February 2023 with the number of research subjects was 120 outpatients who had been diagnosed with Type-2 diabetes from several health centers in Medan city that met the inclusion and exclusion criteria. Each subject fills the identity of the respondent. Blood of 3 cc is taken from the cubital vein. Polymorphism of the Leptin-2548g>a gene was used by the PCR-RFLP technique in the integrated laboratory of the Faculty of Medicine, University Of Sumatera Utara. The average age of patients with Type-2 diabetes mellitus in Medan city is 56.2 years, with an average abdominal circumference of 91.81 cm, based on genotype, leptin heterozygote gene polymorphism GA as many as 41.7% of people, with a proportion of 80% occurring at the age of 51-60 years with the largest tribe is the Batak tribe. Conclusion**:** Leptin gene polymorphism with GA heterozygote genotype occurs in patients with Type-2 diabetes mellitus in Medan City

Keywords: DM type-2; gen leptin; polymorphism

1. Introduction

Diabetes Mellitus is a heterogeneous group of disorders characterized by hyperglycemia due to an absolute or relative deficit in the production or action of insulin. Type 2 Diabetes mellitus (DMT2) is a frequent metabolic change that occurs in about 90% of all cases of diabetes.[1] It is estimated that at least 463 million people aged 20-79 years in the world suffer from diabetes in 2019, equivalent to a prevalence rate of 9.3% of the total population at the same age.[2,3]

International Federation Data in 2020, the number of people with diabetes, including in Indonesia, continues to increase, reaching 6.2 percent with 10,681,400 cases.[4] The prevalence of Diabetes Mellitus in North Sumatra province increased from 2013 by 1.5% to 2% in 2018.[3]

Type 2 Diabetes mellitus is a disease with multifactorial causes, from environmental factors, eating habits, exercise, work, and others in addition to genetic factors. Recent research has found a variant of the gene responsible for assessing the risk of Type 2 diabetes.[5,6] In patients with Type 2 diabetes occurs hyperinsulinemia, insulin resistance occurs so that glucose can not enter the cell. The condition of the body that produces a lot of insulin, causing the body to no longer respond to the concentration of leptin released by adipose tissue. This happens because too much insulin to the brain because it does not get glucose by insulin performance, so the body still needs energy and continues to experience damage.[7]

Leptin is a protein product of the obesity gene (ob) or Leptin gene (LEP) that is expressed and released by adipose tissue in proportion to body weight. Leptin is a hormone produced by fat cells that regulates the metabolism of fat breakdown, and adjusts between hunger and energy expenditure. Hunger is inhibited when fat deposits reach a certain level, leptin is then secreted and circulates in the body and activates leptin receptors in the hypothalamus.[8,9]

One of the DNA sequence variations (gene mutase) that is known to affect the incidence of Type 2 diabetes mellitus is the leptin-2548g>a gene. The leptin-2548g>A gene is related to the mechanisms of weight regulation and energy homeostasis. With the polymorphism in the leptin gene-2548G>A (rs7799039), causing changes in the regulation of food intake with increased appetite, the body will send hunger and will occur continuously so that the intake of glucose, fat, and protein increases.[6,7,10]

Sumatera province which consists of 8 ethnic groups, is very rich in a variety of foods that most of the food ingredients contain carbohydrates, such as coconut milk. This study aims to determine the polymorphism of the gene leptin-2548g>A (rs7799039) in patients with type -2 diabetes mellitus in Medan, Sumatera Utara.

2. Methods

This research is descriptive research with cross-sectional research design. The study was conducted from November 2022 to February 2023. A total of 120 outpatients who have been diagnosed with DM type-2 from several health centers in Medan City, who meet the inclusion criteria were included as research subjects. As for the inclusion criteria of patients aged 30-60 years, do not have complications of DM and are willing to be the subject of research. The exclusion criteria established were having limited movement and being subjected to acute infection. Data on blood pressure, pulse, height, weight, abdominal circumference and blood sugar level are recorded.

2.1. Leptin gene polymorphism research-2548g>A (rs7799039)

A total of 3 cc of blood was taken from the cubital vein, put into the EDTA tube, and centrifuged to separate the plasma and blood elements. DNA isolation was carried out following the procedures of the Promega Genomic DNA Purification Kit (USA). After the DNA was isolated, the Leptin gene was then identified using the PCR method. Leptin Forward Gene 5'-TTT CCT GAA TTT TCC CGT GAG -3'; Reverse 5'AAA GCA AAG ACA GGC ATA AAA A -3'. 12.5 µL Master Mix, 1.0 µL Forward Primer, 1.0 µL Reverse Primer and 1.0 DNA Samples. The PCR machine is set for 30 cycles: denaturation stage 950C for 3 minutes: 30 seconds, annealing 530C for 20 seconds, and extension 720C for 18 seconds. To assess polymorphism, isolates that had been PCR were added with 1.0 µL of RE 10x Buffer, 0.1 µL of acetylated BSA, 0.2 µL of restriction enzyme Dde1, 3.7 µL of nucleus-free water, and 5.0 µL of PCR products. Place the PCR-RFLP into the prepared gel comb. Set the electrophoresis with a voltage of 100 volts for 60 minutes. Then it is inserted into the UV reader for interpretation.

2.2. Statistical analysis

Uni variate data is displayed in the form of a frequency distribution, while to calculate the number of Leptin genes that experience polymorphism, calculations are carried out using the Hardy-Weinbeg Equilibrium principle. If the p-value is > 0.05, it means that there is polymorphism in the study population or in accordance with the Hardy-Weinberg Equilibrium principle.

2.3. Ethical approval

All research subjects were given informed consent before the research was carried out. This study was reviewed and approved by the research ethics committee of the University of Sumatera Utara.

3. Results and Discussion

3.1. Characteristics of research subjects

The characteristics of the research subjects can be seen in the Table. 1. From Table 1. it can be seen that the average age of respondents was 56.2 years with an average abdominal circumference and fasting blood sugar levels above normal.

Table.1 Average age, abdominal circumference, arm circumference, fat percentage, fasting blood sugar levels, and blood pressure of type 2 DM patients

	Mean	SD	Min	Max
Age (years)	56.2	7,14	35	72
Body composition				
Abdominal circumference (cm)	91.81	19.4	33	163
Arm circumference (cm)	30.38	31.5	24	38
Fat percentage (%)	29.5	8.8	14.1	54.0
FBG (mg/dl)	187.07	67.03	101	331
Systole (mmHg)	137.62	20.25	94	180
Diastole (mmHg)	80.28	16.37	68	110

3.2. Distribution frequentation based on ethnicity of type 2 DM patients

Table. 2 Distribution frequentation based on ethnicity of type 2 DM patients in Medan city

Ethnic	Groups total
Batak	42 (35%)
Javanese	29 (24.3%)
Mandailing	14 (11.7%)
Malay	10 (8.3%)
Minang	10 (8.3%)
Karo	7 (5.8)%
Aceh	4 (3.3%)
Simalungun	2 (1.6%)
Dairi	1 (0.8%)
Dayak	1 (0.8%)
Total	**120 (100%)**

From Table 2 there are 10 different ethnic groups of type-2 DM patients who seek outpatient treatment at the Medan city health center. The majority of type-2 DM patients are Batak.

3.3. Leptin gene polymorphism -2548G>A(rs7799039)

In the RFLP electrophoresis results of the leptin gene -2548G>A, the sample group with the homozygous AA genotype was seen with a 242 bp band, the homozygous GG genotype was seen with 181 bp and 62 bp, while the heterozygous GA genotype was seen with 3 bands, namely 242 bp. 181 bp and 62 bp.

Table 3. Distribution of leptin gene polymorphism -2548G>A based on genotype

Leptin gene polymorphism -2548G>A	N	Percentage
AA	55	45.8 %
GA	50	41.7%
GG	15	12.5%

From Table 3, there are 41.7% of type 2 DM sufferers who experience the Leptin gene polymorphism 2548G>A

Table 4. Distribution of GA, AA and GG genotypes of the leptin gene -2548G>A based on ethnicity

Tribes	Genotype AA	Genotype GG	Genotype GA	Total
Batak	20	3	19	42
Javanese	13	4	6	29
Mandailing	6	2	3	14
Malay	5	2	4	10
Minang	6	0	2	10
karo	4	1	2	7
Aceh	0	2	1	4
Simalungun	1	0	1	2
Dairi	0	0	0	1
Dayak	0	1	0	1

3.4. Discussion

The results showed that out of 120 respondents, it was found that 80.8% with an age range of 51-60 years actually suffered from type 2 DM, while only 19.2% in the age range of 35-50 years. The results of this research are in line with the results of previous research. Azam M (2023) who conducted research in Indonesia involving 3.911 DM subjects, most of the respondents on 45-64 years old (57.4%) prevalence of odd ratio is 2.16 and 25.5% were on 15-44 years with prevalence of odd ratio is 3.22.[11] A study published in 2014 in the journal Diabetes Research and Clinical Practice showed that the risk of type 2 DM increases with age. The results show that the risk of type 2 DM increases approximately 1.1 times for every 10 years of age.[12]

Increasing age can reduce insulin sensitivity and insulin production from pancreatic beta cells, thereby increasing the risk of developing type 2 DM.[13–16] Likewise, a significant decrease in physical activity with increasing age results in poor changes in glucose metabolism, increased fasting glucose levels, and hemoglobin A1c.[17,18]

An imbalance between food intake and physical activity can cause the accumulation of visceral fat, because excess carbohydrate metabolism results will be stored in adipose tissue. The accumulation of visceral fat in the abdomen can stimulate the release of inflammatory mediators that affect insulin sensitivity.

In this study, it was found that the average abdominal circumference of type 2 DM sufferers in the city of Medan was above normal with an average fasting blood sugar level above 114 mg/dl (table 1). Fat cells secrete the hormone leptin. Leptin plays a role in energy regulation. Under normal circumstances, the hormone leptin will stimulate the satiety center. The state of hyperleptinemia which

is related to the accumulation of visceral fat and a decrease in insulin sensitivity in muscle cells actually reduces the sensitivity of the satiety center, so that the person still feels hungry.

The research results showed that in the research subjects observed, there were three types of genotypes for the leptin gene -2548G>A(rs7799039), namely AA, GA, and GG. The AA genotype occurred in 55 people (45.8%), the GA genotype occurred in 50 people (41.7%) and the GG genotype occurred in 15 people or 12.5%. The genotype that experienced polymorphism was GA. A meta-analysis study conducted by Huang et.al in 2015 showed that the leptin polymorphism genotype (-2548G/A) was associated with the risk of type 2 DM in Asian populations.[9] From the results of this research, it was obtained that the Batak tribe is the tribe that experiences the most leptin gene polymorphisms.

Gene polymorphism can occur due to various factors, diet, physical activity, environment, etc. Typical North Sumatran food mostly uses coconut milk. Medan city is famous as a culinary center. An unbalanced diet and lack of physical activity may contribute to the occurrence of leptin gene polymorphisms which in turn increase the risk of insulin resistance.

Research weaknesses

Polymorphism is not assessed only in certain ethnic groups but in all patients who come for routine treatment at the health center. And it is not determined whether the two descendants previously married into the same tribe or mixed marriages.

4. Conclusion

The hormone leptin plays a role in energy regulation. Find Leptin gene polymorphisms in type-2 DM sufferers in Medan city.

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Research Article

Effect of physical activity and nutritional status on metabolic syndrome of hajj pilgrims in Palembang

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Abstract: The pilgrimage is the fifth pillar of Islam which is a once-in-a-lifetime obligation for every Muslim who can afford it. Around 221,000 Indonesian pilgrims depart for the Holy Land, of which 60-67% belong to the High Risk (Risti) group and die in Saudi Arabia, mostly caused by heart disease, respiratory, kidney, metabolic, and hypertension. The aim of this study was to analyze the factors that influence the metabolic syndrome of pilgrims from the city of Palembang. This type of research is analytic observational with a cross-sectional research design. The results showed a significant relationship between gender p-value = 0.006, physical activity p value = 0.000, nutritional status p-value = 0.000 and there is no relationship between age p-value = 0.251 and metabolic syndrome. In conclusion, there is a significant relationship between metabolic syndrome and gender, physical activity and nutritional status in pilgrims from Palembang City in 2019.

Keywords: metabolic syndrome; nutritional status; physical activity; pilgrims

1. Introduction

The pilgrimage (Hajj) is the fifth pillar of Islam that is undertaken by 221.000 pilgrims from Indonesia, around which 60 – 67% of members belong to the High Risk (Risti) group. Every year, Indonesian Hajj pilgrims deaths in Saudi Arabia are caused mostly by diseases involving the heart, respiratory tract, kidneys, metabolic, and hypertension.[1] Reports from the Indonesian Hajj Pilgrim Center show that Indonesian pilgrims from 2015-2017 mostly are of the high risk category, and that cardiovascular disease is one of the causes for pilgrims receiving treatment and mortality while on pilgrimage.[2]

Shimemeri, et al reported that cardiovascular disease is the main cause of death in pilgrims and is associated with several comorbidities, including dyslipidemia, hypertension, as well as chronic pulmonary diseases.[2] Meanwhile, Handayani, et al report that smoking, reduced physical activity, circulatory diseases, respiratory diseases, and endocrine and metabolic diseases are the main causes of death of pilgrims. Research done by Harpini, et al. shows that diabetes mellitus and hypertension as risk factors associated with coronary heart disease for pilgrims in 2012.[2] This vulnerability can be a

risk factor for the occurrence of metabolic syndrome, which is a complex metabolic disorder linked to obesity, insulin resistance, dyslipidemia, and hypertension.

In Indonesia, metabolic syndrome affects 23,34% of the population, with a ratio of 26,2% male and 21,4% female.[3] Right now, metabolic syndrome has It is estimated that in the next five to ten years there will be an increase in risk diabetes mellitus (DM) type 2 by five times and cardiovascular disease by two times.[3] Patients with SM have 2-4 times the risk of stroke and 3-4 times the risk of myocardial infarction.[4]

Prevention of metabolic syndrome can be done by knowing in advance what factors can affect metabolic syndrome. This research was conducted with the aim of knowing the factors that influence metabolic syndrome in pilgrims from Palembang City.

2. Methods

This type of research used observational analytical methods with a cross-sectional research design. The research was carried out at Merdeka Health Center (Puskesmas), Sematang Borang Health Center, Dempo Health Center, Campus Health Center, Sukarami Health Center, Kenten Health Center, Pembina Health Center, Kertapati City Health Center, Palembang City.

The population sample of this study is every hajj pilgrims from the observation of health and medical records of Palembang City Siskohatkes (Integrated Hajj Computerized System for the Health Sector) 2019 met the inclusion and exclusion criteria. The inclusion criteria are data from the health examination results and medical records of the Siskohatkes hajj pilgrims in Palembang City in 2019. The exclusion criteria for the sample in this study were incomplete. The dependent variable in this study was the diagnosis of metabolic syndrome. The independent variables are age, gender, blood sugar, blood pressure, physical activity, and nutritional status.

Data collection in this study was carried out using primary data and secondary data. Data collected included age, gender, nutritional status, blood pressure, diagnosis of diabetes mellitus, waist circumference, and physical activity which were processed as needed for this research. Measurement of body weight with a scale and height with a microtoise according to the SOP to obtain the Body Mass Index (BMI). BMI measurements are calculated using the formula for body weight (BW) in kg per the square of body height in meters, while blood pressure measurements use digital blood pressure. The criteria for metabolic syndrome based on IDF 2006 are: 1. Central obesity with waist circumference for men ≥ 90 cm, women ≥ 80 cm, 2. Blood pressure ≥ 130/85 mmHg or currently on hypertension medication, 3. Diagnosed Diabetes Mellitus, or fasting blood sugar > 126 mg/dl or intermittent blood sugar ≥200 mg/dl with classic symptoms or is undergoing DM treatment.[4,5] Physical activity was measured in this study using the IPAQ questionnaire that has been tested for validity and reliability in

12 countries for adults aged 15-69 years designed to measure a person's physical activity used during the last 7 days. Physical activity is divided into 3 categories, namely light, moderate, and heavy.[6]

3. Results and Discussion

In this study, a total of 2,061 research subjects were obtained. The analysis was performed univariately and bivariately. The results of the analysis are then presented in the form of narratives and tables which can be seen in Table 1.

Table 1. Distribution characteristics in hajj pilgrims

Characteristic	Number (n)	Percentage (%)
Metabolic syndrome		
Positive	156	7,6
Negative	1.905	92,4
Age		
>60 Years	660	32
40-60 Years	1.271	61,7
<40 Years	130	6,3
Gender		
Female	1.171	56,8
Male	890	43,2
Physical Activity		
Light	954	46,3
Moderate	957	46,4
Heavy	150	7,3
Nutritional Status		
Obese	1110	53,9
Non-Obese	951	46,1

Based on table 1, it can be seen that metabolic syndrome is seen in 156 people (7,6%), age range of 40-60 years in 1.271 people (61,7%), male gender makes up 1.171 people (56,8%), light activity in 954 people (46.3%), moderate activity in 957 people (46,4%), and obese nutritional status in 1110 people (53.9%). Bivariate analysis using the chi-square test was carried out to determine the relationship between the dependent variable and the independent variable. The results of the study obtained the results of bivariate analysis are as shown in table 2.

Table 2 shows the relationship between characteristics of Hajj pilgrims and metabolic syndrome. Based on the research results for age, it can be seen that the p-value = 0.251 ($p<\alpha$) which shows that there is no significant relationship between age and metabolic syndrome, and 7.7% of Hajj pilgrims aged over 60 years have metabolic syndrome.

Table 2. Analysis of hajj pilgrim characteristics with metabolic syndrome

Variable	Metabolic syndrome Positive n (%)	Metabolic syndrome Negative n (%)	*p-value*	PR (95% CI)
Age				
>60 Years	51 (7,7)	609 (92,3)		
40-60 Years	100 (7,9)	1.171 (92,1)	0,251	-
<40 Years	5 (3,8)	125 (96,2)		
Gender				
Female	104 (8,9)	1.067 (91,1)		1,571
			0,006	(95% CI = 1,112 –2,218)
Male	52 (5,8)	94,2 (890)		
Physical Activity				
Light	104 (10,9)	850 (89,1)	0,000	11,917
Moderat-Heavy	52 (4,7)	13 (41,9)		(2,416-58,767)
Nutritional Status				
Obese	108 (9,7)	1.002 (90,3)		2,028
			0,000	(95% CI = 1,426-2.882)
Non-Obese	48 (5,0)	903 (95,0)		

*Pearson test, Chi-square

Based on the results of gender, it can be seen that the p-value = 0.006 ($p<\alpha$) which shows that there is a significant relationship between gender and metabolic syndrome. Prevalence Ratio (PR) Value shows that women have a 1.571 times higher risk than men of experiencing metabolic syndrome and 8.9% of female Hajj pilgrims have metabolic syndrome.

Based on the category of physical activity, it can be seen that the p-value = 0.000. This shows that there is a significant relationship between physical activity and metabolic syndrome. Prevalence Ratio Value (PR) indicates that light physical activity has a 2.482 times higher risk compared to moderate and heavy activity in causing metabolic syndrome.

Based on research results for nutritional status, it can be seen that the p-value = 0.000 ($p<0.05$) which indicates a significant relationship between nutritional status and metabolic syndrome. The PR value indicates that obesity has a 2.028 times higher risk than non-obesity in causing metabolic syndrome. 9.7% of Hajj pilgrims with obesity have metabolic syndrome

Metabolic syndrome has become a public health problem and clinical challenge throughout the world related to urbanization, excessive energy intake, increasing incidence of obesity, and sedentary lifestyles and their associated impacts.[4] The prevalence of metabolic syndrome is increasing in Asia, especially with the advent of modern lifestyles.[8] In this study, 156 (7.6%) pilgrims had metabolic syndrome. This number is slightly higher than the prevalence of metabolic syndrome among Hajj pilgrims in 2016, namely 1.6% (2450) Hajj pilgrims. Metabolic syndrome is typically characterized by central obesity, atherogenic dyslipidemia such as hypertriglyceridemia and decreased HDL cholesterol, hypertension, and dysglycemia. If only one of these conditions are present, it does not mean that

metabolic syndrome is present, but there is a greater chance of developing a serious illness. Having risks of complications, such as type 2 diabetes and heart disease, then the risk of metabolic syndrome increases.

3.1. Relationship between age and metabolic syndrome

Age is one of the risk factors associated with the occurrence of metabolic syndrome. In this study, it was found that the prevalence of pilgrims with metabolic syndrome was mostly in the age range of 40-60 years with a total of 100 (7.9%) people from all pilgrims. These results are in accordance with research by Liberty IA et al. with the highest prevalence of metabolic syndrome in the age range of 40-49 and 50-59 years, namely 1,512 (3.2%) of all pilgrims.[7] Based on the p value=0,251 ($p<\alpha$), there is no significant relationship between age and metabolic syndrome. These results are in accordance with Pijaryani's 2021 research (p value=0,794) and Driyah et al. in 2019 (p value=0,147).[3,8] This research is reinforced with previous research showing that ages ≥ 40 years and <40 years are not associated with metabolic syndrome, based on PR value = 3,804,it is known that there is a risk of experiencing syndrome at age 40. Bantas, 2012 states that there is an increase risk in developing metabolic syndrome as much as 3,58 times in females above 60 years, as opposed to age below 40 years.[9]

However, this research is not in line with several previous studies, namely Liberty IA et al, on Indonesian pilgrims in 2016 and Li et al. (Mean ± SD 56.45 ± 12.50; p-value < 0.001) in the adult population in China which states that there is a significant relationship between age and metabolic syndrome.[7,10] Guarner-lans et al,2011 states that in general, prevalence of metabolic syndrome increases as age increases. In general, the prevalence of the metabolic syndrome increases with age. The increased risk of metabolic syndrome in the elderly is due to the aging process. Aging is a multifaceted and functional process that affects biological function after it has reached its maximum potential. During aging, steroid hormones decrease so that the binding of globulin increases.[1] Therefore, there was a disturbance of the body's metabolism. This occurs due to changes in body composition in old age, which decreases muscle mass and causes a decrease in basal metabolic rate, decreased activity compared to young.[3]

3.2. Relationship between gender and metabolic syndrome

In this study, it was found that 104 (8.9%) female hajj pilgrims experienced metabolic syndrome, it was found that there was a significant relationship between gender and metabolic syndrome p-value = 0.006 (p-value <0.05) and the PR value of women had a risk 1.571 times higher than men. These results are in line with research, Bantas K, 2012 there was an increased risk of developing metabolic syndrome by 3.58 times in women over 60 years of age.[11] Driyah et al. (2019) and stated that women were 4.78

times more at risk (95% CI 1.11–20.56; p-value = 0.03) of experiencing metabolic syndrome than male.[3,12] The results of the research are tied to the onset of menopause and resistance to insulin in women. At the age approaching menopause, estrogen decreases.[10,13] Estrogen is a factor that affects the levels of sugars and lipids in the body. Estrogen has effects on HDL, with women having higher HDL levels than men on average. Estrogen also affects the distribution of fat, namely in the buttocks and hips rather than in the belly, which is one of the criteria for metabolic syndrome (central obesity). If the amount of estrogen is sufficient, it will help in maintaining normal blood sugar and blood fat levels and avoid an increase in abdominal visceral fat. The difference is not significantly related to changes in male sex hormones that occur during aging. These changes are known to be associated with insulin sensitivity and metabolic syndrome. Higher levels of testosterone and SHBG (sex hormone binding globulin) in aging males are independently associated with higher insulin sensitivity and a reduced risk of metabolic syndrome. This suggests that this hormone can prevent the development of metabolic syndrome.[14]

3.3. Relationship between physical activity and metabolic syndrome

In this study, it was found that 10.9% of hajj pilgrims with light physical activity experienced metabolic syndrome. Based on the results of the chi-square statistical test with a confidence level of 95% and α 0.05, p-value = 0,000 ($p<\alpha$). A p-value of less than 0.05 indicates a significant relationship between physical activity and metabolic syndrome. From the calculation of the prevalence rate, it was found that pilgrims with light physical activity had a 2.482 times greater risk of experiencing metabolic syndrome compared to pilgrims who had moderate and heavy activities. These results are in line with research conducted by Tanrewali which states that there is a relationship between physical activity and the incidence of metabolic syndrome in the adult population in Lambu District, Bima Regency (p-value = 0.001).[15] Ekelund et al., high physical activity plays an important role in risk factors for metabolic.[16] Inadequate physical activity and excessive caloric intake have twice greater risk factors for metabolic syndrome than those who have good physical activity.[17,18] Research in the UK shows that physical activity at moderate and high levels reduces the risk of developing metabolic syndrome.[18] In several studies, the proportion of participants who met the criteria for metabolic syndrome decreased after exercise intervention. Minimal physical activity (150 minutes per week of moderate-intensity activity or 75 minutes per week of vigorous-intensity activity) has consistently been shown to have a significant benefit on the risk of metabolic.[19] The benefits of physical activity include reducing blood pressure, increasing insulin sensitivity, reduces systemic inflammation, improves endothelial function, and reduces visceral fat. According to the American College of Sports Medicine, exercise plays an important role in preventing and controlling insulin resistance, prediabetes,

gestational diabetes, type II diabetes, and complications from diabetes. Both aerobic and weight-lifting exercises increases insulin action and helps control blood sugar.[19] In hypertension, by You Y et al. 2019 research shows light physical activity (p-value=0.006) to heavy physical activity (p-value=0.004) has the effect of preventing hypertension in people aged ≥45 years in China.[20]

3.4. Relationship between nutritional status and metabolic syndrome

Increased body mass index is associated with increased prevalence of metabolic syndrome. Based on bivariate analysis, it was found that 9.7% of pilgrims with obesity also had metabolic syndrome, there was a significant relationship between nutritional status and metabolic syndrome (p-value = 0.000). From the calculation of the prevalence rate, obese people have a 2.208 times greater risk of experiencing metabolic syndrome than those who are not obese. These results are in line with Liberty IA et al., 2019: there is a relationship between the BMI category and the incidence of metabolic syndrome among Indonesian pilgrims in 2016.[7] Driyah et al., 2019, there is a significant relationship between BMI and metabolic syndrome (p value=0,000).[3] According to Kamso et al, 2011 conclusion in executive employees with regression analysis that obese nutritional status has a 7.44 times risk (95% CI 2.48–22.30; p-value=0.000) of experiencing metabolic syndrome compared to normal nutritional status.[21] In addition,Kobo et al., 2019 states that normal nutritional status has a high Negative Predictive Value for reducing the occurrence of metabolic syndrome in men and women (98% and 96% respectively. Obesity is the main component of the incidence of metabolic syndrome. However, the exact mechanism is not yet known.[22] Obesity followed by increased fat metabolism will cause the production of Reactive Oxygen Species (ROS) to increase both in the circulation and in adipose cells. The increase in ROS in adipose cells can cause the balance of oxidation-reduction (redox) reactions to be disturbed, resulting in decreased antioxidant enzymes in the circulation, which is called oxidative stress. Increased oxidative stress causes dysregulation of adipose tissue and is the initial pathophysiology of metabolic syndrome, hypertension, and atherosclerosis.[22]

Excessive food intake accompanied by a lack of activity can cause excessive energy storage in the body in the form of fat. This can affect neurohormonal activation, chronic inflammation, and insulin resistance in the body which can lead to metabolic syndrome.[21] An increase in BMI will result in health problems. Obesity is a risk factor for other diseases such as noninsulin dependent diabetes mellitus, cardiovascular disease, and hypertension, where there is a positive relationship between metabolic syndrome and obesity parameters, namely BMI and the amount of fat under the skin (sum of skin-folds) and BMI, which is positively correlated with systolic and diastolic blood pressure.[23]

4. Conclusion

Based on the research results, it was concluded that 156 (7.6%) Hajj pilgrims experienced metabolic syndrome based on the criteria of central obesity, high blood pressure, and diabetes mellitus. The majority of Hajj pilgrims are in the age range 40-60 years with a total of 1,271 (61.7%) people, female with a total of 1,171 (56,8%). There is a positive relationship between gender, physical activity, and nutritional status with metabolic syndrome so that pilgrims can increase physical activity and balanced nutritional intake.

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Research Article

Effect of trained and untrained intensity submaximal exercise on lymphocyte levels of wistar strain white rat (Rattus norvegicus)

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Abstract: The decline in physical activity, changes in lifestyle, and unhealthy eating habits all contribute to the development of degenerative and infectious diseases. The body's immunity, particularly leukocytes, plays a crucial role in the onset of degenerative diseases. To maintain good immunity and adequate leukocyte levels, there are several things that can be done, including exercising regularly with sufficient intensity. Exercise, which is tailored to the body's ability, can activate the leukocytes, thereby improving immunity and preventing degenerative and infectious diseases. The purpose of this study was to investigate the effect of submaximal exercise with trained and untrained intensity on blood lymphocyte levels in female Wistar rats. This was an experimental research study that utilized a post-test-only control group design. The study involved 25 female white rats (Rattus norvegicus) of the Wistar strain, which were divided into 5 groups. The statistical analysis of the findings using the One-Way ANOVA test showed that $p = 0.859$, indicating no significant differences between the treatment groups. Therefore, the study concluded that there was no significant difference in lymphocyte levels between trained and untrained submaximal intensity exercises.

Keywords: leukocytes; lymphocytes; submaximal exercise; trained; untrained

1. Introduction

Today's life demands that humans are busy all day long, causing reduced overall body mobility. An inactive sedentary lifestyle accompanied by stress can lead to various diseases, both non-infectious (degenerative) and infectious diseases. Technological inventions that help human work also have a negative side. The more advanced and sophisticated technology is, the less physical activity is carried out.[1,2] Degenerative diseases refer to health conditions where the state of the organ or network gradually declines over time. This occurs because the body's cells experience changes that affect the function of the entire organ. The decline in physical activity, change in lifestyle, and unhealthy eating patterns can lead to the development of degenerative

diseases. On the other hand, infectious diseases are common illnesses caused by bacterial and viral infections.[2,3]

Maintaining a strong immune system is crucial for good health. It enables the body to resist or eliminate foreign objects and abnormal cells that can be harmful. One of the essential factors in maintaining a healthy immune system is the balance of physiology in the body, which can be gauged by markers like the number of leukocytes or white blood cells in the bloodstream[2]. Leukocytes play a critical role in the immune system by circulating in the blood, repairing and slowing down the decline of organ function. Exercise is one way to improve leukocyte production. However, it's important to choose the right type and level of physical activity that suits one's body. Excessive physical activity can result in oxidative stress, which is an imbalance between the production of free radicals and the body's ability to neutralize them with antioxidants. This can cause an increase in the number of leukocytes beyond 10,000 cells, which is not healthy. Different sports have varying levels of intensity, which can also affect the immune system. The intensity of sports can be classified as low, medium, or heavy, based on the duration and intensity of the exercise performed.[4]

Sports should be done in accordance with the body's ability to respond to received stress. The body will undergo an adaptation process when the burden of sports is too light. On the other hand, the body can't overcome the burden of sports that are too heavy until it disrupts the body's homeostasis system and damages it. One of the most frequent damages is muscle injury, which can result from excessive sports.[5] Excessive sports can result in the enhancement of leukocytes. The increasing rate of leukocytes is especially related to increasing neutrophils and lymphocytes during sports. Neutrophils increase during the recovery period, whereas lymphocytes increase quickly after the sport is stopped. Several recent studies have shown that an increase in lymphocytes can brief the location of infection and increase the immune system.[6] The theory according to Wahyudi et al. (2019) is that during sports, there is movement of leukocytes, activation, and an increase in the formation of compound radicals by leukocytes.[7] Excessive sports can result in an enhancement of free radicals, which can cause oxidative stress and decrease the flow of lymphocytes in the blood. This is because they get mobilized to the network that is damaged as a consequence of oxidative stress. Routine sports are better than occasional sports because routine sports result in the body experiencing adaptation. This adaptation helps overcome the enhancement of oxidative stress, ensuring that the mobilization of leukocytes happens equally through the blood genre.[8]

Based on the description above, the body's immunity can be improved by engaging in

appropriate physical activities that enable the body to function effectively, without excessive strain on leukocytes. It has been observed that rare physical activity or being sedentary can negatively affect the body's immune functions.[4] Therefore, this research aims to investigate the effect of sports intensity on immunity by measuring the rates of leukocytes, particularly lymphocytes, in white mice (Rattus novergicus). The purpose of this study is to determine whether intensity of physical activity, whether trained or untrained, can increase immunity and prevent infectious or degenerative diseases.

2. Methods

This study utilizes an experimental method called post-test-only control group design and involves using white Benita mice (Rattus norvegicus) of the Wistar strain. The study has been approved by FK Hang Tuah University Surabaya, and it was conducted in the Laboratory of Biochemistry at the Faculty of Medicine, Hang Tuah University Surabaya, over a period of one month, which included one week of adaptation, two weeks of treatment, and one week of rest. The number of animal samples required for the study was calculated using Federer's formula, with five treatment groups, each consisting of five mice, for a total of 25 mice. The mice used in this study were female, white (Rattus norvegicus) strain Wistar, healthy, 2 months old, and weighed between 180-200 grams. The mice were randomly divided into five groups. The details of the groups used in the study are as follows:

K1: Control group

K2: Trained group without intervention

K3: Untrained group without intervention

K4: Trained group with intervention

K5: Untrained group with intervention\

The procedure for research and data collection will be done as follows:

To prepare for the animal experiment, suitable mice were chosen based on certain criteria. They were then adapted to the laboratory environment for 7-14 days, during which they were given food and water. The mice were randomly selected and divided into 5 groups, labelled K1-K5, and placed in separate drums.

Group 1 was the control group, with the mice left in the drum throughout the research process with no intervention.

Groups 2 and 4 underwent training through swimming in a bucket every day for 2 weeks. Before the intervention, a swimming test was conducted to determine the maximum swimming ability of the mice. Once the mice were trained, the process continued for another 2 weeks. In

group 4, the mice received a swimming treatment once with the same duration.

Groups 3 and 5 received the same intervention, but only once a week for 2 weeks. Before the intervention, the swimming test was conducted to determine the maximum swimming ability of the mice. Once the mice were not trained, the process continued for another 2 weeks. In group 5, the mice received a swimming treatment once with the same duration.

To take a blood sample from the mice, they were first anesthetized and then placed on a metal plate while sedated for surgery. The surgery began by opening the skin until the muscles were visible from the epigastric area to the heart. Blood was drawn from the ventricle using a 3 ml syringe, and then entered into an EDTA tube, which was then shaken to mix the blood and EDTA. The mice were then euthanized through exsanguination.

The blood sample mixed with EDTA was analyzed using a hematology analyzer to determine the lymphocyte count from the whole leukocytes in the blood. The data obtained is in the form of a ratio.

Obtained data is rate mice blood lymphocytes from all groups. Data obtained separated between each group (K1-K5). Data was analyzed using statistical tests to see exists different meanings between each group being compared with the group control. analytical method in a manner statistics use :

1. Test Shapiro-Wilk For test normality
2. Test Levene For homogeneity test
3. Test One Way ANOVA For parametric test

3. Results and Discussion

3.1. Research result

The following table lists the rate of lymphocytes in the blood of mice and includes the measurement results that have been whitelisted.

Table 1. The results of examination of lymphocyte levels

No	K1 (%)	K2 (%)	K3 (%)	K4 (%)	K5 (%)
1	72,4	72.0	62.2	60,6	69,6
2	78.5	76,2	53.5	80.1	69.2
3	64,7	61.3	70.6	79.2	64.8
4	83.8	74.7	71,2	60.5	66.5
5	59,7	78.1	82.9	65,9	68,6
Amount	359,1	362.3	340.4	346,3	338.7
Average	71,82	72,46	68.08	69,26	67,74

Note: K1: Control Group; K2: Trained Group without Intervention; K3: Untrained Group without Intervention; K4: Trained Group with Intervention; K5: Untrained Group with Intervention.

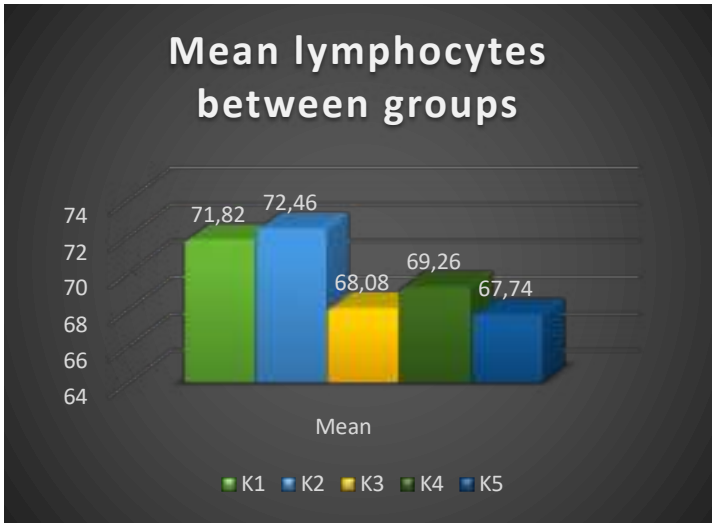

Figure 1. Mean lymphocytes between groups

After analyzing the data, a normality test was conducted using the Shapiro-Wilk method and it was found that all of the data was normally distributed (with a significance level greater than *a*). Following this, a homogeneity test was conducted using the Levene method, which revealed that the data had homogeneous variation (again, with a significance level greater than *a*). Because the data was both normally distributed and homogeneous, a parametric statistical test was conducted using the One-Way ANOVA method. The results showed a significance mark of p = 0.859 (with p> *a*), indicating that there was no significant impact of exercise intensity on the immunity of white female mice (Rattus norvegicus) of the Wistar strain.

3.2. Discussion

Regular exercise can have a positive impact on a person's immunity, but only if it is performed consistently and sustainably. The intensity, duration, and load of the exercise can all play a role in how it affects the immune system. Generally, sports that result in moderate levels of stress and oxidative damage to the muscle cells can stimulate the production of circulating lymphocytes, which help in repairing the damage. However, if the exercise intensity is too high or too low, or if it is not performed consistently, it can have a negative impact on the immune system by increasing oxidative stress. This is because the body does not have a mechanism to adapt to the damage caused by exercise if it is done irregularly.

The best intensity of exercise currently recommended is submaximal, which means exercising at 80-90% of your maximum ability and doing it regularly. Consistent exercise helps the body to adapt to oxidative stress, which leads to reduced muscle damage and increased levels of

lymphocytes and other leukocytes in the circulation. These cells play a crucial role in the immune system. According to the above information, a study is expected to show that there is a significant difference in the rate of lymphocytes between groups that are trained and those that are not trained. The rate of lymphocytes is expected to be higher in the trained groups, both with and without intervention (K4 and K2). The results of the study are expected to show that the trained group has more circulating lymphocytes than the untrained group. This is because, in the untrained group, the damage network is more significant, leading to more lymphocytes being directed toward the injury. In contrast, in the trained group, the level of lymphocytes is expected to be more stable.

Based on statistical tests, there appears to be an average difference between the five groups of treatment with varying bound rate lymphocytes. However, there is no significant difference between the groups when analyzed statistically ($p>0.05$). It has been observed that the trained group has a higher rate of lymphocytes compared to the non-trained group. Previous studies have suggested that physical activity may decrease the rate of lymphocytes due to apoptosis mechanisms [9], as evidenced by the lower levels of lymphocytes in the intervention group. This suggests that the rate of circulating lymphocytes is higher in the trained group than in the non-trained group. In the non-trained group, more damage occurs in the network, leading to more lymphocytes being directed toward the injured network.

When we exercise, it puts physical stress on our body which initially activates the Sympathetic-Adreno-Medullary axis (SAM axis). This axis sends stimuli via sympathetic neurons to the adrenal glands, leading to the release of adrenaline and noradrenaline. These hormones play a crucial role in our body's fight-or-flight response and can increase our heart rate, blood pressure, and breathing rate.

Apart from the SAM axis, exercise also activates the Hypothalamus-Pituitary-Adrenal axis (HPA axis). This axis provides a stimulus to the Limbic-Hypothalamus-Pituitary-Adrenal axis (LHPA axis). The LHPA axis stimulates the hypothalamus to secrete corticotropin-releasing-hormone (CRH), which sends a message to the anterior pituitary gland. This results in the release of Adrenocorticotropin Hormone (ACTH), which influences the secretion of cortisol - a hormone secreted by the adrenal cortex. The cortisol hormone sends negative feedback to decrease activity in inflammation by pushing the rate of lymphocytes in our bloodstream. This process helps our body to cope with exercise-induced stress.[10]

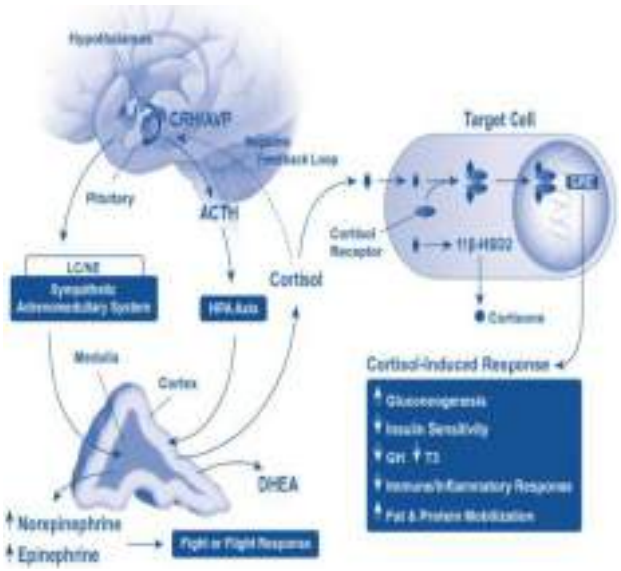

Figure 2. The HPA axis and SAM axis are two systems in the human body that are responsible for the stress response

Regular exercise can lead to adaptations in the body that change the way it responds to stress. Exercise can stimulate changes in the body's capacity to function, including the immune system, specifically lymphocytes. A research study conducted by Shodiq (2016) examined the effect of exercise on the white blood cells of mice.[10] The study found that after two months of exercise, the group that received submaximal exercise had a significantly higher lymphocyte count than the control group. However, the study also found that the difference in lymphocyte count was not statistically significant due to the short treatment period of only two weeks. Additionally, the time of day when the blood samples were taken could have affected the results, as peak levels of plasma glucocorticoids, which suppress the immune system, occur at 8:00 am.

Another study by Mushidah & Muliawati (2019) divided mice into three groups - control, light exercise (swimming once), and heavy exercise (swimming for three days). The study found that the group with heavy exercise had increased cortisol levels, which can lead to tissue damage and hinder the immune system, resulting in lower levels of circulating leukocytes.[11] A study by Laeto, Natsir, and Arsyad (2019) on humans found a significant increase in lymphocyte count after exercise, with the highest count found when the exercise was performed in the afternoon.[2] This is because cortisol levels are relatively higher at night compared to the afternoon.

Further research is needed to determine the pattern of lymphocyte count change in response to exercise, including a time series study that follows the rhythm of circadian cortisol. Additionally, research should be conducted on the effect of exercise on lymphocyte count in normal individuals, especially during high-intensity sports.

4. Conclusion

Based on a study conducted on female white mice (Rattus norvegicus) of the Wistar strain, it can be concluded that there was no significant difference in the rate of lymphocytes in their blood during submaximal exercise, regardless of whether the mice were trained or not trained at a certain intensity level.

5. Acknowledgments

This research was conducted by Maria Angela Velisia Nabita Santoso at the Medical Faculty of Hang Tuah University. Eric Mayo Dagradi, dr., M.Kes. was the main supervisor, and Dr. Dian Ardiana, dr., SpKK, FINSDV were the accompanying supervisors. Dr. Riami, dr., M.Kes. was a thesis examiner.

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Literature Review

Effectivity of anthocyanin in inhibiting Transforming Growth Factor Beta 1 (TGFβ-1) in fibrosis

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Abstract: Fibrosis is a pathological response observed in chronic inflammatory diseases affecting vital organs, including the liver, kidneys, and lungs. The prevalence of fibrosis is observed in up to 45% of patients residing in both developed and developing countries. TGFβ-1 is a key molecule in the pathogenesis of fibrosis. Nevertheless, clinicians have not widely embraced a significant number of efficacious medicines with the purpose of impeding the advancement of fibrosis. The main option for fibrosis management is to target systemic inhibition of TGFβ-1. Furthermore, instead of conventional therapeutic approaches that concentrate on inhibiting TGFβ-1, an alternate approach to managing fibrosis involves the utilization of anthocyanin as a compound with antioxidant, anti-inflammatory, and antifibrotic properties. The objective of this study is to assess the efficacy of anthocyanin in suppressing TGFβ-1 in fibrosis. The research was conducted utilizing the narrative literature review approach. The findings of this study indicate a reduction in TGFβ-1 expression in the experimental models to anthocyanin treatment. Hence, it may be inferred that anthocyanin possesses the capacity to efficiently suppress TGFβ-1 in the context of fibrosis.

Keywords: Anthocyanin; Fibrosis; Transforming Growth Factor Beta 1 (TGFβ-1)

1. Introduction

Fibrosis is a healing process or reaction to tissue injury. This process generally occurs as a result of chronic inflammation which can occur in organs such as the kidneys, lungs, skin, and liver.[1] Fibrosis can be irreversible which eventually causes permanent tissue injury, then causes organ malfunction and may lead to death. This condition is commonly found in chronic inflammatory diseases such as Idiopathic Pulmonary Fibrosis (IPF), end-stage liver disease, and several other diseases.[2]

Fibrosis and other chronic diseases associated with fibrosis have high incidence data in various countries, both developed and developing countries. This disease is also associated with mortality rates in developed countries of up to 45%.[3] Indonesia itself has a high incidence of fibrosis in various

organs. For example, cirrhosis of the liver, which is the occurrence of diffuse pathological changes in liver tissue characterized by fibrosis and the formation of regenerative nodules, has a high incidence in Indonesia. In Indonesia, the prevalence of liver cirrhosis is equivalent to 47.4% of the total hospitalized patients with liver disease.[4]

The high incidence of fibrosis has stimulated interest in the development of therapeutic approaches to inhibit TGF-β1 by disabling and/or stopping fibrosis occurring at various organ sites.[3] One of the potentially beneficial substances on the expression of TGF-β1 is anthocyanin. Anthocyanins are derived compounds found in several types of plants and are abundant in nature in diversity. Anthocyanins have been proven to be used in the health sector.[5]

Anthocyanin

Anthocyanins are natural pigments belonging to the flavonoid group that are soluble in polar solvents and are formed by three carbon atoms linked through one oxygen atom and two benzene aromatic rings (C6H6) that attached to the main structure. As a bioactive compound, there is arrangement of conjugated double bonds in the anthocyanin structure not only functions for the plant itself, but also as a natural free radical scavenging and destroying compound or more commonly known as a natural antioxidant which provides benefit for humans.[6]

The antioxidant function of anthocyanins has many benefits in preventing various degenerative diseases such as anti-diabetic, anti-hypoglycemic, anti-hypertensive, anti-cancer, anti-inflammatory, anti-amnesia and anti-aging (neuroprotective), prevention of dysfunction, and obesity processed by ongoing oxidative inside the body. It can be caused cell damage and uncontrolled cell proliferation into lipid peroxide or malondialdehyde (MDA) and causes cell death in various body tissues.[7]

Transforming Growth Factor β1 (TGF-β1)

TGF- β become an important role in various cellular processes including proliferation, differentiation, migration, and apoptosis which are required to maintain tissue homeostasis. In under normal conditions, TGF-β acts as an inducer of apoptotic effects, this is important for cell differentiation and regeneration, but when TGF-β in high levels it can be cause massive cell death. For example, in liver cells it can be cause fibrosis and cirrhosis.[8]

The relation between Anthocyanin and TGF-β1

In one study it was stated that anthocyanins have an effect on the activation of AMP-activated protein kinase (AMPK). AMPK is a target in metabolic disorders because its activation increases insulin sensitivity, lowers blood glucose levels, and improves lipid profiles. AMPK also works as a tumor suppressor liver kinase 1 (LKB1) to regulate metabolism and growth of cancer cells. AMPK activators, including metformin, phenformin, or A769962, suppress Smad2/3 phosphorylation and expression of

target genes, including PAI-1, CTGF, FN, and IL-6 in response to TGF-β, along with a decreased ability of cancer cells to migrate upon healing wound. Furthermore, AMPK activation works inversely to the stimulation of TGF-β on the expression of EMT markers and changes in the morphology of BEAS-2B cells.

All of these effects depend on LKB1-dependent function, although the inhibitory effect on TGF-β-induced IL-6 expression may also be mediated by the LKB1-independent pathway. This may cause the expression of a dominant negative AMPK mutase or deletion of AMPK subunits increasing TGF--stimulated Smad2/3 phosphorylation, whereas the expression of active AMPK mutations attenuates the TGF- signaling pathway by inhibiting Smad2/3 phosphorylation and transcriptional events thereby reducing the effect of TGF-β. It can be concluded that AMPK activation inhibits TGF-β-modulated EMT and cancer metastasis.[8]

2. Methods

This study performed a literature review and descriptive analysis on Google Scholar, PubMeds, Researchgate, Frontires, and Elsevier. This study use article published in the last 10 years or between 2012 and 2022 with the following keywords to search literature were "anthocyanin or anthocyanidin", "transforming growth factor beta 1 (tgfb1)", "fibrosis", "anthocyanin and transforming growth factor beta 1 (TGF-β1) and fibrosis", and "Effectiveness of Anthocyanin in Inhibiting Transforming growth factor beta 1 (TGF-β1) in Fibrosis".

3. Results and Discussion

3.1. Results

A study conducted by Sohn *et al.* which examined the anti-inflammatory and antifibrosis effects of anthocyanins on *Peyronie's Disease* (PD) using a mouse model showed that the formation of fibrous tissue found in male mouse models given anthocyanin extract (AC) was less than in the control group and PD group. The PD group that did not receive anthocyanin had the most prominent fibrosis among the three experimental model groups. The immune reaction of TGF-β 1 in the control model with normal histology was weaker than the PD and AC groups. In contrast, the PD group showed higher TGF-β1 immunoreactivity, meaning there was an increase in collagen and fibroblast production in the tunica albuginea of PD group. The AC group showed significant differences from the PD group with the results of TGF-β 1 expression decreasing significantly.[9]

On the research model conducted by Wang *et al.*, type II alveolar cells of mice were given exposure to the chemical paraquat (PQ) commonly used as herbicides) and then given anthocyanins. The results

showed that the expression of TGF-β 1 protein decreased significantly after anthocyanin administration compared to control and PQ.[10]

Romualdo *et al*. had done a research regarding anthocyanin extracted from fruit peels of the *Myrtaceae* plant on chemically induced liver fibrosis and carcinogenesis in mice which showed similar results to the two studies discussed, namely there was a decrease in TGF-β 1 levels in mice which was intervened by the administration of DEN / CCL4 and then given S. *malaccense* fruit peel powder which is rich in anthocyanin.[11]

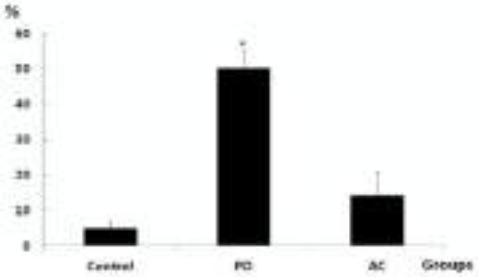
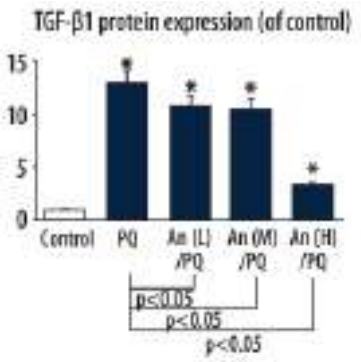
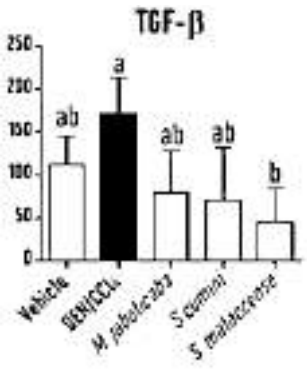
Similar results were illustrated in the study of Morrison *et al.* on the inhibitory effect of bilberry extract towards the progression of non-alcoholic hepatic steatosis and hepatic fibrosis. The three groups of mice studied consisted of a reference group (REF), without cholesterol supplement, a high cholesterol group (HC), a control group given cholesterol supplement), and a *high cholesterol mirtoselect* (HCM), given a cholesterol supplement and anthocyanin). In addition to reducing inflammation, the anthocyanin contained in the bilberry extract has the potential to inhibit the progression of steatosis into fibrosis. The study reported that the HC group showed a significant increase in TGF-β 1 expression, while in the HCM group, TGF-β 1 expression was reduced. Hepatic cell staining showed increased collagen formation in the HC group when compared to the REF group and was significantly reduced in the HCM group. Biochemical examination supports the argument of a significant increase in collagen production in the HC group that was reduced in the HCM group and compared to the REF group.[12]

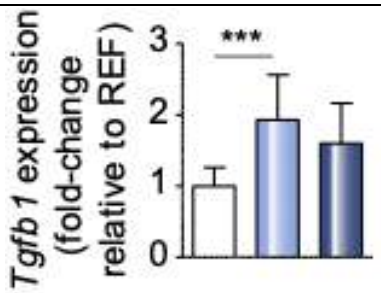
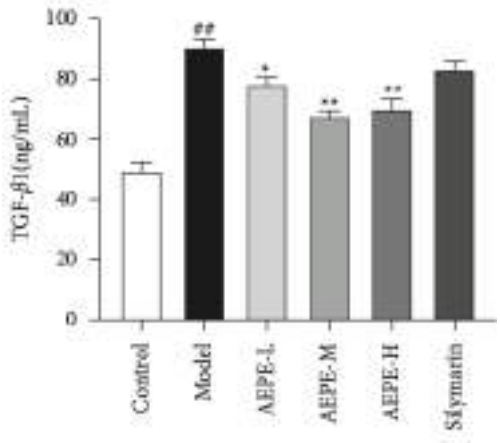
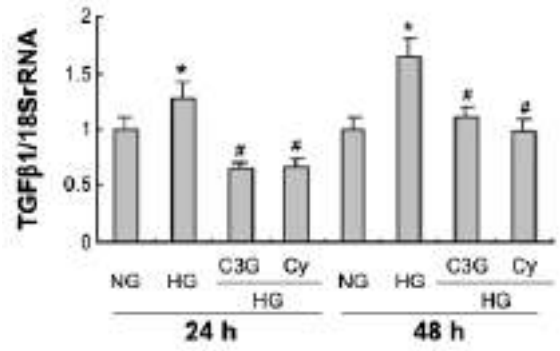
Yin *et al*. stated similar results, namely that there was a dramatic increase in TGF-β 1 expression in mouse models with CCl 4-induced hepar fibrosis that did not get anthocyanin compared to the control group. A significant decrease in the expression of TGF-β 1 in mice with fibrosis was successfully achieved by the administration of anthocyanin extracts.[13]

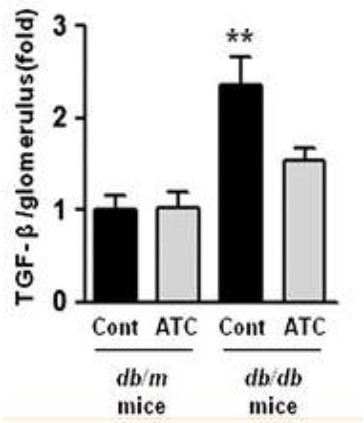
Du *et al*. conducted a study on the effects of anthocyanidins on accumulation and inflammation mediated by high glucose levels in HK-2 cells (human kidney cells). The study found that when cells were given high levels of glucose during the first 24 hours and the next, TGF-β1 protein levels increased compared to controls (NG). After being given anthocyanin derivatives, cyanidine-3-O-β-glucoside chloride (C3G) and cyanidine chloride (Cy), Du *et al* stated that both substances significantly lowered the levels of TGF-β1 protein.[14]

Koh *et al*. examined the effect of anthocyanin administration on improving renal lipotoxicity in the diabetic mice model, stated that the expression of TGF-β 1 in the control group with type II diabetes was higher compared to non-diabetic control mice and diabetic and non-diabetic mice given anthocyanins.[15]

Table 1. Immunohistochemistry findings from different studies on TGFβ1 expression

Researcher	Model	Result	Graph result immunohistochemistry
Sohn *et al.*, 2014	*Peyronie's Disease* (PD) induced by fibrin injection in rats.	The fibrous tissue of the PD group without anthocyanins was thicker than the control group and PD with anthocyanins (AC). TGF-β1 expression in the PD group without anthocyanin increased dramatically and decreased significantly in the PD group with anthocyanin.	 Description: 1. PD: *Peyronie's Disease* 2. AC: Anthocyanin
Wang *et al.*, 2018	RLE-6TN (mice) *lung injury* induced by Paraquat (PQ)	Decreased expression of TGF-β1 An group after being given PQ.	 Description: 1. An: Anthocyanin
Romualdo *et al.*, 2020	C3H/HeJ mice with DEN/CCl induced hepatic fibrosis.	Significant decrease in TGF-β1 expression of group AC (three species of *Myrtaceae* fruit).	

Morrison *et al.,* 2015	Mice with non-alcoholic hepatic steatosis and hepatic fibrosis.	Increased expression of TGF-β1 of the high cholesterol (HC) control group and decreased in the anthocyanin-administered group (HCM). Increased collagen production of HC group compared to HCM and REF groups.	 Description: 1. REF: reference, not given any intervention. 2. White: REF 3. Light blue: HC 4. Dark blue: HCM
Yin *et al.,* 2021	Mice with CCl_4-induced hepatic fibrosis.	Decreased expression of TGF-β1 after administration of anthocyanin in the CCl_4 group.	 Description: 1. AEPE: *Aqueous Extract Protective Effect*
Du *et al.,* 2015	HK-2 cells (human) with cholesterol accumulation and inflammation induced by high levels of glucose (HG).	Increased expression of TGF-β1 in the HG group. Decreased expression of TGF-β1 in groups given anthocyanins (Cy and C3G).	 Description: 1. NG: Normal glucose

Koh *et al.*, 2015	C57BLKS/J are diabetic and non-diabetic mice.	The expression of TGF-β1 control diabetic mice was higher than that of non-diabetic control mice and mice in a given anthocyanin (ATC) model.	 Description: 1. db/db: diabetic mice 2. db/m: non-diabetic mice

3.2. Discussion

Fibrosis is a healing process or reaction to tissue injury. This process generally occurs as a result of chronic inflammation which can occur in organs such as the kidneys, lungs, skin, and liver.[1] The main characteristic of fibrosis is the dysregulation of fibrous tissue formation, which is defined as excessive accumulation of extracellular matrix components. These extracellular matrix components include collagen and fibronectin which are basically essential components in wound healing.[16]

Currently, effective antifibrosis therapy is still hard to accepted by the medical community. The mortality rate produced by fibrosis is 45% in developed countries.[3] Recent studies illustrate that the key to fibrosis lies in myofibroblast cells and their molecular pathways. One of the most important components of myofibroblast activation is the TGF-β pathway.[17] TGF-β under normal conditions has a function as a trigger for apoptosis and has a role in wound healing. However, when repeated injury and inflammation occurs in the long term, the TGF-β group (consisting of TGF-β1, TGF-β2, and TGF-β3 isoforms) is also a pro-inflammatory cytokine. When TGF-β1 binds to its receptor, it will then activate the SMAD signaling pathway. SMAD-2/3 will then regulate profibrotic gene expression. If there is excessive activation of the SMAD pathway mediated by TGF-β, fibrosis can occur.[18] TGF-β, as previously described plays an essential role in fibroblast activation and the epithelial/endothelial-mesenchymal transition. Therefore, the therapeutic strategy of TGF-β inhibition has attracted the attention of researchers.[3]

The strategies pharmacologic therapy such as NIS793 (still in clinical trials (NIH clinicaltrials.gov)) inhibit TGF-β systemically. However, the side effects of these drugs are the great concern. Researchers are concerned that systemic inhibition of TGF-β might actually trigger the formation of cancer cells because essentially TGF-β functions as a tumor growth suppressor. Toxicity to cardiovascular with

histologic changes has been observed and reported in mice and monkey experiments. Persistent bleeding was reported in rat and monkey experiments. It means systemic inhibition can produce a higher toxic effect compared to use of selective inhibition of TGF-β1.[3]

Besides to conventional pharmacological therapy, herbal therapy as a supplement is now increasing the attention of researchers. For an example is the use of anthocyanins from plant materials in inhibiting TGF-β1. Anthocyanin which is one of the compounds belonging to the flavonoid group is known to function as an antioxidant, anti-inflammatory, and in this case, as an antifibrotic although it is still being tested on animals. Based on the literature study by comparing the expression of TGF-1 through immunohistochemical tests from several studies, the researchers observed that anthocyanins can effectively inhibit TGF-1 so that the progression of a disease to fibrosis or already become a fibrosis that has occurred can be inhibited. This is in line with the researchers' expectations of the effectiveness of anthocyanin compounds as TGF-β1 inhibitors in fibrosis.

4. Conclusion

To conclude, the anthocyanin compound has the potential to effectively inhibit Transforming Growth Factor β1 (TGF-β1) in fibrosis. However, the results obtained are mostly the results of animal experiments, thus the same effect on human subjects in greater numbers cannot be known for certain. Therefore, it is necessary to conduct further research on the antifibrotic effect of anthocyanins on the activity of TGF-β1 in humans.

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Research Article

Correlation between cardiovascular disease risk factors andelectrocardiographic (ECG) outcomes in the elderly at PantiWerda Sinta Rangkang, Palangka Raya City

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Abstract: Cardiovascular disease remains a global threat and is the leading cause of death worldwide. The high risk of coronary heart disease (CHD) in the elderly is due to physiologicalchanges in the body, such as decreased elasticity of the aortic wall, thickening and stiffening of heart valves, and reduced cardiac pumping ability. The development of cardiovascular disease can be caused by several factors, including controllable and uncontrollable ones. This research aims to investigate the relationship between CHD risk factors and electrocardiographic (ECG) outcomes in the elderly. This study is a cross-sectional study involving 24 elderly subjects (14 males and 10 females) selected through total sampling. The results of the study showed that 12 subjects (50%) exhibited abnormalities in the ECG with various interpretations such as Old Myocardial Infarction (OMI), occasional Premature Ventricular Complex (PVC), occasional Premature Atrial Complex (PAC), Right Bundle Branch Block (RBBB), and myocardial ischemia. Chi-square test indicated no significant relationship ($p>0.05$) between risk factors (age $p=0.408$, gender $p=1.000$, BMI $p=0.098$, and blood pressure $p=0.660$) and ECG outcomes in the elderly. Therefore, the conclusion of this study is that there is no correlations between age, gender, BMI, blood pressure, and ECG resultsin the elderly residents of Panti Werda Sinta Rangkang, Palangka Raya City.

Keywords: cardiovascular disease; elderly; electrocardiograph (ECG)

1. Introduction

The aging population is a global phenomenon leading to an increase in the elderly population worldwide. As people age, the risk of various chronic diseases, including heart disease, also increases. Heart disease is a serious health issue and a leading cause of death worldwide. In the elderly, heart disease has significant impacts on quality of life, morbidity, and mortality in the elderly population. Physiological changes that occur in the aging process, such as a decline in cardiovascular function, loss of blood vessel elasticity, and the accumulation of atherosclerotic plaques, can contribute to the high prevalence of heart disease in the elderly. According to the Basic Health Research (Riskesdas), the number of people diagnosed with Coronary Heart Disease

(CHD) in Indonesia in 2013 was highest in the 65-74 age group at 5.6%,[1] while in Central Kalimantan Province, the prevalence of CHD patients was highest in the 55-64 age group at 1.28%.[2]

The onset of cardiovascular disease can be caused by several factors, some of which are controllable, and others that are uncontrollable. Uncontrollable factors include age, gender, and genetic factors. Controllable factors include lifestyle factors such as smoking, an unhealthy diet, lack of physical activity, obesity, and hypertension.[3] Based on surveys conducted, nearly 50% of the elderly residents at Panti Werda Sinta Rangkang suffer from hypertension, which raises concerns about its potential negative impact on their quality of life and cardiovascular health conditions in the elderly, warranting special attention.

Abnormalities in the cardiovascular system can be detected through heart examinations using Electrocardiography (ECG). ECG is a commonly used diagnostic tool to measure the electrical activity of the heart and detect disturbances in heart rhythm and function. Research on the relationship between heart disease risk factors and ECG results can provide a deeper understanding of how these factors affect heart electrical activity, rhythm, and overall function. In one study, it was found that ECG abnormalities were three times higher in subjects over 85 years old compared to those aged 65-69.[4]

By understanding the relationship between risk factors and ECG results, medical professionals can identify potential heart disease risks in patients earlier, develop more effective prevention strategies, and formulate appropriate management plans. Therefore, this research aims to delve deeper into the relationship between heart disease risk factors and ECG results, with the hope that the findings from this study will provide valuable information for medical professionals, researchers, and the general public in understanding and effectively addressing heart disease.

2. Methods

The experimental design of the research was an observational cross-sectional study on a sample of elderly (n = 24, mean age 73.87 years, 14 women and 10 men). All subjects gave written informed consent to participate in the study, according to the ethical principle of research with humans. The research was approved by the ethics committee of Fakultas Kedokteran Universitas Palangka Raya. To include the participants in the study, we applied the following selection criteria: age more than 50 years, able to walk and without dementia. The subjects were elderly residents of Panti Werda Sinta Rangkang, Palangka Raya.

Total sampling is used in the sampling technique. Data was collected by interviews to determine age and gender, as well as through direct measurements to determine nutritional status, blood pressure,

and ECG. The gathered data was then entered into a master table using the Microsoft Excel program, processed using the SPSS program, and the results were tabulated.

3. Results and Discussion

We analyzed the relationship between age, gender, nutritional status and blood pressure, with the ECG results of the elderly with the following data results:

Table 1. Frequency distribution based on age, gender, nutritional status (BMI), blood pressure

Variable	Frequency (N)	Percentage (%)
Age		
Middle Age	1	4.2
Elderly	13	54.2
Old	10	41.7
Gender		
Man	14	58.3
Woman	10	41.7
Nutritional Status (BMI)		
Underweight	2	8.3
Normal	8	33.3
Overweight	10	41.7
Obesity Grade 1	2	8.3
Obesity Grade 2	2	8.3
Blood Pressure		
Normal	6	25
Prehypertension	4	16.7
Hypertension Grade 1	4	16.7
Hypertension Grade 2	10	41.7
Total	24	100.0

Table 2 shows that the research respondents totaled 24 individuals, with the majority falling into the elderly category, specifically in the age range of 75 to 90 years, comprising 13 elderly individuals. In terms of gender, there were 14 male elderly respondents. Regarding nutritional status, 10 elderly individuals were found to be overweight, and 10 had blood pressure categorized as grade 2 hypertension.

Table 2. Frequency distribution based on electrocardiograph (ECG) results

ECG Results	Frequency (N)	Percentage (%)
Normal	12	50
Abnormal	12	50
Total	24	100.0

Table 2 shows that one-half of the research population yielded abnormal ECG results, with various different diagnoses such as old myocardial infarction (OMI) occurring in 4 participants, premature ventricular contractions (PVC) in 3 participants, ischemia in 3 participants, and Right

Bundle Branch Block (RBBB) in 2 participants in their ECG results. OMI is a pattern that can be observed in the ECG results of individuals who have experienced a Myocardial Infarction (MI).

MI also referred to as a "heart attack," is brought on by a partial or total cessation of blood flow to the myocardium. Coronary artery occlusion deprives the myocardium of oxygen. Myocardial necrosis and cell death can occur if the myocardium is continuously deprived of oxygen.[5] ECG abnormalities resulting from myocardial ischemia or infarction can be seen in the PR segment, QRS complex, ST segment, or T wave.[6]

Premature Ventricular Contractions (PVCs) or extra heartbeats start in one of the heart's two ventricles, the lower pumping chambers. The regular heart rhythm is disrupted by these extra beats, which can occasionally make the chest feel as though it is fluttering or skipping beats. PVCs can be identified on the ECG by QRS complexes that appear earlier than normal in the cardiac cycle. PVCs have an abnormal shape, are longer than 120 ms in length, and are not preceded by a P wave. The T wave is large and occurs in the opposite direction to the QRS complex.[7]

Ischemia is described as a localized lack of blood flow (circulation) brought on by a blockage of the blood vessels supplying the region. An organ (such as the heart) is said to be ischemic if it is not receiving enough blood and oxygen.[8] Unlike a full thickness myocardial infarction, myocardial ischaemia alters the ST-T wave but does not directly affect the QRS complex (although it may result in bundle branch blocks, which lengthen the QRS complex).[9]

RBBB, a pattern seen on the 12-lead ECG, a characteristic appearance on the ECG caused by RBBB is manifested by a widened QRS complex and changes in the directional vectors of the R and S waves. This is because the normal sequence of activation is altered. This ECG pattern is typically benign and seen frequently in clinical practice.[10]

Table 3 shows that the participants with the highest number of abnormal ECG results were in the elderly age group (75-90 years), comprising 29.2% of the total, followed by the old age group (>90 years) at 16.7%. The remaining 4.2% of participants fell into the middle-aged category. This is consistent with previous research findings from a study involving 171 geriatric subjects aged over 70 years, where 65.6% of the population exhibited ECG abnormalities.[11] This trend can be attributed to the fact that the proportion of normal ECGs decreases with age, reaching a minimum of 20.0% in males and 22.7% in females after the age of 75.[12] However, in this study, it was found that there was no significant difference in the number of elderly individuals with normal and abnormal ECG results based on gender. Based on the chi-square test results, a p-value > 0.05 was obtained for both variables, indicating no correlation between age and gender with ECG results. This lack of correlation is likely because age and gender are non-modifiable risk factors for heart

disease, with the majority of risk being attributed to lifestyle and behavior patterns that can be modified.

Table 3. Relationship between risk factors age, gender, nutritional status (BMI), blood pressure with ECG results

Variable	ECG Results		Total N (%)	P Value
	Normal N (%)	Abnormal N (%)		
Age				
Middle Age	0	4.2	4.2	
Elderly	25.0	29.2	54.2	0.408
Old	25.0	16.7	41.7	
Gender				
Man	29.2	29.2	58.3	1.000
Woman	20.8	20.8	41.7	
Nutritional Status (BMI)				
Underweight	4.2	4.2	8.3	
Normal	8.3	25.0	33.3	
Overweight	29.2	12.5	41.7	0.098
Obesity Grade 1	0	2	8.3	
Obesity Grade 2	2	0	8.3	
Blood Pressure				
Normal	16.7	8.3	25	
Prehypertension	4.2	12.5	16.7	0.660
Hypertension Grade 1	4.2	12.5	16.7	
Hypertension Grade 2	25	16.7	41.7	

In terms of nutritional status, a p-value > 0.005 was obtained, indicating no relationship between nutritional status and ECG results in the elderly. However, based on the cross-tabulation results, most participants who were overweight had normal ECG results, accounting for 29.2%. This differs from previous research suggesting that changes in body composition can impact ECG results. Cardiovascular risk is known to be higher in individuals with obesity, which can affect ECG outcomes.[13] The discrepancy may be due to differences in the age of the sample; this study focused on individuals over 50 years old.

This study aligns with research conducted by Maulina which found that 67.9% of elderly patients with Coronary Heart Disease (CHD) had normal body weight, while 25% were obese.[14] Obesity is one of the modifiable risk factors for CHD. Nutritional status is not the sole risk factor leading to CHD; other factors such as age, gender, genetics, hypertension, hypercholesterolemia, hypertriglyceridemia, diabetes mellitus, smoking, lack of physical activity, and stress also play significant roles.[15]

Obesity increases the risk of developing Coronary Heart Disease (CHD) in individuals, regardless of whether they lead a healthy lifestyle. Conversely, a healthy lifestyle in obese

individuals does not significantly reduce the risk of CHD. Prolonged obesity can lead to diabetes (diabetogenic) due to insulin resistance. Insulin resistance is often associated with various other cardiovascular risk factors, such as hypertension, dyslipidemia, and this condition is known as metabolic syndrome. Epidemiological studies have shown that metabolic syndrome can double the risk of cardiovascular diseases, especially CHD, compared to individuals without metabolic syndrome.

Metabolic syndrome, involving insulin resistance, is also associated with various issues affecting triglycerides and glucose metabolism, increased blood pressure, and inflammation in blood vessels. Insufficient physical activity and high-calorie intake can affect metabolic profiles by reducing the rate of fat and glucose burning in the body's muscles, including the heart muscle, leading to fat accumulation in the body and insulin resistance. Additionally, various cytokines, such as Tumor Necrosis Factor (TNF) α and interleukin (IL)-6, produced by adipose tissue, can affect insulin suppression of glucose production by the liver, increase fatty acid production and cholesterol synthesis, and increase Very Low-Density Lipoprotein (VLDL) production by the liver and adipose lipolysis. Increased lipolysis will raise the supply of Non-Esterified Fatty Acids (NEFA) to the liver, which can, in turn, affect lipid metabolism, including increased fasting plasma triglyceride levels, decreased High-Density Lipoprotein (HDL) concentrations, and increased Low-Density Lipoprotein (LDL) concentrations.

The decrease in HDL cholesterol levels is considered to increase the risk of CHD for several reasons: HDL plays a role in preventing the formation of atherosclerotic plaques, low HDL levels indicate increased lipoproteins containing atherogenic apolipoprotein B, and low HDL levels are usually associated with other non-lipid risk factors of metabolic syndrome. Many prospective studies have shown that metabolic syndrome increases the risk of CHD, and this relationship appears to be due to changes in metabolism affecting lipids, glucose, blood pressure regulation, thrombosis, fibrinolysis, and inflammatory responses. Increased inflammation can make atherosclerotic plaques more vulnerable to rupture. Recent findings also indicate that metabolic syndrome is associated with a decrease in thrombolysis function and an increase in coagulation due to increased plasminogen activator inhibitor-1 (PAI-1) and fibrinogen. Atherosclerotic plaques can rupture and trigger thrombus formation, which is difficult to dissolve. Acute changes in atherosclerotic plaque morphology, thrombosis, and vasospasm in coronary arteries are major factors underlying the occurrence of CHD.

Regarding blood pressure, the results showed that the majority of participants (25%) with grade 2 hypertension had normal ECG results, while 16.7% had abnormal ECG results. Univariate test

results indicated no relationship between blood pressure and ECG results in the elderly. This contradicts previous research suggesting that abnormal ECG results are closely related to an increase in systolic and diastolic blood pressure.[16] The results of this study differ from previous research due to limitations in the number of participants and the variables studied.

The increase in blood pressure with age is largely associated with changes in the stiffness of arteries and arterioles. The stiffness of large arteries (LAS) is primarily due to structural changes caused by atherosclerosis and calcification. This results in pressure waves being reflected back earlier from arterioles towards the heart during the propagation of blood pressure waves. These pressure waves return during systole, increasing central systolic blood pressure (SBP) and widening pulse pressure (PP).[17]

The increase in diastolic blood pressure (DBP) up to the age of 50 is mainly due to increased peripheral vascular resistance (PVR) in small blood vessels. However, both LAS and PVR contribute to the increase in SBP, whereas DBP increases with PVR but decreases with increasing LAS. Although PVR can trigger hypertension, it is the acceleration of LAS that leads to a sharp increase in SBP after the age of 50.[18]

Structural changes in large arteries observed in systolic hypertension are very similar to changes due to the aging process. This makes it difficult to distinguish between arterial changes due to the disease and those due to aging.[18]

Other pathophysiological factors that influence the rise in blood pressure with age include decreased baroreceptor sensitivity, increased responsiveness to sympathetic nervous system stimulation, changes in kidney and sodium metabolism, and alterations in the renin-aldosterone relationship.[18]

4. Conclusion

There is no correlation between cardiovascular risk factors (age, gender, nutritional status, and blood pressure) and the ECG findings in the elderly.

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Literature review

Bioinformatics of the progesterone receptor

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Abstract: Progestins are the newest medical treatments and to be successfully indicated to treat endometriosis. Therapeutic effects of progesterone and progestins in human endometrium and endometriosis such as; effect on hypothalamic-pituitary-ovary axis, effects on estrogen receptors and estrogen synthesis effects on tissue morphology, growth, vascularisation and regeneration, effects on local immune response, differential effects of progesterone and progestins between normal endometrium, eutopic endometrium of females with endometriosis, and endometriotic lesions. We review here the action mechanisms of progesterone receptor ligands in endometriosis, identify critical differences between the effects of progestins on normal endometrium and endometriosis and envisage pathways to escape drug resistance and improve the therapeutic response of endometriotic lesions to such treatments. We performed a systematic Pubmed search covering articles published since 1958 about the use of progestins, estro-progestins and selective progesterone receptor modulators, to treat endometriosis and its related symptoms. Two reviewers screened the titles and abstracts to select articles for full-text assessment. Progesterone receptor signalling leads to down-regulation of estrogen receptors and restrains local estradiol production through interference with aromatase and 17 beta-hydroxysteroid dehydrogenase type 1. Progestins inhibit cell proliferation, inflammation, neovascularisation and neurogenesis in endometriosis. However, progesterone receptor expression is reduced and disrupted in endometriotic lesions, with predominance of the less active isoform (PRA) over the full-length, active isoform (PRB), due to epigenetic abnormalities affecting the PGR gene transcription. Oxidative stress is another mechanism involved in progesterone resistance in endometriosis. Among the molecular targets of progesterone in the normal endometrium that resist progestin action in endometriotic cells are the nuclear transcription factor FOXO1, matrix metalloproteinases, the transmembrane gap junction protein connexin 43 and paracrine regulators of estradiol metabolism. Compared to other phenotypes, deep endometriosis appears to be more resistant to size regression upon medical treatments. Individual genetic characteristics can affect the bioavailability and pharmacodynamics of hormonal drugs used to treat endometriosis and, hence, explain part of the variability in the therapeutic response. Medical treatment of endometriosis needs urgent innovation, which should start by deeper understanding of the disease core features and diverse phenotypes and idiosyncrasies, while moving from pure hormonal treatments to drug combinations or novel molecules capable of restoring the various homeostatic mechanisms disrupted by endometriotic lesions.

Keywords: endometriosis; endometrium; hormonal treatments; innovation; new drugs; progestins; selective progesterone receptor modulators; therapeutic failure

1. Introduction

Endometriosis-like symptoms have been alluded to in ancient medical records dating from about 4000 years ago. Chinese medicinal herbs have been prescribed to alleviate disabling pelvic pain and severe systemic symptoms related to the menstrual period since ancient times, when the pharmacological rationale for the medical treatment of dysmenorrhea was still unknown.[1]

Some of the first recommendations for endometriosis prophylaxis were early marriage and frequent childbearing.[2] In fact, the attempt at using hormonal therapies for symptomatic management of endometriosis, already performed occasionally, gained an impulse when the idea of inducing a state of 'pseudo pregnancy' came to the light.[3,4]

Progestins, either alone or conjugated with estrogens, continue to be successfully indicated to treat endometriosis.[5] However, some patients have only partial improvement or do not respond to this therapy at all.[6] Estrogen-suppressive therapies, such as gonadotropin-releasing hormone (GnRH) agonists and antagonists or aromatase inhibitors, are also far from being a panacea and are still expensive and have bothersome side effects.[7]

2. Methods

In this narrative review, we performed a historical bibliographic search for references to the medical treatment of endometriosis and a systematic Pubmed search covering articles in any language published since 1958 with the following terms: ('Endometriosis'[Mesh] OR 'Endometrium'[Mesh]) AND ('Progestins'[Mesh] OR 'Medroxyprogesterone Acetate'[Mesh] OR 'Norethindrone'[Mesh] OR 'Desogestrel'[Mesh] OR 'dienogest' [Supplementary Concept] OR 'Dydrogesterone'[Mesh] OR 'Levonorgestrel'[Mesh] OR 'Mifepristone'[Mesh] OR 'ulipristal acetate' [Supplementary Concept] OR vilaprisan [Supplementary Concept]). Two reviewers screened the titles and abstracts to select articles for full-text assessment. We also searched clinicaltrials.gov and EudraCT for study protocols on hormonal treatments of endometriosis with the status of 'not yet recruiting', 'recruiting', 'active, not recruiting' or recently (until December 2017) 'completed' or 'terminated

3. Results and Discussion

3.1. Progesterone receptors in human endometrium and endometriosis

The progestational effects of natural progesterone and synthetic progestins are mediated by three categories of specific receptors: the classical nuclear PRs that include subtypes A and B [8], a mitochondrial isoform (PR-M) also derived from the same PGR gene [9], and the cell membrane receptors that comprise progesterone receptor membrane components (PGRMC) and membrane

receptors (mRPs) belonging to the progestin and adipoQ (PAQR) protein family.[10]

In myometrial cells, progesterone represses interleukin (IL)-1β-induced proinflammatory genes through co-repressor molecules recruited by the PR DNA-binding domain in cooperation with the amino-terminal region that is unique to PRB.[11] An increased abundance of PRA also represses PRB-mediated transcriptional activity.[12,13]

Table 1. Summary of studies that have evaluated progesterone receptor expression and localisation in endometriotic lesions

Reference	Sample	Phenotype	Findings
Bedaiwy et al, 2015	C, E, L	SUP, OMA	PRA (western blot): C < E > OMA > SUP PRB (Western blot): C = E > OMA > SUP
Beliard et al, 2004	E, L	SUP	PRA + PRB (immunohistochemistry): C = E > L Down-regulation in the secretory phase in C and E, but not in L
Bono et al, 2014	L	OMA	PRA + PRB (immunohistochemistry) : detected in 10/10 samples PRB (immunohistochemistry): detected in 6/10 samples
Eaton et al, 2013	C, L (stromal cells)	OMA	PRA (western blot) : C > L PRB (western blot): C > L
Hayashi et al, 2012	C, L	OMA	PRA (immunohistochemistry): C=L PRB (immunohistochemistry): C > L
Wu et al, 2006	C, E, L	SUP, OMA	PRB mRNA (real-time PCR): C > E > L Hypermethylation or PGR at the PRB promoter region in L
Attia et al, 2000	E, L	"extra-ovarian"	PRA (western blot): E > L PRB (western blot): only E
Beranić and Riźer, 2012	L (Z-12 cell line)	SUP	Expressed PRB mRNA (real time PCR) and protein (western blot)
Vinci et al, 2016	L	DIE	PRA + PRB (immunohistochemistry): detected in 34/112 samples
Zanatta et al, 2015	L	DIE	PRA + PRB (immunohistochemistry): detected in 17/18 samples PRB (immunohistochemistry): detected in 17/18 samples
Liu et al, 2018	C, L	OMA, DIE	PRB (immunohistochemistry): C > DIE > OMA

3.2. Therapeutic effects of progesterone and progestins in human endometrium and endometriosis

Progestins are synthetic compounds that mimic the effects of progesterone. The features that all progestins share are the ability to bind PR and induce the secretory transformation of estrogen-primed uterine endometrium, the so-called progestogenic effect. Steroids with progestational activity have differences in their chemical structures that affect their profile and potency of action on hypothalamic–pituitary axis, reproductive and breast tissues and metabolic processes.[14]

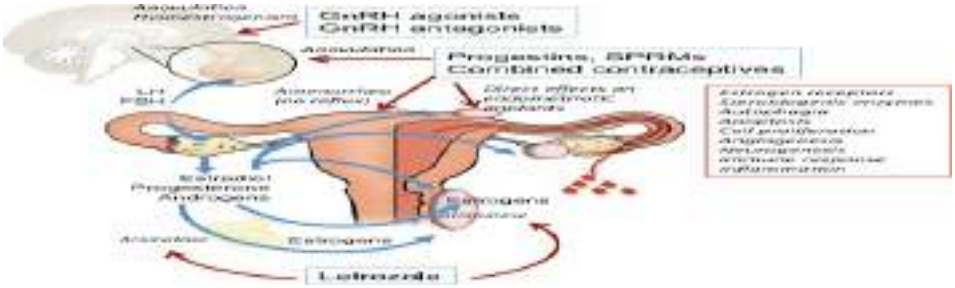

Figure 1. Therapeutic mechanisms of hormonal treatments for endometriosis. Progestins, selective progesterone receptor modulators (SPRMs) and combined contraceptives block ovulation through central inhibition of gonadotropin release, induce amenorrhea which prevents repeated menstrual reflux and have many direct effects on endometriotic implants

Gonadotropin-releasing hormone (GnRH) agonists and antagonists block ovulation and induce hypoestrogenism, while aromatase inhibitors such as letrozole reduce androgen aromatization into estrogens, both in the adipose tissue and within endometriotic lesions.

The long-standing hypoestrogenic and hypergestagenic state supports the use of progestins for treating endometriosis. In fact, the condition induced by these compounds causes decidual transformation of the eutopic endometrium and the same effect is observed to some extent in ectopic lesions.[5]

3.3. Effect on hypothalamic–pituitary–ovary axis

Hormonal therapies for endometriosis inhibit gonadotropin release, reduce ovarian steroid secretion and can induce amenorrhea, thereby preventing repeated endometrial reflux. Combined oral contraceptives (COCs) inhibit the pituitary production and secretion of both FSH and LH.[15,16]

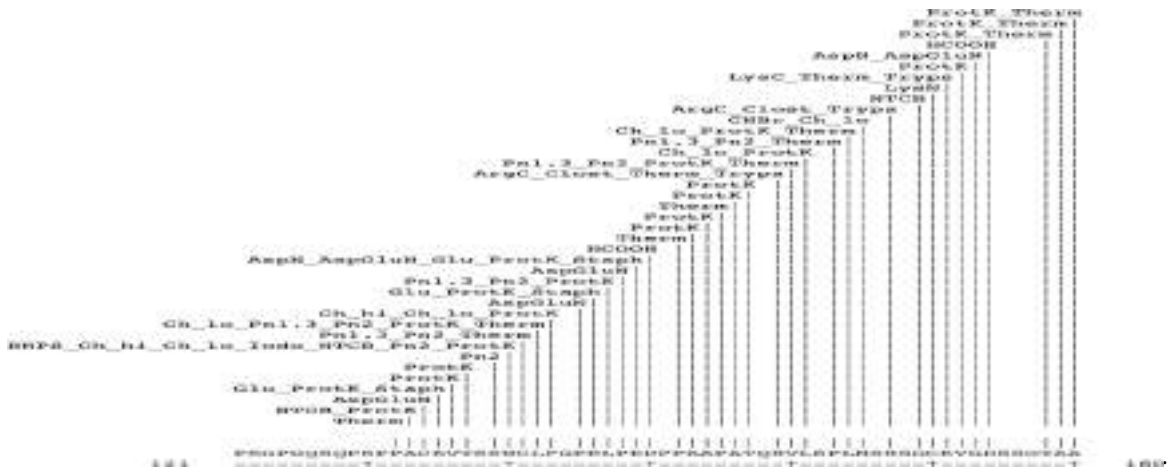

Figure 2. Cleavage site of chosen enzymes

3.4. Prediction of protein location (TARGETP)

Danazol has anti-gonadotropic activities, and daily oral administration of 400 mg of danazol inhibits both ovulation and LH surge.[17–19] However, ovulation is not inhibited by daily vaginal administration due to the lower serum concentration of danazol reached by this route.[20,21]

3.5. Effects on estrogen receptors and estrogen synthesis

Perhaps the most remarkable therapeutic mechanisms of progestins in endometriosis derive from the fact that PR signalling leads to down-regulation of estrogen receptors (ERs). This effect has been clearly demonstrated not only in the eutopic endometrium but also in the glands and stroma of endometriotic lesions following in vivo treatment with different progestational formulations.[22–24] Another front of anti-estrogen action of progestins is the restraint of local estradiol production through interference with certain enzymes. As shown by experimental evidence, dienogest inhibits aromatase expression and, consequently, local estrogen production in immortalized human endometrial epithelial cells.[25]

3.6. Effects on tissue morphology, growth, vascularisation and regeneration

Endometrial tissues undergo physiological continuous changes resulting from endogenous ovarian hormonal secretion. The use of exogenous steroids with estrogenic, androgenic and progestogenic activity induces a spectrum of histologic changes in endometrial glandular and stromal architecture, blood vessels and cytology.[26] These changes are observed not only in eutopic endometrium but also in endometriosis. The direct effects of progestins include the attenuation of the inflammatory state and the creation of a pseudo-pregnancy condition with increased apoptosis and atrophy of the endometriotic implants.[27,28]

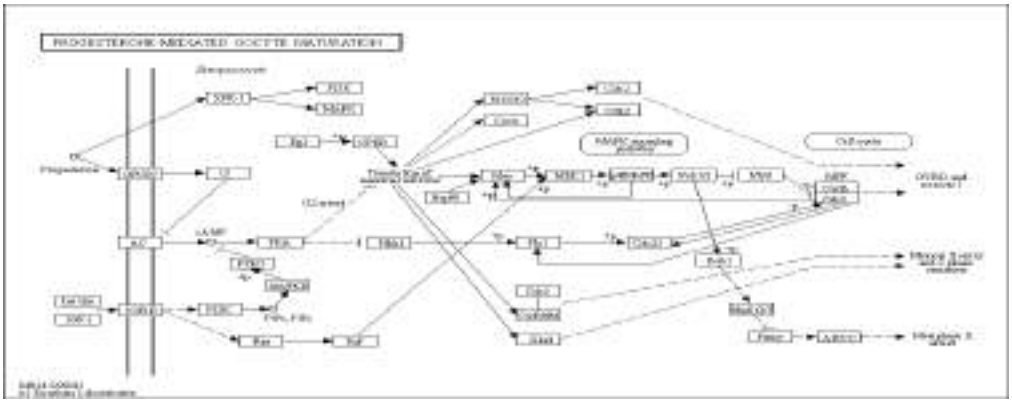

Figure 3. Pathway of progesterone receptor in oocyte maturation

3.7. The role of PR protein in metabolism

During treatment with dydrogesterone in endometriosis, the ectopic tissue undergoes different changes, including decidualisation, undifferentiation or involution.[29] Histological evaluation after a short-term treatment of endometriosis with gestrinone or danazol showed a degree of cellular inactivation and degeneration of the endometriotic implants.[30]

In vitro, dienogest induces a dose-dependent inhibition of human endometrial stromal cell (hESC) proliferation together with morphological and functional changes, including the production of prolactin, a typical marker of decidualization.[31] Furthermore, dienogest treatment of endometriotic cells suppresses protein kinase B (AKT) and extracellular signal- regulated kinase (ERK)1/2 activity, inhibiting mammalian target of rapamycin (mTOR), inducing autophagy, and promoting apoptosis.[32].

3.8. Effects on local immune response

Endometriosis is associated with changes in both cell-mediated and humoral immunity.[33] Progesterone, dienogest and danazol attenuate the expression of IL-8 by reducing tumour necrosis factor (TNF)-induced nuclear factor-kappa B (NF-κB) activation in endometriotic stromal cells.[34,35] In short, the therapeutic effects of progestins, COCs and other hormonal drugs used in endometriosis include inhibition of estrogen synthesis and action through down-regulation of steroidogenic enzymes and ER, inhibition of endometriotic cell survival and proliferation, limitation of local angiogenesis and neurogenesis and, linking all these mechanisms, attenuation of the immune-inflammatory response.

3.9. Differential effects of progesterone and progestins between normal endometrium, eutopic endometrium of females with endometriosis and endometriotic lesions

In animal models. In spite of the limitations of murine models of endometriosis to emulate the human disease, they help to understand the morphological and functional changes induced by hormonal treatments in endometriotic implants. In nude mice transplanted subcutaneously with human endometrial fragments, treatment with depot MPA induced low or atrophic surface epithelium, decidual reaction and either narrow or dilated glands. For obvious reasons, the effects of hormonal treatments on heterotopic endometrial xenografts cannot be compared with the effects of the same treatments on the eutopic endometrium of the human donors or of the mouse host.

In cultured human cells and tissues. Primary cultures of hESCs and epithelial cells provide an insightful model to understand the differential effects of hormonal treatments between normal endometrium, eutopic endometrium in the presence of endometriosis and endometriotic lesions. With

this approach, our group found out that both stromal and epithelial cells from endometriotic lesions produce aberrantly high amounts of reactive oxygen species (ROS) such as hydrogen peroxide and, unlike normal endometrial cells, endometriotic cells fail to reduce their hydrogen peroxide production in response to danazol.[36]

As summarized, a number of morphological and functional changes that follow progestin or progesterone stimulation of normal endometrial cells are not seen when the same stimulus is applied to endometriotic cells or eutopic endometrial cells from women with endometriosis.[37–43]

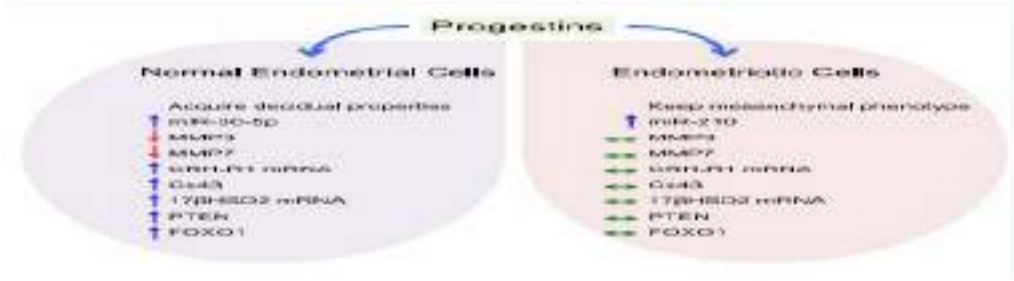

Figure 4. Effects of progestin treatment in vitro on normal endometrial cells compared to endometriotic cells cultured in parallel and under the same conditions. The arrows indicate that progestin treatment stimulates (↑), inhibits (↓) or has no effect (⇔) on the target gene, RNA or protein

In human studies in vivo. Clinical studies evaluating human endometriotic lesions before and after medical treatments are rare, as current practices recommend avoiding multiple surgeries. Nevertheless, two independent clinical trials obtained samples of both eutopic endometrium and endometriotic lesions before and 6 months after the insertion of LNG-IUS in symptomatic women with endometriosis.[23,24] These results suggest that levonorgestrel treatment delivered into the uterus is able to induce progestin-like effects in pelvic endometriosis that are surprisingly similar to the effects induced in the eutopic endometrium that is in close contact with the drug releasing system.

Why do progestins sometimes fail in endometriosis? Characteristics of the type of hormone

All progestins used to treat endometriosis have been labelled for contraception and/or menopausal hormone therapy in doses and regimens sufficient to induce endometrial decidualisation and prevent endometrial hyperplasia.[5] This evidence places letrozole as a second-line therapy for selected patients who fail to respond to progestins (Table 2).

3.10. Characteristics of therapeutic regimen and route of administration

A retrospective cross-sectional study of deep endometriotic lesions compared women who were using different medical treatments preoperatively (COC, oral progestins or GnRH agonists) to a group without treatment, and found a large intra-lesion, intra-patient and intra-treatment variance in the expression levels of ER, which was more evident than any difference between treatments or between

treated and non-treated lesions.[22]

Table 2. Potential approaches for patients who have failed initial therapy for endometriosis and the currently available evidence

Cause of progestin failure	Potential approaches	Evidence
Hormone type	To change the type of progestin	Observational study: NETA and dienogest may have similar effects (Vercellini et al., 2016a). In-vitro studies: different progestins may have different mechanisms of action (Grandi et al., 2016; Nirgianakis et al., 2016; Roth et al., 2019).
	To avoid estrogen association	RCTs did not show meaningful differences between isolated progestins and progestin-estrogen associations (Cheewadhanaraks et al., 2012; Razzi et al., 2007b; Vercellini et al., 2002; Vercellini et al., 2005). Prospective self-control study: for patients who do not respond to COC, changing to NETA may be beneficial (Vercellini et al., 2018c).
	To inhibit estrogen synthesis	Non-randomised open-label trials: for patients who fail to respond to progestins, letrozole can be tried as a second line therapy (Ferrero et al., 2009; Ferrero et al., 2014).
Therapeutic regimen	To change therapeutic regimen	Systematic review: continuous administration may be better than cyclic regimens (Seracchioli et al., 2009).
Route of administration	To change the route of administration	RCT: systemic and local progestins may be similar (Carvalho et al., 2018).
Progesterone resistance	To associate NSAIDs to progestin therapy	No high-quality evidence for NSAIDs effectiveness on endometriosis symptoms, but the association with hormonal treatments might diminish the inflammatory response that boosts progesterone resistance (Brown et al., 2017).
	To associate antioxidants	Multi-centre open-label non comparative clinical trial: antioxidant preparations containing N-acetyl-cysteine may mitigate symptoms (Lete et al., 2018).

3.11. Characteristics of the disease phenotype

Endometriotic implants are very heterogeneous in clinical features and evolution, being currently classified into three distinct phenotypes: superficial peritoneal endometriosis, ovarian endometrioma and deep-infiltrating endometriosis.[44,45]

Morphological endpoints. Structurally and functionally, endometriotic lesions are not all the same.[46] Nerve fibers expressing protein gene product 9.5 are much more abundant in deep-infiltrating than in superficial peritoneal endometriosis.[47]

Clinical endpoints. In a patient preference, parallel cohort trial comparing oral progestin (NETA) therapy versus second-line laparoscopic surgery for women with persistent or recurrent endometriosis after a first surgery, the outcomes varied according to the treatment chosen but also depending on the type of endometriosis.

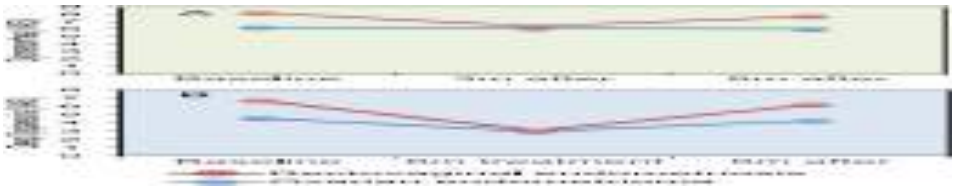

Figure 5. Endometriosis phenotype may affect the therapeutic response to progestins. The graphs summarize dysmenorrhea (A) and deep dyspareunia (B) scores obtained by visual analogue scale (VAS) in women with rectovaginal endometriosis (Ferrero et al., 2009) and in women with ovarian endometrioma (Ferrero et al., 2014) treated with norethisterone acetate for 6 months. Data were extracted from published tables of two separate studies and plotted together for better visualization

3.12. Characteristics of individuals: the pharmacogenomics

Individual genetic characteristics can affect the bioavailability of hormonal drugs used to treat endometriosis and, hence, explain part of the variability in the therapeutic response. A semiquantitative study of deep endometriosis biopsies from women undergoing hormonal treatments (progestins, COC or GnRH agonist) revealed a large heterogeneity of ERα and PR.[48]

3.13. Progesterone resistance in endometriosis: an insurmountable barrier?

Progesterone resistance is defined as subnormal cellular response to the effects of natural progesterone, but the concept can be extended to encompass a poor response to therapeutic progestins. It is surprising that the effectiveness of non-steroidal anti-inflammatory drugs to treat endometriosis symptoms remains uncertain as no high-quality evidence is available to date.[7] Oxidative stress is another mechanism involved in progesterone resistance in endometriosis, as endometriotic lesions and their surrounding peritoneal fluid are rich in ROS.[49] Antioxidants like N-acetyl-cysteine inhibit ROS production and cell proliferation in endometriotic lesions and mitigate symptoms.[50,51]

3.14. Selective progesterone receptor modulators to treat endometriosis: rationale and state of the art

Selective progesterone receptor modulators (SPRMs) comprise a relatively recent class of synthetic molecules capable of interacting with PR. The interest in these new drugs is based on their affinity to PR, especially considering their antagonist effects.[52–54] The final action also relies on a complex machinery of co-regulators and co-repressors, whose proportion will determine a greater or lesser

transcriptional activity in target genes.[52,55]

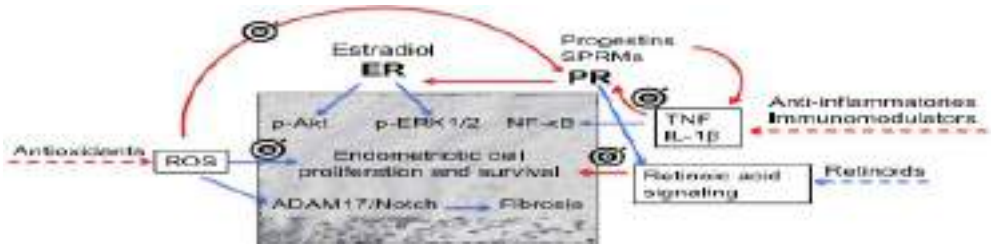

Figure 6. Hypothetical targets and strategies to overcome progesterone resistance in endometriosis based on currently available drugs. Progesterone receptor (PR) expression is inhibited by gene hypermethylation induced by proinflammatory cytokines and reactive oxygen species (ROS), which could be inhibited by anti-inflammatory drugs/immunomodulators and antioxidants, respectively. The same drugs and others, such as retinoids, may activate mechanisms downstream PR signalling that prevent endometriosis proliferation and fibrosis. Blue arrows: stimulation; red arrows: inhibition. ER: estrogen receptor; p-Akt: phosphorylated serine/threonine protein kinase B; p-ERK1/2: phosphorylated extracellular signal regulated kinase 1/2; NF-κB: nuclear factor-kappa B; ADAM17: A disintegrin and metalloproteases metallopeptidase domain 17; TNF: tumour necrosis factor; IL: interleukin

3.15. Ulipristal acetate

UPA is currently approved for emergency contraception and as a presurgical therapy for symptomatic women with uterine fibroids. UPA inhibits human endometrial cell proliferation in vivo [56], and suppresses the growth and development of endometriotic implants in mice.[27] In 2018, the European Medicines Agency (EMA) provided an alert about a serious risk of liver injury caused by UPA and recommended some measures to minimize the negative outcomes. The medication is contraindicated in patients with liver disease; liver tests are required before, during and after the drug administration; repeated therapeutic courses can be offered only to women not eligible for surgery; and patients have to be widely oriented about the risks.

3.16. Vilaprisan

Vilaprisan is a new SPRM that differs from other drugs of the same class by a distinct metabolic clearance, with the potential benefit of not presenting liver toxicity. A 2B phase trial evaluated the safety and efficacy of the drug in patients with uterine fibroids during 12 weeks of treatment and a 24-week post-treatment follow-up. In summary, although some studies performed with SPRMs in endometriosis showed a potential clinical use for symptom relief [57], there is no drug representative of this class currently approved for clinical use in endometriosis therapy.

3.17. Strategies to overcome the therapeutic shortcomings: innovative drug design, better phenotyping, personalized medicine

A number of PR-related treatments having endometriosis as inclusion criterion and its symptoms as primary outcomes are under investigation in registered clinical trials (Table 3). The recognition that endometriosis requires a multisided medical therapy contemplating its multifaceted pathophysiology may be the first step towards a real breakthrough in the field.

Table 3. New progesterone receptor modulating drugs for endometriosis treatment with study protocols registered in ClinicalTrials.gov

Class	Substance	Route	Trial number	Completed
Progesterone	Progesterone	Subcutaneous	NCT02793908	No
Progestin	Etonogestrel (Nexplanon)	Subcutaneous implant	NCT02669238	No
SPRM?	Danazol	Vaginal ring	NCT001 17481	Yes
PR antagonist	PF-02413873	Oral	NCT00800618	Yes
Analgesic + progestin	Low dose naltrexone + norethindrone acetate	Oral	NCT03970330	No

Although the process of decidualization involves many PR targets [58], the transcription factor named promyelocytic leukemia zinc finger (PLZF) emerged as a critical mediator with a pivotal role in inducing the transcriptional reprogramming of endometrial stromal cells that ultimately leads to the decidual phenotype.[59,60]

4. Conclusion

Progestins have been used to treat endometriosis for over 60 years with large success rates, but still some patients do not respond to this therapy as expected. Progestins depend on the expression of PR to exert their actions in the target cells, but PR expression is often blunted and disrupted in endometriotic foci, which cannot be resolved by simple dose increment or by small modifications in the molecular design of the drug. Combined hormonal contraceptives have the same limitations of isolated progestins, plus a possible counterproductive effect of the estrogenic compound.

Understanding the mechanisms of therapeutic success and failure is essential to guide clinical decisions and inform future research in this field. The development of new molecules for medical treatment of endometriosis should aim at strategies to overcome the resistance mechanisms and aim at new targets. However, current clinical trials consist of old drugs with new delivery (danazol vaginal ring), new drugs with old mechanisms (oral GnRH antagonists) or new mechanisms with old concepts (steroidogenic enzyme inhibitors). Innovation is urgently needed and should start by deeper

understanding of the disease core features, diverse phenotypes and idiosyncrasies while moving from pure hormonal treatments to drug combinations or novel molecules capable of restoring the various homeostatic mechanisms disrupted by endometriotic lesions

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Research Article

Xanthine oxidase inhibitory activity of ethanol fractions of *Arcangelisia flava*

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Abstract: Xanthine oxidase is an enzyme that catalyzes the formation of uric acid. This enzyme must be inhibited so that excessive uric acid formation can be avoided. Inhibition of xanthine oxidase can be carried out by secondary metabolites in herbalssuch as flavonoids, alkaloids, terpenoids, saponins, and tannins. From the latest research, ethanol extracts of the stem of *Arcangelisia flava* contains flavonoids, alkaloids, terpenoids, saponins and tannins, so it has antihyperuricemic potential. Flavonoid and alkaloids are generally polar and can dissolve in solvents that have thesame level of polarity such ethanol. The aim of this research is to determine the ability of the ethanol fraction from ethanol extracts of *Arcangelisia flava* stems as aninhibitor of the xanthine oxidase. This research is an in vitro study by carrying out phytochemical tests to determine the secondary metabolites and measuring the inhibitory ability of xanthine oxidase from the ethanol fraction of *A. flava* stems using UV-Vis spectrophotometer with a wavelength of 293 nm and xanthine oxidase inhibitory activity as seen from the IC50 value. Ethanol fraction of *A. flava* stems contains flavonoids, alkaloids, terpenoids, and quinones, but it has not steroids, saponins and tannins. Ethanol fraction of *A. flava* stems has IC50 value as 31.27 ppm. The ethanol fraction of *Arcangelisia flava* stems has strong ability to inhibition xanthin oxidase.

Keyword: *Arcangelisia flava*; xanthine oxidase inhibitory

1. Introduction

Xanthine oxidase (XO) is an enzyme required in the formation of uric acid through the metabolism of purine bases. Guanine monophosphate and adenosine monophosphate are transformed into hypoxanthine and xanthine, respectively, during the purine breakdown process. Additionally, the XO plays a part in catalyzing the conversion of hypoxanthine to xanthine, which results in the formation of uric acid.[1]

A rise in uric acid levels in the body that is higher than usual is known as hyperuricemia, and it is most common in postmenopausal men and women (3.5-7.0 mg/dl) and premenopausal women (2.6-5.7 mg/dl).[1] Patients with hyperuricemia are more likely to develop gouty arthritis diseases due to the formation of monosodium urate crystals in the joints. Additionally, these crystals have the potential to

emerge as kidney stones, increasing the risk of urinary tract stones.[2] Based on this, XO levels can influence the development of hyperuricemia. XO inhibition has been shown to be one of the effective treatments drug strategies for the treatment of gout.[3]

The prevalence of hyperuricemia in Asia is still rising. According to studies on the genetics and epidemiology of hyperuricemia, Asians are discovered to be 2.7 times more susceptible than Caucasians.[4] For 2018, China had a prevalence of hyperuricemia ranging from 6% to 25%, Taiwan had a prevalence of 10% to 52%, and Indonesia had a prevalence of 18%. In comparison to other Southeast Asian nations, Indonesia has the second-highest prevalence of hyperuricemia, with the Philippines having a prevalence of 25%, Thailand having a prevalence of 10.6%, and Singapore having a prevalence of 4.1%.[5] The influence of lifestyle changes, especially the consumption of high-purine diets and physical activity that can increase the risk of hyperuricemia, so the World Health Organization (WHO) recommends investigating various plants that have an effect antihyperuricemia.[5][6]

Handling high uric acid in the blood can be done through medication, both medically and traditionally. Medical treatment that is often done is by administering the drug allopurinol. The way the drug allopurinol works in reducing uric acid levels is by inhibiting the xanthine oxidase enzyme.[7][8] More than 80% of the world's population still relies on plant extracts and active components for traditional medicine, which means there is still a very great potential for employing plants as an alternative treatment.[9]

One common Indonesian plant, *Arcangelisia flava* or yellow wood, is used to cure a number of ailments including allergies, fever, malaria, bacterial infections, diarrhea, diabetes mellitus, kidney stones, and is thought to have antihyperuricemia properties.[10] It is known that the stems of the *A. flava* have the potential to inhibit the xanthine oxidase enzyme and found the secondary metabolites namely flavonoids, alkaloids, triterpenoids, and quinones, that have the potential to be antihyperuricemia based on research of the ethanol extract from *A. flava* stems to inhibit the xanthine oxidase enzyme in vitro.[11]

Alkaloids and flavonoids are examples of secondary metabolites that are often polar because they have a number of sugar groups attached. The ethanol solvent was chosen because it possesses the characteristics of a polar solvent; in theory, a molecule dissolves in a solvent that has the same degree of polarity.[12][13][14] The extract's active fraction, which was isolated from other fractions to produce a purer fraction, was examined to see if it could block the xanthine oxidase enzyme.

Previous research found that the content that has potential as antihyperuricemia is flavonoids with a competitive inhibitory mechanism and it has been proven that ethanol extract of *A. flava* stems can

inhibit xanthine oxidase enzyme with an inhibition value that is classified as active, so it needs to be proven how the level of inhibition ability of the ethanol fraction of ethanol extract of *A. flava* stems against xanthine oxidase enzyme.

2. Methods

2.1. Material

The materials used in this study included *A. flava* stems from Musi Rawas District, ethanol (technical), ethyl acetate (technical), n-hexane (technical), dimethyl sulfoxide (DMSO), HCl, distilled water, sodium phosphate buffer (pH 7.5), xanthine substrate (Sigma/X0626), xanthine oxidase from bovine milk lyophilized powder (Sigma/X4376) and allopurinol.

2.2. Preparation of ethanol fraction

Maceration was the extraction technique used in this study. Making crude extract of *A. flava* stems by1600 mL of 96% ethanol was used to soak 750 g of *A. flava* simplicia for three separate 24-hour periods while stirring occasionally.[15] Until a clean filtrate was obtained, this process was continued. Use a rotary evaporator to dry the extract filtrate that was produced. Utilizing an analytical balance, weigh the dried filtrate before storing and covering with aluminium foil. For fractionation, ethanolic extract dissolved in 100 ml of aquadest and liquid-liquid extraction with three different solvents were used: n-hexane, ethyl acetate and ethanol. The ethanol fraction from the ethanol extract of *A. flava* was then obtained by evaporating the ethanol filtrate.

2.3. Phytochemical screening

Phytochemical screening was performed on viscous extract and fraction of *A. flava* to determine the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, quinones and teroids/triterpenoids.[16]

2.4. Inhibitory of xanthine oxidase

The inhibition test of the extract against xanthine oxidase in vitro was carried out at the optimum conditions obtained and measured spectrophotometrically by the indirect method, namely measuring the amount of residual xanthine oxidase. indirect method by measuring the amount of residual unreacted xanthine. First prepare the test solution, positive control (Allopurinol 100 µg/mL) and negative control (DMSO). The ethanol fraction test solution of the ethanol extract of *A. flava* stem was made with concentrations of 6.25; 12.5; 25; and 50 ppm. It is done by comparing the absorbance of uric

acid formed between the test solution (xanthine + test sample + xanthine oxidase) and the negative control absorbance (xanthine + xanthine oxidase) and the positive control absorbance (xanthine + allopurinol + xanthine oxidase). This was measured by spectrophotometry at a wavelength of 293 nm. The assay solution consisted of 300 µL of 50 mM sodium phosphate buffer (pH 7.5), 100 µL of the test solution in DMSO, 100 µL of the enzyme solution (0.2 units/mL xanthine oxidase in phosphate buffer) and 100 µL of distilled water. The above assay solution was incubated at 37°C for 5 minutes in an incubator. Next, 200 µL substrate solution (0.15 mM xanthine) was added to the assay solution and incubated at 37°C for 30 minutes. Then the reaction was stopped by addition of 200 µL of 0.5 M HCl. The absorbance was measured by Perkin Elmer Lambda 25 UV/VIS spectrophotometer using a blank enzyme solution in phosphate buffer.[17]

2.5. Calculation of Percent Inhibition and IC_{50} value

Calculation of the ability to inhibit xanthine oxidase enzyme is done using the formula:[18]

$$\%\text{Inhibition}=\frac{n-m}{n}.100$$

n : the activity of the enzyme without plant extract (⊚abs. with enzyme- ⊚abs. without enzyme)
m : the activity of the enzyme with plant extract (⊚abs. with enzyme - ⊚abs. without enzyme)

Plotted on the x and y axes of a linear regression equation. The equation obtained the form y = a + bx. Furthermore, this linear regression equation is used to find the value of lC_{50} (inhibitor concentration 50%) by stating the value of y by 50 so that the equation is obtained:[16]

$$IC_{50}=\frac{50-a}{b}$$

Description:
x: sample concentration
y: percentage of inhibition
a: intercept, which is the point of intersection between a line and the y-axis on a diagram or cartesian axis when the value of x = 0
b: slope, which is a measure of the slope of a line

3. Results and Discussion

3.1. Extraction and fractionation of A. flava stem

Extraction of 750 g of *A. flava* stem was carried out using ethanol solvent to obtain 23.542 g of ethanol extract of *A. flava* stem. Furthermore, 23 g of ethanol extract was fractionated by liquid-liquid extraction method using solvents with different levels of polarity. The results of the extraction and fractions is shown in table 1.

Table 1. Extraction and fractionation results of *A. flava stem*

Sample	Weight (g)	Yield (%)
Ethanol extract of A. flava stem	23.542	3.139
n-hexane fraction of ethanol extract	9.561	41.567
Ethyl acetate fraction of ethanol extract	1.522	6.617
Ethanol fraction of ethanol extract	12.783	55.577

Table 1 shows the ethanol fraction produced from the ethanol extract of *A. flava* has the highest yield (55.577%) than the other fractions. This is because the extract used for fractionation is an extract obtained from extraction with polar solvents with ethanol solvent, so that the secondary metabolite dissolved in the ethanol fraction are compounds that dissolve in polar solvents as well.

3.2. Results of phytochemical screening

Phytochemical screening is carried out based on color tests and foam formed after treatment of ethanol extract of *A. flava* stems and ethanol fractions. Results of this phytochemical screening is shown in table 2.

Table 2. Phytochemical screening of sample

Test	Sample	
	Ethanol extract	Ethanol fraction
Alkaloid	+	+
Flavonoid	+	+
Triterpenoid	+	+
Steroid	-	-
Saponin	-	-
Tanin	-	-
Kuinon	+	+

Table 2 shows that the content of secondary metabolites contained in ethanol extract from A. flava stems and ethanol fraction from ethanol extract contained flavonoids, alkaloids, triterpenoids and quinones.

3.3. Percent inhibition of xanthine oxidase and IC_{50} value

Inhibition testing against xanthine oxidase enzyme was carried out to determine the ability of the ethanol fraction of *A. flava* stem to inhibit xanthine oxidase enzyme. The test was conducted by measuring the absorbance value of ethanol extract and ethanol fraction of *A. flava* stem, allopurinol, and blank using spectrophotometry at a wavelength of 293 nm with three repetitions.

The absorbance value obtained was used to calculate the percent inhibition. The percent inhibition results obtained were used to obtain a linear regression equation between the fraction concentration on the x-axis and the percent inhibition on the y-axis. The linear regression equation is used to calculate

the IC_{50} value which shows the concentration of the sample that can inhibit 50% of xanthine oxidase enzyme activity. The results of the calculation of percent inhibition, regression equation, and IC_{50} value of ethanol extract, ethanol fraction of *A. flava* stem and allopurinol is shown in table 3.

Table 3. Results of percent inhibition xanthine oxidase and IC_{50} value

Sample	Concentration (mg/mL)	% inhibition XO	Linear regression	IC_{50}
Ethanol extract	6.25	6.495	y = 1.5272x + 4.1243	30.039
	12.50	31.326		
	25.00	42.692		
	50.00	79.161		
Ethanol fraction	6.25	1.150	y = 1.4851x + 3.5566	31.273
	12.50	29.093		
	25.00	50.677		
	50,00	72.530		
Allopurinol	6.25	26.252	y = 1.3036x + 27.634	17.157
	12.50	48.241		
	25.00	70.433		
	50.00	87.821		

Table above shows that the IC_{50} value of the ethanol fraction is slightly lower than the IC_{50} value of ethanol extract but it is different from the IC_{50} value of allopurinol. This is due to the results of phytochemical tests carried out, secondary metabolite compounds contained in extracts and ethanol fractions are no different. This is possible because the levels of secondary metabolites that can inhibit XO in the ethanol fraction are less than in the ethanol extract. So it is necessary to quantitatively examine the secondary metabolites contained in the extract and its fraction.

Comparison of line equation graphs between ethanol extract, ethanol fraction of *A. flava* stem and allopurinol is shown in figure 1.

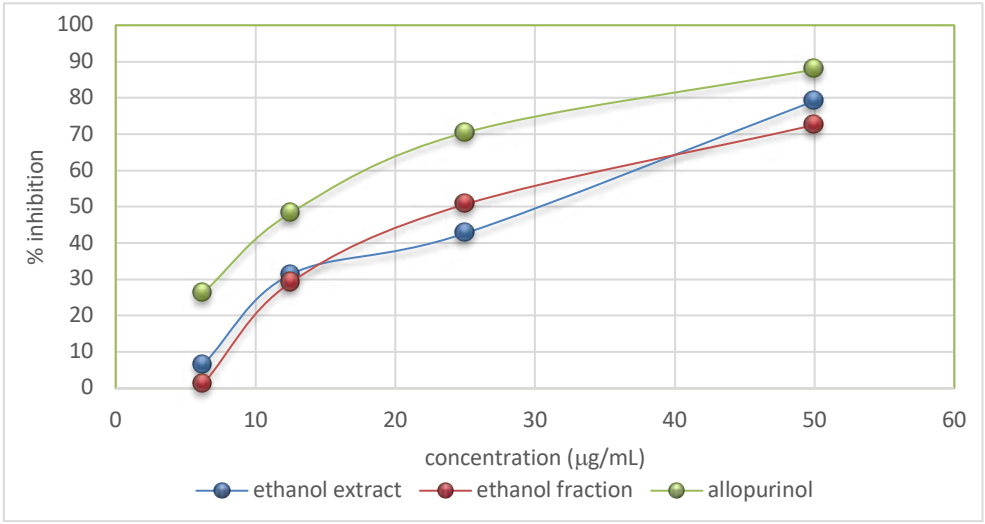

Figure 1. Comparison of percent inhibition of XO based on concentration for each sample

Bioactive compounds in plants are known to have the ability to inhibit xanthine oxidase by binding to xanthine oxidase residues via hydrophobic bonds and occupying the enzyme's active site. This prevents the conversion of substrates (hypoxanthine and xanthine) to uric acid and suppresses the production of uric acid. The second metabolites in plants have known that can inhibit xanthine oxidase, such as luteolin, quercetin, isorhamnetin, galangin, chrysin, prosapogenin, and cajanin stibenoic acid, have similar inhibitory effects and are even more potent than the comparator drug (allopurinol).[19]

Flavonoid compounds are known to be able to reduce uric acid levels in the human body and eliminate superoxide activity, and thus can be used as ischemia and gout medicines. Alkaloid compounds such as colchicine, which can inhibit xanthine oxidase, can be used as antihyperuricemia, thereby inhibiting the breakdown of hypoxanthine and xanthine to uric acid. In addition, colchicine can inhibit the chemotaxis of inflammatory cells, thus healing inflammation caused by high levels of uric acid in the blood. Other alkaloid compounds, such as rombifolin, are thought to inhibit xanthine oxidase and xanthine dehydrogenase, thereby preventing hepatic hyperuricemia in vivo.[20]

Flavonoid can inhibit the enzyme xanthine oxidase because they are structurally similar to xanthine. Based on previous literature, the classification of flavonoids that inhibit the enzyme xanthine oxidase includes flavonols, flavones and isoflavones.[21] The flavonoid structure has the ability to inhibit the enzyme xanthine oxidase because in its structure there are hydroxyl groups (-OH group) at C-5 and C-7 and the double bond between C-2 and C-3 facilitates absorption. interacts with xanthine oxidase and forms hydrogen bonds.[22] This causes flavonoid compounds to act by competitive inhibition by binding to the active side of the enzyme, thereby inhibiting xanthine oxidase enzyme activity and uric acid formation.[23] It is believed that alkaloids, terpenoids, and quinones also inhibit xanthine oxidase enzyme activity, but the mechanism is unknown.

4. Conclusion

The ethanol fraction from ethanol extracts of *A. flava* stems can inhibit xanthine oxidase enzyme with a relatively strong level of ability (IC_{50} = 31.273 mg/mL) and its ability to inhibit XO is slightly lower than the ethanol extract (IC_{50} = 30.039 mg/mL).

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6. References

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Research Article

Bioinformatic analysis of human beta defensin 2

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Abstract: Antimicrobial peptides (AMP) are cationic molecules with antimicrobial activity, including 11 defensin, such as HβD2. This protein contributes in the innate immune system as an immunomodulator, protector against microbial as well as being involved in stimulation of skin commensal microbes. However, research on this protein in a number of diseases is still limited. Therefore, informatic analysis i s needed regarding the characteristics of this protein, especially in supporting the sample processing. The NCBI server and a number of website tools, i.e PROTPARAM, PROTSCALE, TMHMM, PEPTIDECUTTER, NETNGLYC and TARGETP, was performed to analyze the bioinformatic aspects of HβD2 protein. The gene ID is NC_000008.11 and protein ID is NP_004933.1. This study found that stability index was 41,32, aliphatic index is 92,81, and the GRAVY value is 0,605. The Hβd2 protein characteristic is unstable, hydrophobic, a signal peptide, as well as located in inside transmembrane and outside of the cell. This bioinformatic characteristics need to be considered in HβD2 protein research techniques. A number of HβD2 induction factors may be different for each disease agent and their structure may affect the membrane integrity of the agent.

Keywords: analysis; bioinformatic; defensin; HβD2

1. Introduction

Antimicrobial peptides (AMP) are cationic molecules with antimicrobial activity. There are 3569 AMPs that have been identified from bacteria, archae, protists, fungi, plants, animals, and synthetic peptides. In humans, there are 153 AMPs including cathelicidins, defensins, protein S100, and ribonuclease. The AMPs can be found in various tissues and cells, including skin, eyes, ears, as well as in the mucosa of the respiratory, digestive and urogenital tracts, while in cells, it produced by eccrine gland, keratinocytes, mast cells, phagocytes and sebocytes.[1,2]

Defensins consist of 3 classifications according to disulfide bonds and gene mapping, namely α or Human neutrophil peptide (HNP) 1 – 4 and Human α-defensins 5-6; β, and θ. There are totals 28 β defensins in human, but only Human beta defensins (HβD) 1 – 4 have been deeply studied.[2] Epithelial cells secrete Hβd 1 – 6, i.e. Hβd 1 – 4 are produced by epithelial cells such as keratinocytes, dendritic cells, monocytes or activated macrophages, while Hβd5 and Hβd6 are produced by the male genitalia

epididymis tract.[3,4]

There are a number of roles for HβD2, especially in the innate immune system, including as an immunomodulator, protector against bacteria, viruses, fungi and parasites, as well as being involved in stimulation of skin commensal microbes.[3,5–8] Therefore, HβD2 has been widely studied in various diseases, but still limited to diseases caused by parasites. Therefore, informatic analysis is needed regarding the characteristics of this protein, also, it can assist in the HβD2 collection, preparation and examination.

2. Methods

A number of web-based tools used in this study. Genomic information regarding HβD2 was obtained from website National Center for Biotechnology Information's (NCBI) (https://www.ncbi.nlm.nih.gov/). This server provides genetic information about gene ID NC_000008.11, its location and sequences, as well as protein ID NP_004933.1 and its sequences. The protein sequences used to explore the bioinformatic characteristics at another tools, ie: PROTPARAM (https://web.expasy.org/cgi-bin/protparam/protparam) contains physical, chemical and computed parameters; PROTSCALE Hydropath./Kyte & Doolittle scale perform hydrophobicity and hydrophilicity profile; TMHMM (https://services.healthtech.dtu.dk/services/TMHMM-2.0/) to predict transmembrane helices in proteins; PEPTIDECUTTER (https://web.expasy.org/peptide_cutter/) to predict potential site of protein sequences and its proteases or chemicals; NETNGLYC (https://services.healthtech.dtu.dk/services/NetNGlyc-1.0/) to predicts N-Glycosylation sites; and also TARGETP (https://services.healthtech.dtu.dk/services/TargetP-2.0/) to predict subcellular location of proteins.

3. Results and Discussion

3.1. Location and structure

In humans, HβD2 or *Skin-Antimicrobial Peptide 1* (SAP-1) is coded by HβD2 gene, or by another name BD-2, SAP1, DEFB2, DEFB4, HBD2, DEFB-2, and DEFB102. The ID of this gene was NC_000008.11 (784677..7896716).[9] This gene was located in chromosome 8p23.1, meaning that the carrier of HβD2 genetic information was located on chromosome number 8, short arm position (p), at second region and third band (Figure 1).[10]

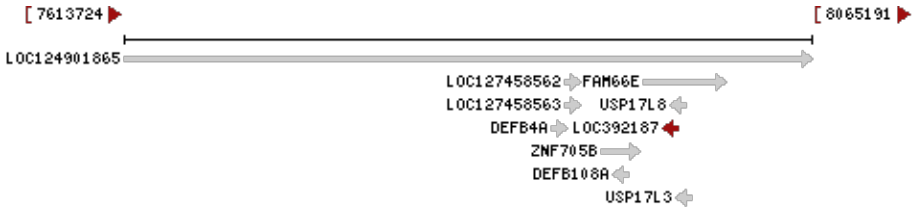

Figure 1. Genomic position of HβD2 gene.[10]

The HβD2 gene has 2040 base pairs (bp) in length, starting from positions 7,894,677 to 7,896,716. This gene consists of two exons flanking one intron. The first exon encoded a hydrophobic leucine-rich signal sequence and second exon encodes the propeptide and mature peptide. The promoter region consisted of several response element sequences to NF-IL 6β and NF-κB.[11,12]

3.2. The mRNA expression

Quantitative transcriptomic analysis (RNA-Seq) and Human protein atlas (HPA) of HBD2, combined with antibody-based profiling was carried out on 27 of tissues and organs of 25 normal individuals. These organs include adrenals, bone marrow, colon, endometrium, fat, heart, liver, lymph nodes, pancreas, prostate, skin, spleen, testes, and urinary bladder. The HBD2 gene expression was reported to be highest in the gallbladder, as well as in the proximal parts of the digestive tract such as the stomach and esophagus. In other tissues, HBD2 was expressed in the appendix, endometrium and skin.[12] The Hβd2 protein is constitutively secreted in small amounts, including in the stratum corneum of skin, mainly by keratinocyte in hair follicles.[8] as well as in the tract of respiratory, gastrointestinal (enterocytes) and genito-urinary. This protein was increased in some diseases at various organs such as psoriasis and dermatitis atopic, even in endometriosis rat models.[13]

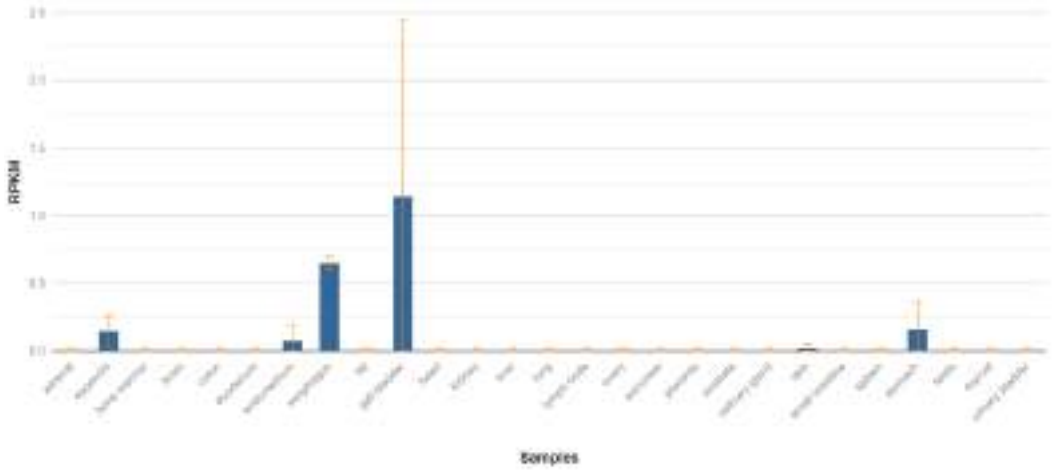

Figure 2. RPKM (Reads per kilobase of transcript per Million mapped reads) of HBD2.[12]

3.3. Sequences of protein

The HβD2 protein only has one precursor form with identity code NP_004933.1 and annotation code NM_004942.4.[12] The amino acid sequence of this protein precursor can be seen in Figure 3.

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  1 mrvlyllfsf lfiflmplpg vfggigdpvt clksgaichp vfcprrykqi gtcglpgtkc
 61 ckkp
```

Figure 3. Amino acid sequences of HβD2.[12]

The gene product is a prepropeptide, which consists of a presequence region with the function of coding a signal peptide, an anionic pre-segment and a C-terminal cationic region. Post-translational proteolytic cleavage occurs at the signal sequence and propeptide which then releases the C-terminal region of the prosegment (Figure 4). Release of this region in the cell environment will express the peptide and evoke its antimicrobial activity (mature peptide), for example in epithelial cells.[14] The enzymes responsible for the cleavage of α-defensins are elastase, metalloproteinase, matrilysin, and trypsin, but in β-defensins, this is still poorly understood.[14,15]

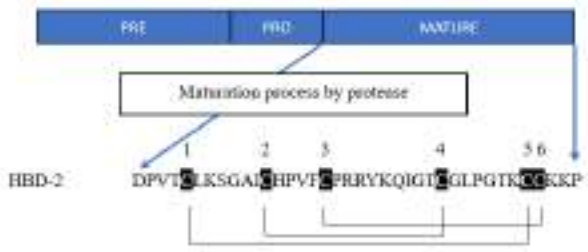

Figure 4. The forming process of HβD2 protein.

3.4. Structure and model

The structure of the HβD2 protein consisted of 64 amino acids with a low molecular weight and is rich in cysteine residues. Its structure is monomeric at concentrations < 2.4mM. There are folds on the β sheet inside the structures, where six cysteine residues are involved in the formation of thress disulfide bonds, namely in Cys 1-5, 2-4 and 3-6 (Figure 4). This bond plays an important role in maintaining structural integrity.[4] The HBD2 structure contains several key components, i.e. a triple-stranded, antiparallel beta sheet with strands 2 and 3 in a beta hairpin conformation (Figure 5).[16,17]

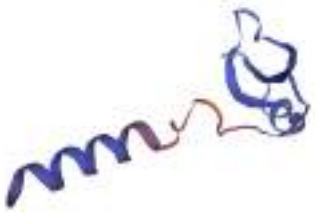

Figure 5. The structure of HBD2 protein (alphafold model).[17]

The method used can determine the structural components. Use of X-ray crystallography technique observed the N-terminal R-helical region and the uniform surface distribution of positively charged residues. Antimicrobial activities come from electrostatic interaction resulting in disintegration of bacterial membrane.[18] Performing of Nuclear Magnetic Resonance (NMR) did not show the alpha-helix structure and the allocation of positive charges are ununiform. This technique also suggest that hydrophobic end component would attach in the bilayer of bacterial membran, whereas the hydrophilic part interact with liquid sphere.[16]

3.5. Physical-Chemical characteristics

The physical and chemical analytic performed of PROTPARAM showed that HβD2 consist $C_{327}H_{515}N_{81}O_{75}S_8$ atom, with the total amount 1006. The instability index of HBD 2 was 41,32, indicated that this protein was unstable. Aliphatic index of this protein was 92,81. It influenced the stability of thermal, suhu, i.e. the higher the index, the more stable at high temperatures. Amino acids with a high aliphatic index are naturally hydrophobic and less than 100.000 in molecular weight.[19] This corresponds to a GRAVY value of 0,605, which was a positive value indicating that the HBD2 protein is hydrophobic or fat soluble.[20]

Table 1. Physical-chemical characteristics of HβD2 protein[20]

Characteristics	HBD2 protein							
Number of amino acids	64							
Molecular weight	7037.66							
Theoretical pI	9.46							
Amino acid composition	Ala	(A)	1	1.6%	Lys	(K)	5	7.8%
	Arg	(R)	3	4.7%	Met	(M)	2	3.1%
	Asn	(N)	0	0.0%	Phe	(F)	6	9.4%
	Asp	(D)	1	1.6%	Pro	(P)	7	10.9%
	Cys	(C)	6	9.4%	Ser	(S)	2	3.1%
	Gln	(Q)	1	1.6%	Thr	(T)	3	4.7%
	Glu	(E)	0	0.0%	Trp	(W)	0	0.0%
	Gly	(G)	8	12.5%	Tyr	(Y)	2	3.1%
	His	(H)	1	1.6%	Val	(V)	4	6.2%

	Ile	(I)	4	6.2%	Pyl	(O)	0	0.0%
	Leu	(L)	8	12.5%	Sec	(U)	0	0.0%

Total number of negatively charged residues (Asp + Glu)	1		
Total number of positively charged residues (Arg + Lys)	8		
Atomic composition	Carbon	C	327
	Hydrogen	H	515
	Nitrogen	N	81
	Oxygen	O	75
	Sulfur	S	8
Extinction coefficients	3355		
Abs 0.1% (=1 g/l)	0.477, assuming all pairs of Cys residues form cystines		
Ext. coefficient	2980		
Abs 0.1% (=1 g/l)	0.423, assuming all Cys residues are reduced		
Formula	$C_{327}H_{515}N_{81}O_{75}S_8$		
Total number of atoms	1006		
Instability index	41.32 (unstable)		
Aliphatic index	92.81		
Grand average of hydropathicity (GRAVY)	0.605		

Based on its physical and chemical characteristics, the Hβd2 protein was unstable, hydrophobic, cationic molecule and was a signal peptide. An instability index of more than 40 (41,32) indicated unstable and will decompose more easily in the test tube. Unstable proteins also require storage at cold temperatures. However, on the other hand, this protein may not require an extractor on its processing. The aliphatic index (92,81) was considered as a factor increasing the thermostability of proteins such as alanine, valine, isoleucine and leucine. The higher the aliphatic index, the greater the thermostability of a globular protein.[14,21]

The HβD2 protein that has 3 disulfide bonds on 6 cysteines, is very cationic at neutral pH due to the high number of Lys and Arg residues.[22] Based on the 3D Nuclear Magnetic Resonance (NMR) structure, HβD2 is in monomeric form. The monomer structure has a lower charge density than the dimer so that the antimicrobial activity is also smaller. HβD3 with a dimer structure, has a higher charge density so that its antimicrobial activity is also greater. HβD3 has the highest positive charge (+11),

HβD2 +6, and the lowest is HβD1 (+4). Positive charge describes the antimicrobial efficacy of a specific peptide by using electrostatic bonds to attach the negatively charged molecules present on bacterial cells such as sialic acid.[4,6,23]

In innate immunity, a microbial killing effect of Hβd2 through the ability of cationic molecules to bind to the anionic membrane of bacteria, especially gram-negative bacteria. In intracellular parasites such as protozoa, an abundance of GPI protein causes a negatively charged membrane and affinity for Hβd2. It causes significant integrity changes of the parasite membrane, osmotic imbalans, edema, even cell lysis. [24] Hβd2 also modulate the production of other cytokines such as IL-8, which are chemotactic and initiate the inflammatory process.[3] This protein involved in immune cell chemotaxis and activates Tool like receptor (TLR)s on their surface, and binds strongly to complement C1q.[25]

3.6. Hydrophobicity

The hydrophobicity analysis used PROTSCALE, Hydropath scale/ Kyte & Doolittle. This scale identified 20 amino acids, with value of Alanine 1,800 and Glycine -0,400 (Figure 6).[26]

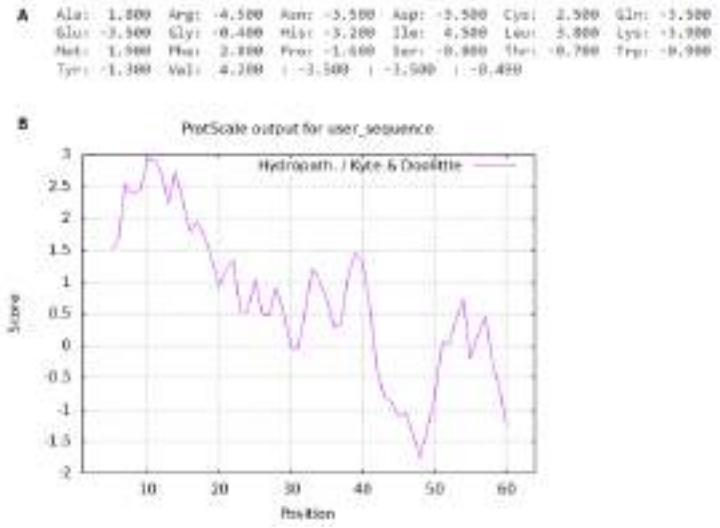

Figure 6. (A) Amino acid value; (B) Hidrophobicity of HBD2 protein.[26]

3.7. Transmembrane helices in protein

The membrane protein topology prediction analysis used Transmembrane Hidden Markov Models (TMHMM) to obtain results as shown in Figure 7. The HBD2 protein was located inside, transmembrane and outside of the cell. Locations inside the cell were in the sequence of number 1 and 2, in the transmembrane were numbered 3 to 25, while positions outside the cell were numbered 26 to 64.[27]

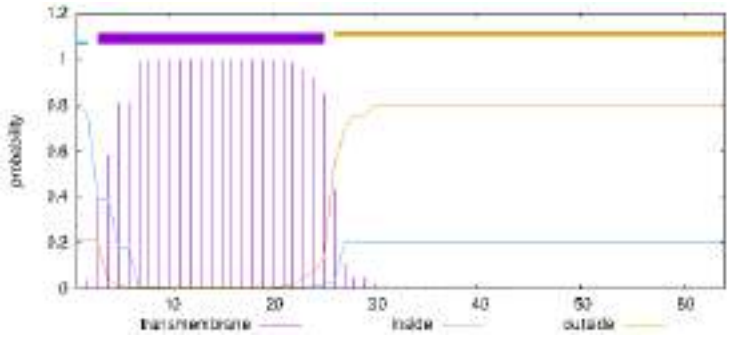

Figure 7. TMHMM result of HBD2 protein.[27]

3.8. Cutting prediction by protease

Peptide cutter is a tool to predict the proteases or chemicals that can cut certain protein sequences at potential cleavage sites. There were 17 enzymes that can cut the HBD2 protein, out of the 37 enzymes listed. The 17 enzymes were Arg-C protease, Asp-N endopeptidase, Asp-N endopeptidase + N-terminal Glu, CNBr, Chymotrypsin low and high specificity, Clostripain, Formic acid, LysC, LysN, NTCB, Pepsin, Proline-endopeptidase, Proteinase K, Thermolysin and Trypsin. Apart of these enzymes include BNHPS-Skatole, Caspase 1 – 10, Glutamyl endopeptidase, Granzyme B, Hydroxylamine, Iodosobenzoic acid, Staphylococcal peptidase I, Thrombin, Tobacco etch virus protease, could not cut HBD2 protein (Figure 8).[28]

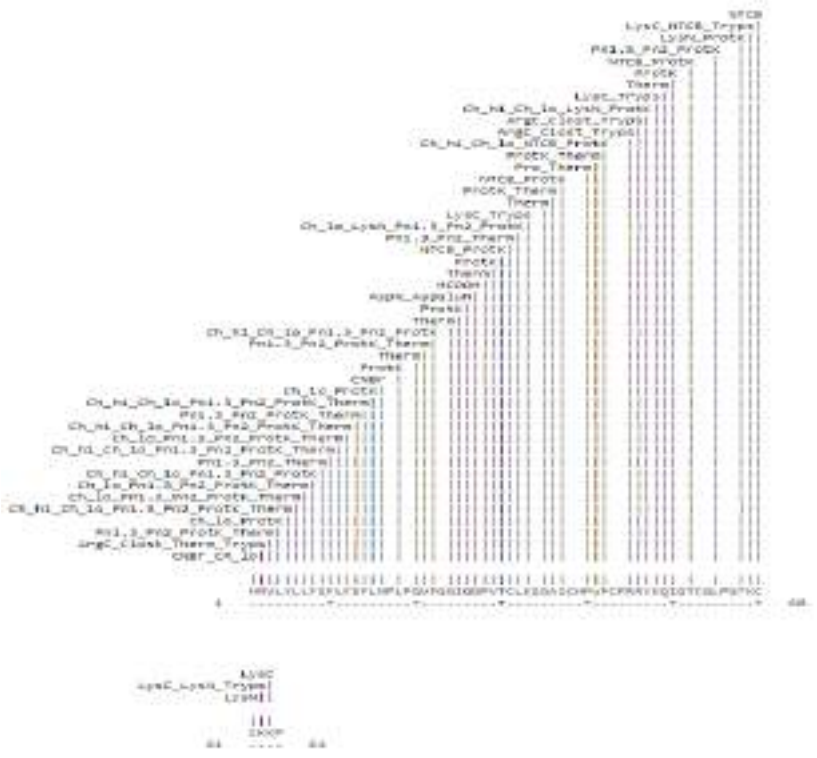

Figure 8. The potential protease to cut HβD2 protein.[28]

3.9. Predict the potential of glycosylation sites

NetNglyc is a tool to predicts N-Glycosylation (NETNGLYC) sites in human proteins using artificial neural networks that examine the sequence context of Asn-Xaa-Ser/Thr sequons. But unfortunately, N-Glycosylation is only predicted on Asn residues. In the HBD2 amino acid sequence, this residue was not found so it could not be predicted the N-glyc sites.[29]

3.10. Prediction of protein location

TargetP-2.0 (TARGETP)is a tool to predicts the location of subcellular protein in mitokondria, chloroplastic, secretory pathway or other. This tool performed predictions through the presence of N-terminal presequences, i.e signal peptide (SP), mitochondrial transit peptide (mTP), chloroplast transit peptide (cTP) or thylakoid luminal transit peptide (lTP). Predicted of TARGETP to HBD2 was as a signal peptide (Figure 9).[21] Signal peptide is released as a protein and important in initiating the process of transporting protein out of the cell membrane. Signal peptide is cut off by some protease after after being transferred across the membrane.[30]

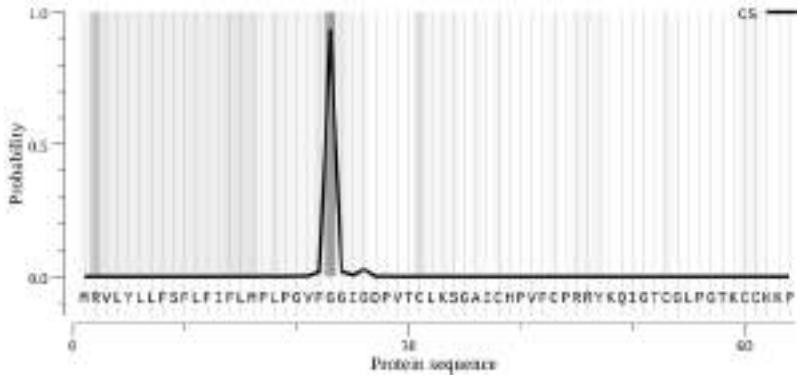

Figure 9. Target-2.0 prediction of HBD2 protein.[21]

3.11. The role of HBD2

Kyoto Encyclopedia of Genes and Genome (KEGG) pathway (https://www.genome.jp/kegg/pathway.html) is collection of pathway map that depicted manually. Each pathway describe knowledge about molecular interaction, reaction and relation. The network describes about metabolism, genetic and environmental information processing, cellular process, organismal systems, human diseases, as well as drug development. There were three pathways in the KEGG server with HBD2 involved i.e Nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs) signaling pathway (Figure 10), IL-17 signaling pathway and *Staphylococcus aureus* infection mechanism.[25]

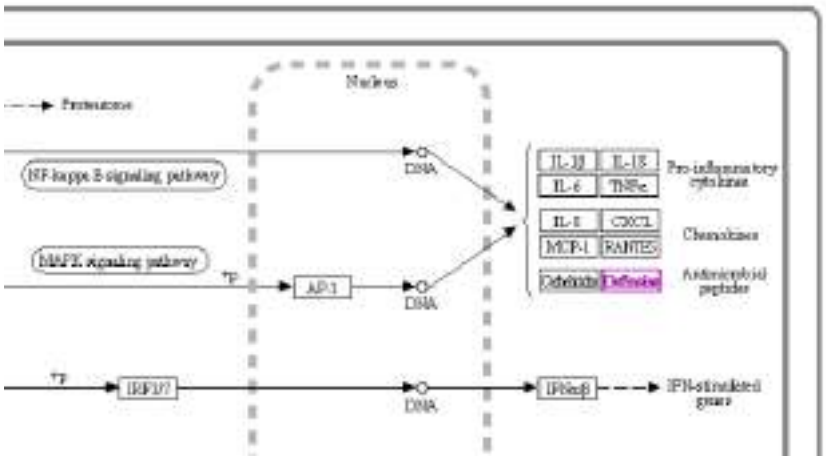

Figure 10. One of the role of HβD2 in KEGG server that describes NOD-like receptor signaling pathway.[25]

From this KEGG pathway, the increasing of this production mainly due to the induction of infection with microorganisms such as live *S. aureus*, bacterial elements (peptidoglycan), lipopeptides or lipoteic acid, as well as inflammatory cytokines such as IL-1, IL-17 and TNF-α.[25] Some study showed glico phosphatidil inositol (GPI) of intracellular parasite,[24] and damage of epithelial cell.[2,3] also induced the secretion of this PAM. This protein can be considered as a solution in the era of antibiotic resistance, post-operative healing and cancer because it has antimicrobial effects, is involved in the wound healing process and reduces angiogenesis which is important in tumorigenesis.[13,31,32]

4. Conclusion

The characteristic of Hβd2 protein was unstable, hydrophobic and a signal peptide. This needs to be considered in HβD2 protein research techniques. A number of HβD2 induction factors may be different for each disease agent and their structure may affect the membrane integrity of the disease agent.

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Literature Review

High exposure to fluoride aggravates the impairment in cognitive function

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Abstract: Fluoride is one of the most abundant elements naturally found in drinking water, and levels around 0.7mg per liter have been proven to reduce tooth decay. Fluoride exposure also impacts neurological problems. Many researchers found that damage to mental ability might exist for communities that use water with fluoride above recommended values. Experimental studies given exposure to sodium fluoride in mice that were tested using cognitive behavioral. Prolonged and high exposure fluoride can result in neuron cell damage in the elderly. This affects nerve injury at the level of proteins, cells, and organelles that cause structural damage to the brain, especially the hippocampus.

Keywords: behavioral; cognitive; fluoride

1. Introduction

Age is one of the factors that play a role in the impairment in cognitive function. Studies show the prevalence of cognitive impairment is around 5.1% to 41%, or equivalent to 22 to 76.8 per 1000 people per year at ages ranging from 50 years to >70 years.[1] Mild cognitive impairment may progress until the elderly experience more severe cognitive decline diseases, such as Alzheimer's disease and dementia.[2]

When individuals reach elderly (>65 years), the aging process begins to occur in the physiology of the body's organ systems, including the brain which regulates cognitive function in humans.[3] The aging process in every person results in different variations in cognitive impairment. one of the factors that can impair cognitive function is the environmental factors related to chemical exposure that can be toxic to the brain.[4] One of the substances that can affect cognitive function is fluoride.

Fluoride is one of the most abundant minerals in nature, it derived from the rocks in the Earth's crust and can be found in varying amounts in foods, beverages, supplements, and health products such as toothpaste. [5–9] The most common source of fluoride is water. Fluoride in water can be found naturally in mountainous areas.

The World Health Organization (WHO) states that there are endemic areas of fluorosis associated with high fluoride exposure that exceeds the fluoride content in drinking water, which is 1.5 mg/L.[10,11] In previous studies, fluoride exposure at the optimal dose was aimed at decreasing the incidence of dental caries. However further studies, excessive fluoride intake will increase the incidence of dental and bone fluorosis.[6] However further studies, excessive fluoride intake will increase the incidence of dental and bone fluorosis. The incidence of fluorosis is associated with the incidence of neurotoxicity as proven by studies that assess cognitive function in the age group > 60 years which states that there is a decrease in cognitive function in humans who consume high fluoride by measuring the MoCA-B, AD-8, and MMSE measurement methods.[12,13] Experimental studies given exposure to sodium fluoride in mice that were tested using cognitive behavioral tests including the novel object recognition test, Morris water test, and elevated-plus maze test showed a decrease in cognitive function in mice.[14]

2. Fluoride

Fluoride (F-) is an inorganic ion that is distributed in nature, has a reactive ion, and is often found in the form of minerals that bind to other compounds[6,7,15] The WHO policy on fluoride in water is regulated. It is said that fluoride doses below 1.5 mg/L are recommended.[16]

Fluoride compounds such as sodium fluoride can be absorbed easily. From the gastrointestinal tract, fluoride absorbed in the stomach and intestines through a passive diffusion mechanism. Fluoride is distributed to organs, such as teeth, bones, heart, lungs, kidneys, liver, and brain.[17]

Fluoride is commonly used to reduce dental damage due to the process of dental caries. Caries appears by preventing demineralization of enamel caused by acid produced by bacteria and remineralization of enamel caries.[18–20] But, throughout the research on fluoride exposure, fluoride has toxic effects on human health. Effects on teeth and bones cause fluorosis. Neurological effects lead to decreased intelligence levels (IQ tests) in children and cognitive impairment in the elderly.[21]

3. Cognitive function and memory

Cognitive function is the mental process and cognition in the regulation of behavior and intelligence through perception, reasoning, knowledge acquisition, information processing, and problem solving.[22] Cognitive function is controlled by the limbic system, including the cingulate gyrus, parahippocampal subcalosal gyrus, hippocampus, amygdala, hypothalamus, thalamus, basal ganglia, and prefrontal cortex. The connecting pathways of the limbic system include the fornix, tractus mammillothalamicus, and striae terminalis.[23]

The domain of cognitive function is divided into five functions, namely attention, visuospatial, language, executive function, and memory. The cognitive domain most affected by fluoride exposure is memory. Stimulus will be captured by the sensory system and delivered to the cerebral cortex. Impulses will be relayed to the limbic system for processing in encoding, storing, and recalling memory.[24]

Memory is formed by sensory impulse that is captured and processed in the cerebral cortex, then transmitted to the hippocampus where a new memory will be formed. Impulse processing that occurs continuously will be transmitted back to the cortex for storage. Brain has millions of neuron cells that are connected by synapses created synaptic plasticity. The more frequently a synapse transmits impulses, the easier it forms a new connection. New memory will be formed if there are new connections and synapse strengthening between neurons. Repetition will strengthen the connection between synapses. This process is called long-term potentiation (LTP).[25]

Glutamate is an excitatory neurotransmitter that works in increase the density of neuronal interactions by regulating brain excitability. It helps in memory consolidation which is triggered by the long-term potentiation (LTP) process due to its interaction with receptors. This process will initiate action potentials in impulse transmission in the learning and memory process. LTP occurs by signaling, especially in the hippocampus.[26] Glutamatergic neurons are stimulated release glutamate into the synapse gap.[27]

Glutamate receptors such as alpha-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid (AMPA) and N-methyl-d-aspartate (NMDA) as Na+ and Ca2+ ion channels. If impulses are relayed continuously and repeat, as more glutamate binds to receptors, ion channels will open and Na+ enter the post-synapse. This will cause depolarization of the dendrite, triggering the release of Mg2+ that blocks the ion channel on the NMDA receptor and followed by the entry of Ca2+ into the post-synapse. In the early phase, Ca2+ initiates a signaling process that activates protein kinases. This will initiate the attachment of a phosphate group to the AMPA receptor. The process will increase the conduction between AMPA and Na+ to the post-synapse and initiate the movement of AMPA receptors from the intracellular to the plasma membrane of the post-synapse.[26,27]

4. Fluoride and impairment of cognitive function

Excessive fluoride intake can cause damage to body tissues. Previous studies have shown that fluoride can cause neurotoxicity and neurodegeneration due to its ability to cross the blood brain barrier. Chronic and high exposure of fluoride can cause fluoride levels in mice brain to be 300x higher than mice that are not exposed to fluoride.[28]

Fluoride can affect the structure of CA1, CA3, and dendate gyrus in the hippocampus seen in the involution of neuronal cell membranes, mitochondrial inflammation, and chromatin clumping in the results of motor coordination and maze tests in experimental animals decreased.[29,30] In histopathology, glial cells and pyramidal cells in the dendate gyrus is decreased and have vacuolar degeneration with irregular patterns.[31]

Prolonged fluoride exposure also leads to neuronal injury through mitochondrial dysfunction and oxidative stress mediated by Sirt3 inhibition, affecting short-term memory and long-term memory.[32,33] The structure of microtubuli proteins in neuronal cells, such as microtubule-associated protein 2 (MAP2), synaptophysin (SYP), and drebrin (Dbn) at the mRNA level has decreased protein expression so that interactions between neuronal cells are impaired.[34]

Fluoride affects neuroplasticity in the learning and memory retention process. The secretion of pro-inflammatory cytokines can suppress the induction of LTP and reduce the levels of postsynaptic density protein 95 (PSD-95). Fluoride exposure also decreases the expression of glutamate by changes the ratio of protein in the glutamateric receptor.[31,35]

The neurodegenerative effects of fluoride are multidimensional. It disrupting the regulation of brain metabolism, synaptic function, blood brain barrier integrity, oxidative stress, and induction of inflammation in neuronal cells. This is associated with increased of reactive oxygen species (ROS) triggering stress and cellular dysfunction resulting in neuronal cell damage and apoptosis.[36]

5. Summary

Prolonged and high exposure fluoride can result in neuron cell damage in the elderly. Many studies say fluoride can cause degenerative cognitive function linked to learning and memory. This affects nerve injury at the level of proteins, cells, and organelles that cause structural damage to the brain, especially the hippocampus.

6. Conclusion

High exposure to fluoride for a long time can result in nerve cell damage in the hippocampus, causing a impairments cognitive function in the elderly.

7. Reference

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Literature Review

The role of necroptosis in the process of CD4 cell death in HIV/AIDS patients

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Abstract: HIV infection is a chronic RNA virus infection that has the ability to integrate with human DNA, which causes CD4 T cell death, long-term immune suppression, and immune system dysfunction so that the host is prone to opportunistic infections. The cause of the decrease in CD4 count is due to the high process of CD4 cell death through apoptosis. However, when the apoptotic pathway is blocked, CD4 T cell death can occur due to necroptosis, a subtype of programmed necrosis with activation of necrosomal components consisting of Receptor- Interacting Protein Kinase 1 (RIPK1), Receptor-Interacting Protein Kinase 3 (RIPK3) and Mixed-Lineage Kinase domain-Like protein (MLKL) after stimulation with the proinflammatory cytokine TNF-α. In this process, cells undergo controlled destruction, releaseof intracellular and inflammatory contents. However, there is little clinical evidence on the role of necroptosis in HIV and CD4 T cell death. Various studies are being developed to optimize the understanding of the necroptosis process and future HIV treatment opportunities.

Keywords: CD4 T cells; HIV; necroptosis

1. Introduction

Worldwide, the current estimated prevalence of HIV patients is 36.9 million individuals, with 2 million new HIV cases recorded in 2014. HIV (Human Immunodeficiency Virus) infection is a chronic RNA virus infection, in which the virus can integrate with the host (human) DNA, resulting in modifications to the immune system that lead to the death of CD4 T cells and long-term immune suppression. The ultimate outcome of this virus infection is immune system dysfunction, progressive virus replication, and the persistence of the virus in the body, making the host susceptible to opportunistic infections.[1]

The decrease in the number of CD4 cells directly leads to a weakened immune system, increasing the risk of opportunistic infections that can result in patient deaths. The cause of the decrease in CD4 cell count is due to the high rate of CD4 cell death through apoptosis. However, besides apoptosis, some studies have also shown that cell death can occur through a process called necroptosis, triggered

by the activation of necrosome components, including Receptor-Interacting Protein Kinase 1 (RIPK1), Receptor-Interacting Protein Kinase 3 (RIPK3), and Mixed-Lineage Kinase domain-Like protein (MLKL).[2–4]

Apoptosis is a form of programmed cell death characterized by the regular destruction of cells into small vesicles or apoptotic bodies. Understanding of programmed cell death has expanded to include regulated or programmed forms of necrosis. Necroptosis, one subtype of programmed necrosis, occurs after stimulation with proinflammatory cytokines such as TNF-α when the apoptosis pathway is blocked. Necroptosis occurs through the formation of the necrosome, which is a complex of three types of proteins: RIPK1, RIPK3, and MLKL. Auto- and transphosphorylation of RIPK1 and RIPK3 are required for necrosome assembly, which contains RIPK1/3 and activates necroptotic signaling. RIP3 then recruits and phosphorylates mixed lineage kinase domain-like protein (MLKL) to promote oligomerization and translocation to the plasma membrane, leading to necrotic cell death. In addition, necrosomes containing RIPK1/3 have been reported to activate mitochondrial phosphatase phosphoglycerate mutase family family 5 (PGAM5), which recruits and activates mitochondrial fission factor dynamin-related protein 1 (DRP1). DRP1 activation leads to mitochondrial fragmentation and the generation of reactive oxygen species, resulting in necrotic cell death. Cell rupture and necrosis release damage-associated molecular patterns (DAMPs) such as high-mobility group box 1 protein (HMGB1), triggering inflammation and secondary tissue damage.[5–10]

Enzymes RIPK3 and MLKL associated with necroptosis have been shown to play a significant role in some experimental models of HIV-related CD4 cell count decline. However, there is still limited clinical evidence regarding the role of necroptosis in HIV and CD4 T cell death in connection with the necrosome factor. Various studies have been developed to optimize understanding of the necroptosis process and potential HIV treatments that can be derived from this process.[11–14]

Considering the limited research on the role of necroptosis in the CD4 T cell death process in HIV/AIDS patients, the author raises this issue.

2. Epidemiology

Currently, the estimated worldwide prevalence of HIV patients is 36.9 million individuals, with 2 million new HIV cases recorded in 2014. Since the identification of HIV, it has caused 39 million deaths. The prevalence of HIV/AIDS has increased in recent years due to advances in treatment that allow individuals to live longer with HIV.[2]

In Indonesia, as of 2020, an estimated 543,100 people were living with HIV. This number has decreased from the previous figure of 643,443 in 2016. New HIV infections in Indonesia continue to decline, in line with the global decrease in new HIV infections. However, the reduction in new

infections is not as significant as hoped. In certain key populations (Men who have Sex with Men (MSM) and transgender individuals), there is an increase in new HIV infections.[15]

Most HIV cases are found in "non-key populations," which are groups at risk of HIV infection outside of key populations. These groups include sexual partners of drug users, bisexual male sexual partners, former sex workers, pregnant women, TB patients, sexually transmitted infection (STI) patients, hepatitis patients, and individuals showing signs of weakened immune systems.[15]

3. HIV (Human Immunodeficiency Virus)

HIV is a cytopathic virus classified in the Retroviridae family, Lentivirinae subfamily, Lentivirus genus. HIV is an RNA virus with a molecular weight of 9.7 kb (kilobases). Its structure consists of an outer envelope composed of glycoprotein gp120, which attaches to glycoprotein gp4. Inside, there is a second layer composed of protein p17. Beyond that, there is the core of HIV formed by protein p24. Inside the core, there are two crucial components: two RNA strands and the enzyme reverse transcriptase. The envelope, consisting of glycoproteins, plays a crucial role in infection due to its high affinity for the specific CD4 receptor on host cells. The RNA molecule is surrounded by a double-layered capsid and a membrane envelope containing proteins.[16,17]

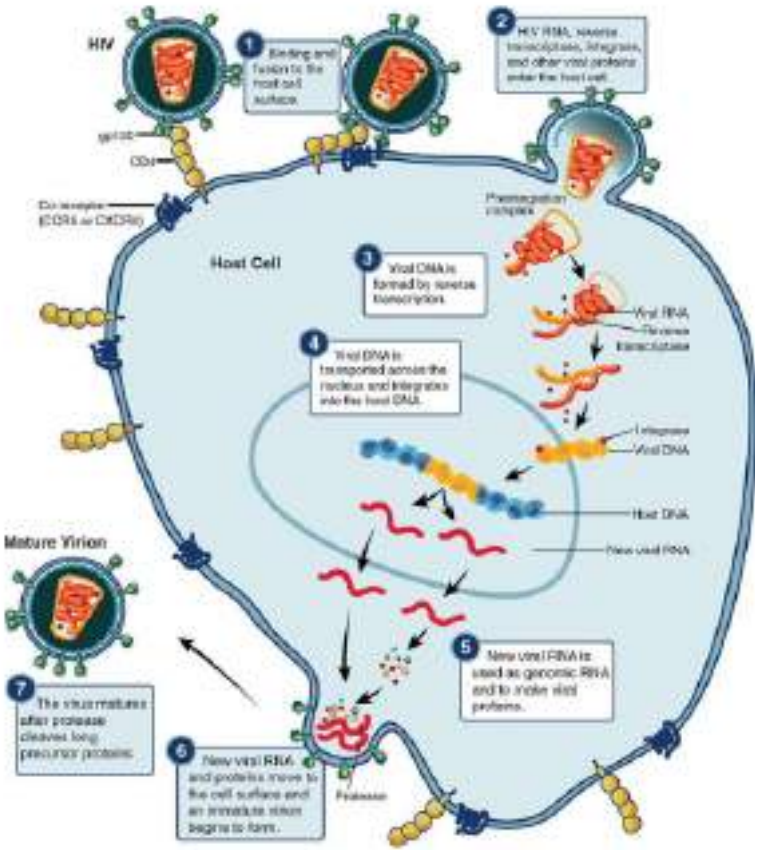

Figure 1. Replication of HIV[16]

4. The role of necroptosis in CD4 cell death in HIV patient

4.1. The decline of CD4 T cells in HIV infection

HIV infection and AIDS are infectious diseases caused by the HIV virus, which attacks and weakens the immune system. CD4 T cells are central mediators of the immune response that coordinate cellular and humoral responses against infections.[17]

The decline of CD4 T cells in three natural phases of HIV infection is depicted in Figure 2. Phase I, known as the window period, occurs when the body is already infected with HIV, but anti-HIV antibodies are not yet detected in blood tests. This phase is characterized by a very high HIV viral load and a sharp decrease in CD4 T lymphocytes. It usually lasts for about two weeks to three months after initial infection. Phase II is a latent period that can be asymptomatic or accompanied by mild symptoms. It is marked by a decrease in viral load and a relatively stable condition, but CD4 levels gradually decline. This phase is typically asymptomatic and lasts an average of 2-3 years, while the mildly symptomatic phase can extend for 5-8 years. Phase III, AIDS, is the terminal phase of HIV infection with a significantly weakened immune system, a higher viral load, and very low CD4 counts, leading to the development of various opportunistic infections.[17,18]

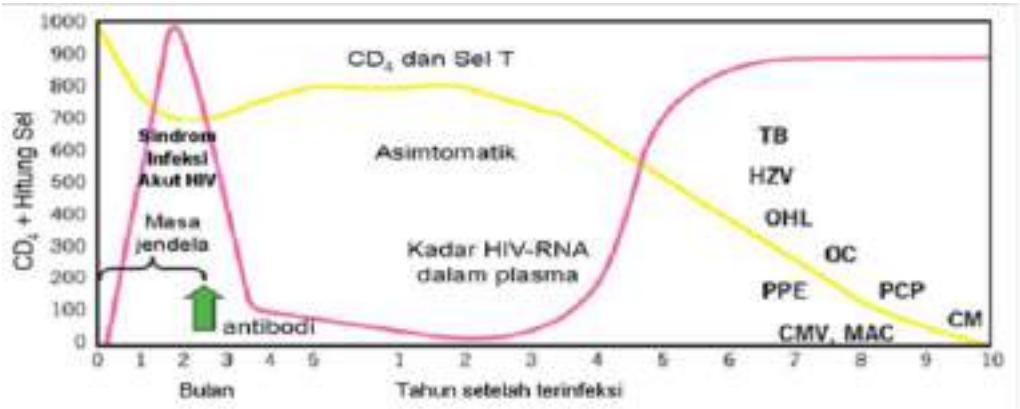

Figure 2. History of HIV and AIDS infection

4.2. Mechanisms of CD4 T cell reduction

In vitro studies have revealed that HIV infection leads to the lysis of CD4 T cells or the formation of syncytia, which is the fusion of uninfected and infected CD4 T cells. Animal models of SIV infection show significant depletion of CD4 T cells in the gut-associated lymphoid tissue (GALT), which is a major source of CD4 T cells in the body.[19]

According to the theory proposed by Ho et al., there is a homeostatic response to the decrease in CD4 T cells, where the body compensates by producing more CD4 T cells. However, this balance is

disrupted when homeostasis becomes exhausted, leading to a decline in CD4 T cells. HIV infection accelerates both the production and destruction of CD4 T cells. In the early stages of HIV infection, there is a constant replacement of CD4 T cells with T cells originating from the thymus. During the early stages of HIV infection, it is reported that 1 billion HIV particles are produced, and as more CD4 T cells become infected and eliminated, there is a progressive decline in CD4 T cells. Although the direct destruction of CD4 T cells by HIV/cytolysis is a cause of CD4 T cell loss, it cannot fully explain CD4 T cell death during the asymptomatic phase.[19]

Another proposed pathogenesis is the "hyperimmune activation hypothesis." There is a high proliferation of CD4 T cells, CD8 T cells, NK cells, and B cells, which is associated with increased activation markers. This hyperimmune activation induces the proliferation of memory T cells. Yates et al. found that activated CD4 T cells have a short lifespan and induce apoptosis.[19]

In general, chronic immune system activation associated with HIV is characterized by high levels of proinflammatory cytokines and chemokines in circulation, including IFNs, IL-6, TGFβ, IL-8, IL-1α, IL-1β, MIP-1α, MIP-1β, and RANTES. Plasma proteins like neopterin, β2-microglobulin, TNF-α, and IFN-γ are also increased. Elevated cytokine production is referred to as a cytokine storm and marks acute and chronic infections, contributing to immune activation and CD4 T cell decline.[19]

During the early stages of infection, the virus disrupts the immune function of the mucosa. The loss of gastrointestinal mucosal integrity and microbial translocation are involved in local inflammation and chronic immune activation, which further contribute to disease progression and CD4 T cell decline.[19,20]

Researchers have debated for years about the causes of CD4 T cell death, whether it is due to apoptosis or necrosis. Pyroptosis, known as an independent cell death pathway until 1992, shares morphological similarities with both apoptosis and necrosis. Pyroptosis is a programmed cell death that specifically depends on caspase-1 activation, leading to cell lysis and the release of cellular contents into surrounding tissues. In 2000, necroptosis was recognized as a cell death pathway. The limited differences between these pathways have posed a challenge for researchers in determining the pathway responsible for HIV pathogenesis.[19,20]

In general, the decline in CD4 T cell numbers is considered a result of apoptosis. However, in recent years, non-apoptotic necrotic cell death known as necroptosis has been found to be involved in the CD4 T cell decline. Pan (2014) reported that the decline in CD4 T cells is not solely due to apoptosis but also involves necroptosis.[4]

Various factors play a role in the decline of CD4 T cell numbers. These factors include direct cytopathic effects of HIV on CD4 T cells and their progenitors, cytotoxicity of cytokines, destruction of

lymphoid tissues, including the thymus gland, induction of apoptosis, pyroptosis, and necroptosis through chronic immune activation. [4,21]

4.3. Primary cell death pathways

The Nomenclature Committee on Cell Death (NCCD) has developed guidelines for the morphology, biochemistry, functional definitions, and interpretation of cell death. The mission of the NCCD is to provide widely accepted nomenclature for cell death to support further advancements in this field. Various programmed cell death programs have been classified based on their biological functions, including intrinsic apoptosis, extrinsic apoptosis, necrosis, and pyroptosis, influenced by mitochondrial permeability transition (MPT).

a. Apoptosis

Apoptosis is a natural process that plays different roles in morphological and biochemical changes. It is also known as programmed cell death (PCD) and can occur through two different pathways: intrinsic and extrinsic apoptosis. Some key characteristics of apoptotic cells include chromatin condensation, DNA fragmentation, exposure of phosphatidylserine on the outer leaflet of the plasma membrane, and the formation of apoptotic bodies. Caspase enzymes are key mediators of apoptosis. Apoptosis is morphologically characterized by membrane blebbing, chromatin condensation, intra-nucleosomal DNA fragmentation, and apoptotic body formation and is mediated by a family of specific cysteine protease enzymes called caspases.

b. Pyroptosis

Piroptosis is a newly discovered programmed cell death, studied in the context of cancer and neurological diseases. Morphologically, piroptosis differs significantly from other cell death mechanisms like apoptosis and necrosis in terms of occurrence and regulatory mechanisms. Piroptosis differs from apoptosis due to its high level of inflammation, involvement in the formation of membrane pores, its lytic nature, and its activation by caspase-1, previously considered an "IL-1β-converting enzyme," unrelated to apoptosis. Furthermore, it differs from other programmed cell deaths, such as necroptosis, because it involves the gasdermin (GSDM) protein family as membrane pore-forming agents. It manifests through the activation of one or more caspases, particularly caspase-1 in humans and mice, caspase-4/5 in humans, and caspase-11 in mice, and can thus be broadly classified into canonical caspase-1-dependent piroptosis and non-canonical caspase-1-dependent piroptosis. However, both types remain morphologically similar.

c. Necroptosis

Necroptosis is a programmed cell death initiated in response to cellular stress, such as infection, inflammation, or trauma. It is characterized by regulated necrosis, where cells undergo controlled destruction, leading to the release of intracellular contents and inflammation.[22]

Necroptosis is triggered by pathogen invasion and mediated by ligand-receptor interactions. Some apoptosis ligands that can induce necroptosis include Fas, TNF, and TRAIL. TLR3 and TLR4 also play similar roles in necroptosis. At the molecular level, necroptosis is mediated by sequential activation of the receptor RIP3 and MLKL. Both factors form a complex known as the necrosome. When TNF/TNFR1 interacts, RIP3 is activated by RIPK1. Active RIPK3 catalyzes the phosphorylation of MLKL, which forms MLKL oligomers that insert into the plasma membrane, forming ion channels and triggering plasma membrane permeabilization.[23]

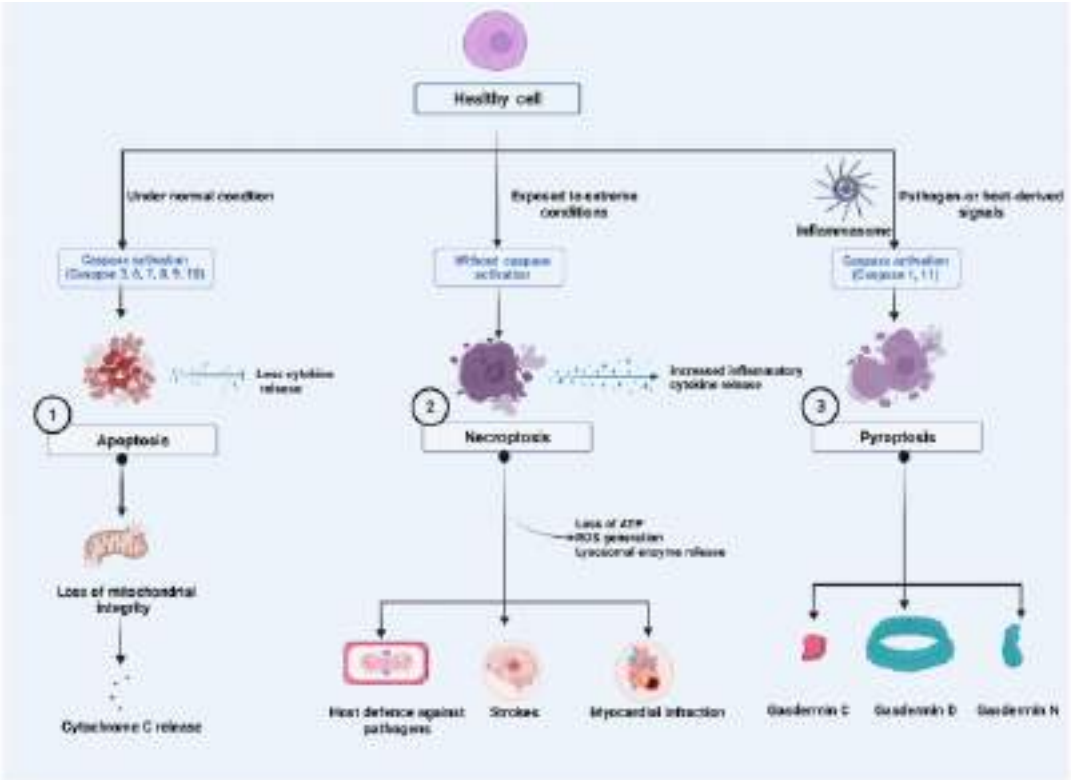

Figure 3. Primary cell death pathways

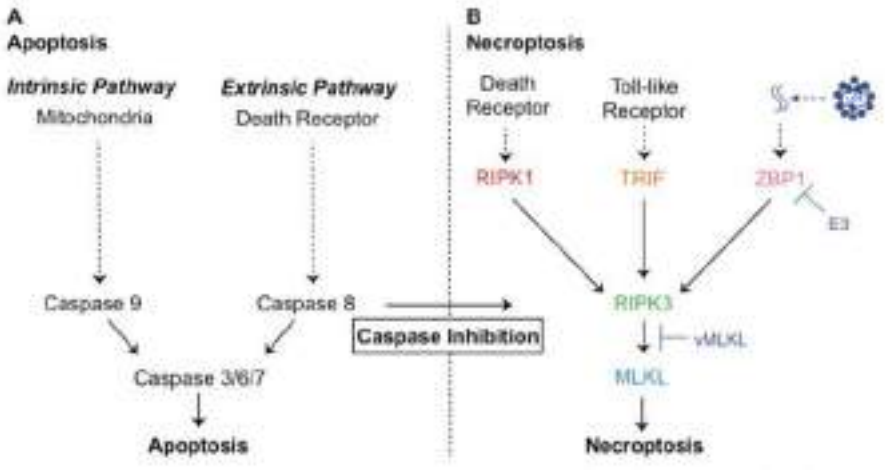

Figure 4. The difference between apoptosis and necroptosis. (A) Apoptosis is a regulated cell death pathway that serves as a key host defense against pathogens, mediated by caspases through either the intrinsic or extrinsic pathway. B. Necroptosis is an alternative cell death pathway triggered by the inhibition of apoptosis death receptors by pathogens.[24]

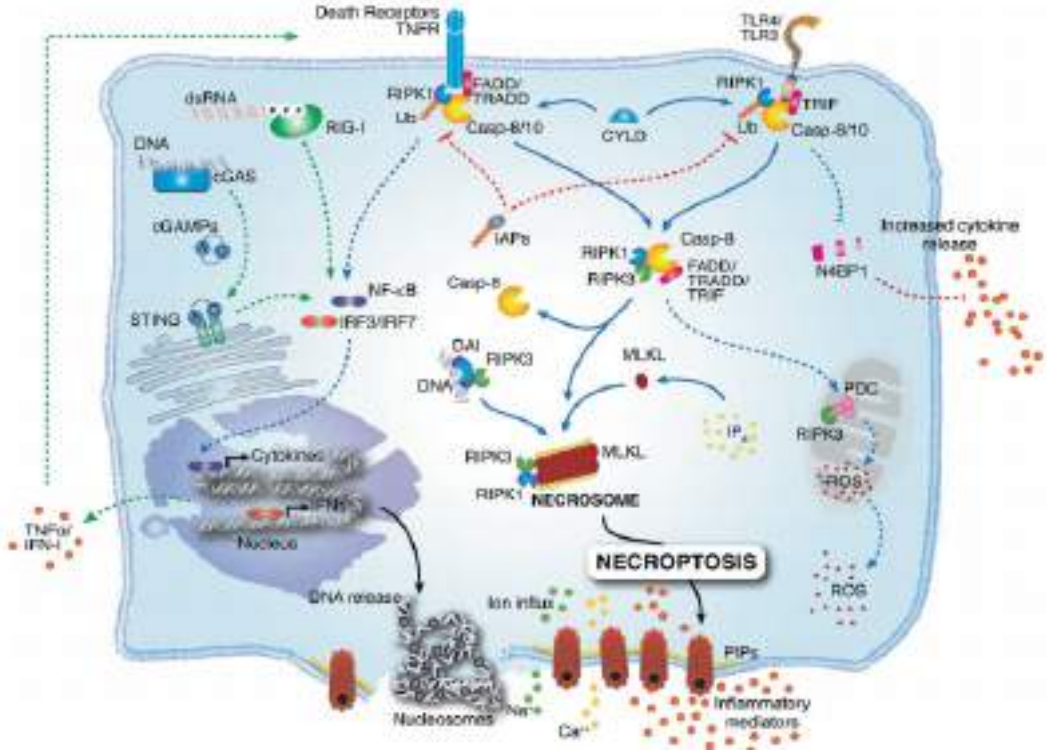

Figure 5. Necroptosis

Table 1. Necroptosis in virus infection

Author	Methods	Results
Zhang et all (2020)[9]	Experimental	Coxsackievirus A6 virus infection induces necroptosis linked to an increase in RIPK3. Necrostatin-1 inhibits CA6 virus production with the ability to inhibit VP1 virus protein and genomic RNA.
Soliman et all. (2021)	Experimental	Transfection of RVA NSP4 induces necroptosis through the RIPK1/RIPK3/MLKL pathway. Inhibition of necroptosis molecules during RVA or S4 infection reduces necroptosis and increases cell viability and apoptosis, resulting in a decrease in virus quantity in RVA-infected cells.
Sharif et all. (2023)[25]	Experimental	Chemical inhibitors that inhibit necroptosis suppress Porcine virus protein expression.
Nogusa et all. (2016) [26]	Experimental	Mice deficient in RIPK3 are more susceptible to influenza A virus infection.
Huang, et all. (2015)[27]	Experimental	HSV-1 induces necroptosis, mainly dependent on RIP1 and completely dependent on RIP3 and MLKL.
Solima, et all. (2021)[28]	Experimental	Anti-RIP1, RIP3, and MLKL therapy reduces Rotavirus A (RVA) virus expression and significantly decreases RVA titer.

4.4. The role of necroptosis in the death process of CD4 cells

HIV infection is characterized by the progressive decline of CD4 T cells and immune system dysfunction. The number of CD4 T cells in the body is maintained constantly by homeostasis mechanisms, which fail during HIV infection, mainly due to apoptosis. In recent years, a non-apoptotic cell death program has started to be studied in many biological and pathological processes. [29]

4.4.1 Mechanism of action and regulation of necroptosis

Necroptosis or programmed necrosis is considered a "trap door" to eliminate pathogens when the caspase system is disrupted. The process of necroptosis involves the activation of specific signaling pathways initiated by the interaction of receptor molecules on the cell surface with ligands, such as TNF-α, even though TNF-α has long been considered an inducer of apoptosis. Activation of other cellular receptors also triggers necroptosis. These receptors include death receptors (e.g., Fas/FasL), toll-

like receptors (TLR4 and TLR3), and cytosolic nucleic acid sensors like RIG-I and STING, which induce type I interferon (IFN-I) and TNFα production, thereby promoting necroptosis.[30]

Most necroptosis pathways trigger proinflammatory and prosurvival signals dependent on NFκB. However, additional inhibition of the proteolytic enzyme Caspase-8 by microbes or pharmacological agents also activates the necroptotic pathway. The subsequent signaling cascade involves the recruitment of RIPK1 into an oligomeric complex that includes FADD, caspase-8, and caspase-10. In the absence of caspase-8 activity, RIPK1 recruits and phosphorylates RIPK3, forming a complex called the ripoptosome. The RIPK1/RIPK3 complex recruits and phosphorylates MLKL, forming the necrosome. Phosphorylation of MLKL induces conformational changes leading to the exposure of the 4-helix bundle domain (4HBD). Early studies speculated that the RIPK1/RIPK3/MLKL pathway induces cell death by destabilizing mitochondria. This effect occurs through the phosphoglycerate mutase 5 (PGAM5) family and dynamin-related protein 1 (DRP1)-dependent pathway. However, in mice with genetic deficiency in PGAM5, DRP1 did not show changes in TNF-induced necroptosis, challenging this theory.[31]

Two theories explain how MLKL compromises cellular integrity: (1) MLKL acts as a platform on the plasma membrane to open calcium or sodium ion channels, allowing ion influx, cell swelling, and rupture, and (2) MLKL itself forms pores in the plasma membrane. Furthermore, MLKL oligomerization and membrane translocation appear to depend on specific inositol phosphate (IP) codes.[31]

Ultimately, these molecular interactions lead to the activation of a series of intracellular signaling events that result in the activation of a protein complex called the necrosome, leading to cell death. Morphologically, necroptosis is characterized by increased cell volume, membrane rupture, organelle swelling, cellular collapse, and the release of cellular contents.[32]

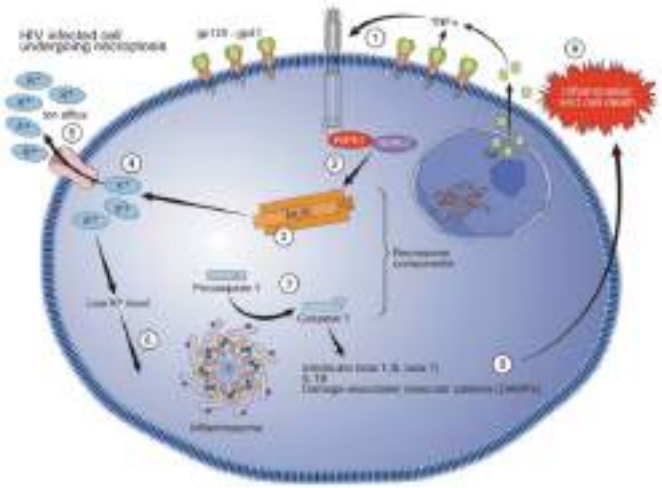

Figure 6. Necrotopsis in HIV

4.4.2 HIV and necroptosis

The Necroptosis pathway begins with the interaction between TNF/TNFR1, followed by RIP3 activation by RIPK1. Active RIPK3 catalyzes the phosphorylation of MLKL, forming MLKL oligomers that insert into the plasma membrane, creating ion channels and triggering plasma permeabilization. Ion disregulation promotes inflammasome formation, which activates caspase-1 and the secretion of IL-1β, IL-18, and DAMP. The end products are inflammatory responses and necrotic cell morphology, characterized by swollen cells, diffuse fragmentation, and membrane integrity loss. Quoted from Ana, et al.[33]

Several studies have attempted to determine whether infected cells also undergo necroptosis in HIV infection.

Table 2. Necroptosis in HIV infection

Author	Methods	Results
Pan et all. (2014)[4]	Experimental	CD4 T cells infected with HIV virus in vitro, underwent flow cytometry examination, experiencing 7.35% necroptosis and 24.72% apoptosis. TNF-alpha plays a crucial role during necroptosis and increases during HIV infection.
Terahara et all . (2020)[29]	Experimental	Necroptosis was found to occur in CD4 T cells induced by HIV virus infection in a mouse model during the early phase of HIV infection.
Campbell GR, et all. (2020)[34]	Experimental	The observed cell death was not related to apoptosis and necroptosis. In this study, treatment with necrostatin-1 resulted in an 88.9% reduction in the death of HIV-infected macrophages.
Zhang G, et all. (2019).[35]	Experimental	The observed death of CD4 T cells in this study was not related to apoptosis, and necrostatin-1 did not affect the death of CD4 T cells.
He, et all (2022)[36]	Experimental	In the SIV model, necroptosis was obtained in CD4 T cells, with only about 0.5% expressing RIP3 protein obtained from blood and tissues.

5. Conclusion

Necroptosis is a programmed cell death initiated in response to cellular stress such as infection, inflammation, or trauma. It is characterized by regulated necrosis, where cells undergo controlled destruction, leading to the release of intracellular contents and inflammation. Cell death through necroptosis differs from cell death through apoptosis, which has been known for a longer time.

The necroptosis pathway begins with the interaction between TNF/TNFR1, followed by the activation of RIP3 by RIPK1. Active RIPK3 catalyzes the phosphorylation of MLKL, forming MLKL oligomers that insert into the plasma membrane, creating ion channels and triggering plasma membrane permeabilization. Necroptosis is characterized by increased cell volume, membrane rupture, organelle swelling, cellular collapse, and the release of cellular contents.

Necroptosis can occur in virus infections that lead to the death of infected cells. In HIV infections, necroptosis has also been observed. Based on experimental research on cells inoculated with the HIV virus, necroptosis was found to occur in HIV-infected CD4 T cells, leading to the death of these cells.

Since current research has been limited to experimental cells, further research is needed to examine the occurrence of necroptosis in HIV/AIDS by directly studying humans as research subjects, in order to gain a clearer understanding of the role of necroptosis in HIV/AIDS.

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Research Articles

Effect of intermittent fasting and moderate physical activity on systolic pressure and creatinine in hypertension patients

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Abstract: Hypertension is a silent killer already causing 10.44 million people to die yearly. Prevalence hypertension based on results Riskesdas 2018 too experienced an increase, as well as in Jambi Province. Studies pre-clinical and clinical intermittent fasting and activity physique is non-pharmacological therapy on hypertension and decline factor risk cardiovascular disease. Study this aims to know the effect of intermittent fasting and activity physique currently on blood systolic And creatinine sufferer hypertension in Jambi City. Study This is a study quasi-experiment with design cross-sectional research. A study was done on as many as 45 patients who suffer from hypertension treated at the Community Health Center in Jambi City and those with no heart, failure kidneys, a history of stroke, and diabetes mellitus. Research sample: Inspect urea And creatinine before And after research, conduct intermittent fasting 2 times a week for 1 month, and measure blood pressure every three days. Respondents did guidance about activity physique with guidance sports and sports videos. The research results show that intermittent fasting and moderate physical activity in hypertensive patients can reduce systolic pressure and systolic blood pressure after intermittent fasting (mean=135.2 + 19.9) compared to before (mean=144.1 + 21.1) (t-test; p=0.001) as well as reducing creatinine levels, after the intermittent fasting diet, the average creatinine level was 0.8 (0.05-1.95) compared to previously 0.9 (0.7-1.58) (t-test; p=0.001). This study concludes that intermittent fasting and moderate physical activity can reduce systolic blood pressure and creatinine in hypertension sufferers.

Keywords: Blood Pressure; Creatinine; Hypertension; Intermittent Fasting; Physical Activity

1. Introduction

According to the World Health Organization data, around 1.13 billion residents worldwide suffer from Hypertension, And the majority experience it in moderate countries. Prevalence hypertension based on results Riskesdas 2018 too experienced enhancement from results Riskesdas 2013, where sufferer hypertension increased from 25.8% to 34.1%. Hypertension is a disease of 10 diseases, most of them are in community health centers in Jambi Province; in 2016, the total sufferers medicated

Hypertension to health centers in Jambi Province was around 13.69%, and in 2020, the total sufferers of Hypertension already reached 23.63%.[1]

Intermittent fasting and activity physique is possible non-pharmacological therapy helps Good in a way studies pre-clinical and clinical Hypertension and factor risk disease cardiovascular.[2] Intermittent fasting, i.e., diet modification with fasting is possible to increase sensitivity from insulin, reduces oxidative stress, cholesterol blood, as well can reduce inflammatory factors.[3] Intermittent Fasting performed in the study: intermittent fasting type 5:2. This type of 5:2 Intermittent Fasting can lower visceral fat and increase adiponectin. Increased visceral fat Also influences the improvement of blood pressure. This 5:2 type of intermittent fasting is a fasting diet done twice a week, Whereas on-moment fasting only gets the intake of as much as 500 calories. The 5:2 type intermittent fasting method can lower visceral fat and increase adiponectin, namely intermittent fasting type 5:2, where done fast 2 times a week. During fasting, only consume food as much as 500 calories.[4] Research about the impact of intermittent fasting on humans was done by Rynders in 2019, which obtained results that heavy body as much as 3.8%.[5]

Moderate Physical Activity, i.e., the moment you do an activity physique, the body will experience a little sweating, enhanced pulse heart, and frequency more breath, but still can talk. The energy released during the activity physique is currently 3.5 up to 7 Kcal/ minute.[6] Based on the research, it is known that activity physique light is a factor risk of hypertension. Activity physique is easy to do somebody cause frequency heart taller because muscle heart work harder moment contraction heart.[7] Research this objective is to know the current effect of intermittent fasting and activity physique on pressure systolic and creatinine levels in patients with hypertension.

2. Methods

Study this is a quasi-experimental with design study, i.e., Cross-Sectional as place study, i.e., carried out at the Jambi City Health Center as place inspection pressure blood and do guidance activity physique currently and Laboratory Emerald Jambi for inspection rate creatinine. Study done from March 2023 to month July 2023. Inclusion criteria for patients, namely sufferers of HypertensionHypertension who are treated at the Jambi City Health Center and are willing to follow the instructions on research and exclusion criteria, namely not participating in activities until the completion of the research and having a history of other previous illnesses, i.e., suffer disease heart; experience fails kidneys, history of stroke and experience diabetes mellitus. Participants study intermittent fasting twice a week, i.e., Monday and Thursday, as well as moment fast consume as much as 500 calories, blood pressure checks every 3 days, creatinine assessment before and after the study, and members of an exercise video. Activity physique currently and guidance in doing sport.

3. Results and Discussion

This research was conducted on 45 hypertensive patients willing to follow an intermittent fasting diet and moderate physical activity. The patient sample consisted of 40 female and 5 male hypertensive patients.

3.1. Systolic blood pressure

Blood pressure checks are carried out on hypertensive patients every three days. The average value of systolic blood pressure for hypertensive patients who follow an intermittent fasting diet and moderate physical activity is as follows;

Table 1. Systolic blood pressure

		Mean (mmHg)	Difference	CI 95%	*p*-value *
Systolic	Pre	144.1 ± 21.1	-9.2 ± 17.3	4.04 - 14.41	0.001
	Post	135.2 ± 19.9			

*T-Test

In hypertensive patients who follow an intermittent fasting diet and moderate physical activity for 1 month, it is known that there is a decrease in the average systolic blood pressure in these hypertensive patients. Based on the T-test, it is known that there is a significant relationship between systolic blood pressure before and after intermittent fasting diet treatment and moderate physical activity. Intermittent fasting is known to reduce the risk of cardiovascular disease. High blood pressure is a marker of cardiovascular disease.[8] In the same research, it is known that alternative day fasting carried out for 8 to 12 weeks can reduce systolic blood pressure.[9] Another study conducted on a 6-month intermittent fasting diet type 5:2 can also reduce systolic and diastolic blood pressure.[10]

3.2. Creatinine levels

In hypertensive patients who follow an intermittent fasting diet and moderate physical activity, creatinine is checked before and after treatment. Creatinine assessment is carried out to determine kidney function in hypertensive patients. The creatinine results for hypertensive patients who follow an intermittent fasting diet and moderate physical activity are as follows;

Table 2. Creatinine levels of hypertensive patients before and after intermittent fasting diet

		Average	Min-Max	*P - value* *
Creatinine	Pre	0.9	0.7 - 1.58	< 0.001
	Post	0.8	0.52 - 1.95	

*Wil coxon test

In this study, it was found that there was a decrease in the average creatinine value before and after doing an intermittent fasting diet and moderate physical activity in hypertensive patients. Based on the Wil Coxon rank test, it is also known that there is a significant difference in the average before and after the intermittent fasting diet and moderate physical activity in hypertensive patients. Creatinine examination is one of the parameters of kidney function because the concentration of creatinine in plasma and its excretion in urine is relatively constant for 24 hours.[11] Kidney damage is one of the complications that occurs in hypertensive patients, which is why creatinine measurements were carried out in this research sample. The same results are known from research on Ramadan fasting by patients with stage II-IV chronic kidney failure who experienced moderate improvements in creatinine values.[12] Another study by Abdullah, 2023, revealed increased creatinine in patients with chronic kidney failure who fasted during Ramadan. Increased creatinine may occur due to the patient's lack of compliance in taking medication and lack of fluids during fasting during the day.[13]

4. Conclusion

This research concludes that Intermittent Fasting and Moderate Physical Activity in Hypertension Sufferers can reduce systolic blood pressure and blood creatinine levels.

5. Acknowledgements

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Research Article

The effect of Dawood's fast on body mass index, waist circumference and waist-to-hip ratio in students at PondokPesantren Hidayatullah Surabaya

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Abstract: Obesity is a major risk factor associated with various diseases. One of the managements of obesity is anthropometric assessment for screening and reducing calorie intake. Several studies have shown that the Ramadan fast significantly reduces BMI, waist circumference, and waist-to-hip ratio, whereas Dawood's fast is still rarely studied. Therefore, this study aims to examine the effect of Dawood's fasting on body mass index (BMI), waist circumference (WC), and waist-to-hip ratio (WHR) in a group of students at the Hidayatullah Islamic Boarding School, Surabaya. This research is quasi-experimental research with pre-test and post-test design. Subjects consisted of 36 people and were divided into two groups, namely the fasting group (18) and the control group (18). The Dawood's fast was observed for six consecutive weeks. Anthropometric measurements were carried out before and after fasting. Statistical data analysis used the Wilcoxon signed rank test and the Mann-Whitney test after analyzing the data for normality and homogeneity. Based on statistical tests, the difference in BMI between the fasting and control groups was not significantly different (p=0.601). The difference in WC between the two groups was not significantly different (p=0.599). The difference in WHR between the two groups was not significantly different (p=0.861). The conclusion is that there are no significant differences in BMI, WC, and WHR between the fasting and control groups in young adults who do Dawood's fast.

Keywords: body mass index; Dawood's fast; intermittent fasting; waist circumference; waist-hip circumference ratio

1. Introduction

The World Health Organization states that obesity is a world health problem. Obesity causes an increase in morbidity and mortality rates in developed and developing countries. Obesity is a risk factor for coronary heart disease, diabetes mellitus, stroke, metabolic syndrome, musculoskeletal disorders, cancer, etc. Currently, the prevalence of obesity continues to increase worldwide from year to year. According to WHO, in 2016, more than 1.9 billion adults (> 18 years) were overweight or obese. More than 650 million of them are obese. In children and adolescents,

more than 340 million are overweight or obese.[1] According to Basic Health Research, in 2010, nutritional problems in the adult population (> 18 years) in Indonesia, including 12.6% were underweight and 21.7% in the combined category of overweight and obesity.[2] The prevalence of obesity in men is 16.3% and women are 26.9%. The prevalence of obesity tends to be higher in urban areas, in the adult population with higher education and working as civil servants/military/police/guardians. Obesity management can include obesity screening, reducing energy intake, and increasing energy expenditure. Obesity screening with anthropometry is important as an early detection of a person's health status. The anthropometric measurements include the BMI, WC, and WHR.[3] Fasting is a way of limiting energy intake for a certain period of time. When fasting, the body will metabolize fat accumulated in the body as energy fuel. In addition, fasting can reduce levels of bad cholesterol in the body which causes dangerous diseases. Indonesia is a country with the largest Muslim population in the world. Muslims often observe fasting. The most commonly observed fast is the Ramadan fast, which is a mandatory fast for Muslims. Research on Dawood's fasting is still rare. Based on this background, the researchers wanted to know the effect of Dawood's fasting on the BMI, WC, and WHR of Islamic boarding school students at Hidayatullah Surabaya.

The general objective of this study was to determine the effect of Dawood's fasting on the BMI, WC, and WHR of Islamic boarding school students at Hidayatullah Surabaya. The specific objective of the study was to analyze the effect of Dawood's fasting on the BMI, WC, and WHR of Islamic boarding school students at Hidayatullah Surabaya.

2. Methods

This study was an experimental design with pre-test and post-test with a control group, to assess body mass index (BMI), waist circumference (WC), and waist-hip ratio (WHR) for students at Hidayatullah Islamic Boarding School who fasted Dawood for six consecutive weeks and who did not fast Dawood. The population in this study were male students aged between 17 and 30 years at the Hidayatullah Islamic Boarding School, Surabaya. The sample in this study was male students aged 17 to 30 years who met the inclusion criteria. The sampling procedure in this study used a non-probability sampling technique, namely purposive sampling until the number of samples that met the inclusion criteria was met, then used a probability sampling technique, namely simple random sampling, to determine whether the research sample was included in the treatment group or control group. The treatment group is the sample group that is willing and able to undergo Dawood's fast for six consecutive weeks. The control group is a sample group that does not undergo Dawood's fasting for six consecutive weeks.

The inclusion criteria for this study include (1) Muslim; (2) Male; (3) Age between 17 to 30 years; (4) Willing and able to fast Dawood for six consecutive weeks; (5) Healthy, with no history of metabolic diseases such as diabetes mellitus as assessed by filling out a medical history questionnaire, normal random blood glucose laboratory tests (<200 mg/dl) and no history of anemia as assessed by normal hemoglobin levels (male: 13 .5 g/dl -17.5 g/dl) using the Easy touch GcHb tool; (6) Not taking drugs to reduce or increase weight; (7) Willing to become a research sample by signing an informed consent sheet after being explained the benefits, objectives and procedures of this study. Respondents' exclusion criteria included: (1) Having a history of metabolic diseases such as diabetes mellitus and anemia; (2). Not willing to be a research sample. The criteria for dropping out of respondents in this study included: (1) The research sample did not follow the research procedure in an orderly manner; (2) The research sample withdrew during the research; (3) Sick so that he stopped doing Dawood's fast; (4) lost to follow up.

Determining the sample size in this study used the Higgins Kleinbaum formula so that it was found that the sample needed was 13 and added a correction factor of 30% to anticipate respondents dropping out. The minimum sample size was 13 people for each group and added with a correction factor of 30% to 17 people. for each group with a total sample of at least 34 people. The sampling technique in this study used non-probability sampling, namely purposive sampling until the number of samples that met the inclusion criteria was met, then using probability sampling techniques, namely simple random sampling, to determine whether the research sample was included in the treatment group or control group. The treatment group is the sample group that is willing and able to undergo David's fast for six consecutive weeks. The control group is a sample group that does not undergo David's fasting for six consecutive weeks.

The research was conducted at the Islamic boarding school Hidayatullah Surabaya, Jalan Kejawan Putih Tambak VI/1 Keputih Sukolilo Surabaya 60111 and the Clinical Pathology Laboratory of RSUD Dr. Soetomo Surabaya in September 2019 to November 2019. The procedure for collecting data on height, weight, waist circumference, and hip circumference was carried out before doing the Dawood's fast and at the beginning of the 7th week after completing the Dawood's fast. The data were tested for normality first using the Shapiro-Wilk test because the number of samples was ≤ 50. Normal data distribution if $p > 0.05$. If the data is normally distributed, the analysis is carried out using the paired t-test, while the data distribution is not normal, the Wilcoxon signed rank test is used to test the differences in BMI, WC, and WHR values before and after treatment in the two groups. Then, if the distribution is not normal, an analysis is carried out with the Mann-Whitney test and the independent test on normally distributed data to

determine differences in changes in BMI, WC, and WHR values in the two groups. Data were analyzed using statistical analysis software.

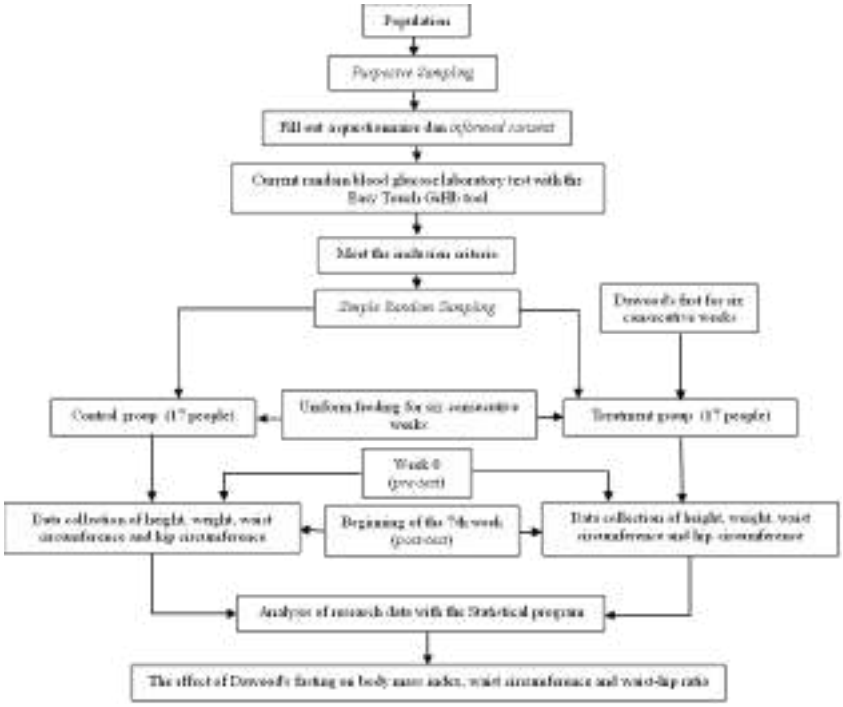

Figure 1. The operational framework of the research

3. Results and Discussion

The research was conducted at the Hidayatullah Islamic Boarding School, Surabaya. The research subjects obtained through purposive sampling were 47 people but as many as six people met the exclusion criteria and five people dropped out so the population obtained was 36 people who were divided into 2 groups, namely the fasting group of 18 people and the control group of 18 people.

3.1. The respondent's characteristics

Based on the data below in Table 1, it is known that the percentage of respondents in the age group of 18 - 20 years is found to be higher in the control group (50.0%) compared to the fasting group. The percentage of respondents in the age group 21 - 24 years was found to be higher in the fasting group (61.1%) than in the control group. The greatest percentage of hemoglobin levels 13,9 g/dL – 1,9 g/dL was found in the fasting group (66,7%) while hemoglobin levels of 16,0 g/dL – 18,3 g/dL were found more in the control group (72,2%). Random blood glucose percentages of 71 mg/dL - 99 mg/dL were more common in the fasting group (33,3%). Random blood glucose levels of 102 mg/dL -119 mg/dL were more common in the fasting group (61,1%). Random blood glucose levels of 124 mg/dL -160 mg/dL were more common in the control group (44,4%). Based on the

independent t-test using Levene's test method, there was no significant difference in age, Hb levels, and random blood glucose (RBG) levels between the fasting group and the control group with a p > 0,05. This shows that the initial state of the research subjects was homogeneous.

Table 1. Respondent's characteristics based on age, Hb levels, and RBG

Respondent's characteristics	Fasting treatment group		Control groups		P
	N	%	N	%	
Age					
• 18-20 years old	7	38,9%	9	50,0%	0,791
• 21-24 years old	11	61,1%	9	50,0%	
Hemoglobin levels					
• 13,9 g/dl - 15,9 g/dl	12	66,7%	5	27,8%	1,117
• 16,0 g/dl - 18,3 g/dl	6	33,3%	13	72,2%	
Blood glucose level					
• 71 mg/dl - 99 mg/dl	6	33,3%	4	22,2%	2,899
• 102 mg/dl - 119 mg/dl	11	61,1%	6	33,3%	
• 124 mg/dl - 160 mg/dl	1	5,6%	8	44,4%	
Total	**18**	**100%**	**18**	**100%**	

3.2. Baseline data on the fasting group and the control group

BMI, WC, and WHR measurements in both groups were carried out twice before and after Dawood's fast. The results of measurements of BMI, WC, and WHR are presented in the following figure and table.

Table 2. Pre-test and post-test BMI

BMI category	Fasting treatment group		Control group		Fasting treatment group		Control group	
	N	%	N	%	N	%	N	%
Underweight	8	44,4%	6	33,3%	5	27,8%	1	5,6%
Normal	8	44,4%	11	61,1%	11	61,1%	16	88,9%
Overweight	1	5,6%	1	5,6%	*1*	5,6%	1	5,6%
Obesity	1	5,6%	-	-	*1*	5,6%	-	-

Based on Figure 2 and Table 2, it is known that before Dawood's fasting study began the majority of the fasting group's BMI was underweight and normal (44.4%) while in the control group, the majority was normal (61.1%). After the study was completed, the BMI increased of the fasting group (61.1%) and the control group (88.9%) were mostly normal.

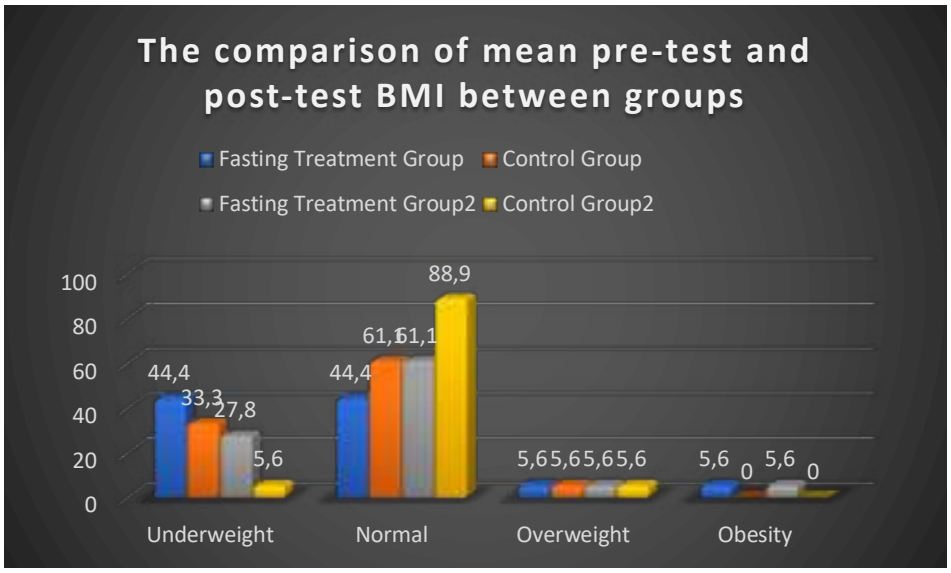

Figure 2. Figure comparison of mean pre-test and post-test BMI between groups

Table 3. Pre-test and post-test WC

WC category	Pre-Test				Post-test			
	Fasting treatment group		Control group		Fasting treatment group		Control group	
	N	%	N	%	N	%	N	%
Normal	18	100%	*18*	100%	*18*	100%	18	100%
Obesity	-	-	-	-	-	-	-	-

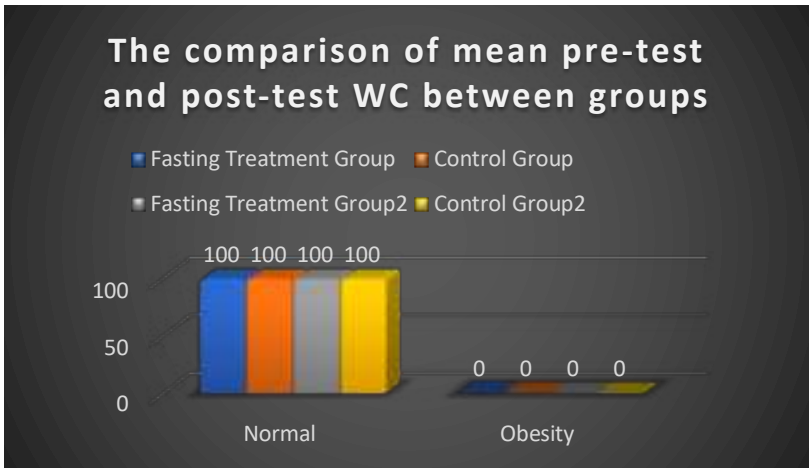

Figure 3. Figure comparison of mean pre-test and post-test WHR between groups

Based on Figure 3 and Table 3, it is known that before Dawood's fasting study began the waist circumference of the fasting group (100%) and the control group (100%) were normal. And after the study was completed the waist circumference of the fasting group (100%) and the control group (100%) remained within normal limits.

Table 4. Pre-test and post-test WHR

WHR Category	Pre Test				Post-test			
	Fasting treatment group		Control group		Fasting treatment group		Control group	
	N	%	N	%	N	%	N	%
Normal	16	88,9%	17	94,4%	12	66,7%	15	83,3%
Obesity	2	11,1%	1	5,6%	6	33,3	3	16,7%

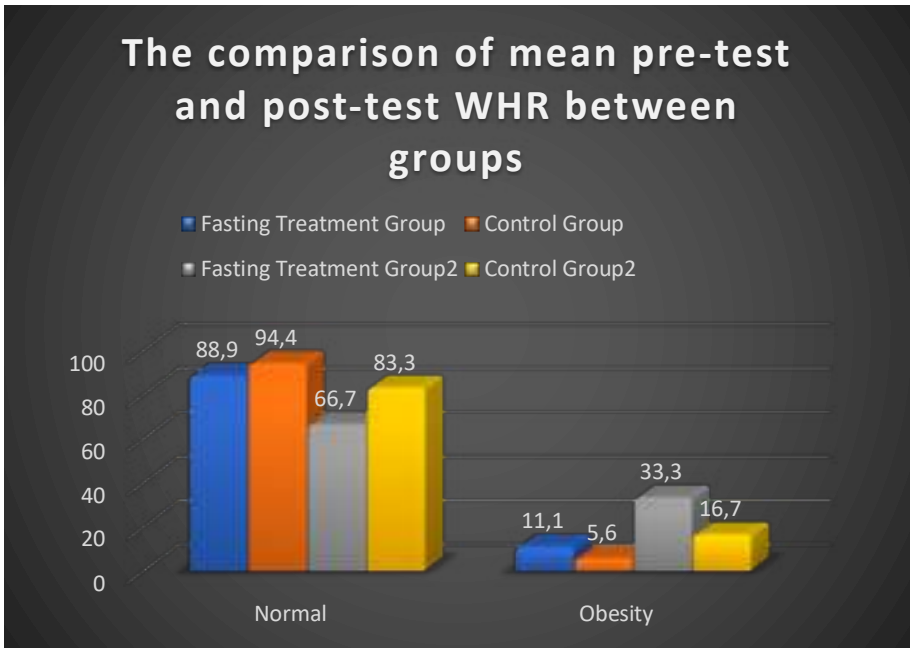

Figure 4. Figure comparison of mean pre-test and post-test WHR between groups

Based on Figures 4 and Table 4, it is known that before Dawood's fasting study began, the waist-to-hip ratio of the fasting group (88.9%) and the control group (94.4%) were mostly normal. After the study was completed, the waist-to-hip ratio of the fasting group (66.7%) and the control group (83.3%) remained normal, however, there was an increase in obesity RLPP in the fasting group (33.3%) and control group (16,7%).

3.3. Descriptive analysis, normality test, and Levene's test

Descriptive analysis

Univariate analysis was carried out on each group to determine the average, standard deviation, minimum, and maximum values. Based on the data presented in Table 8 above, it is known that before Dawood's fasting treatment (pre-test), the average BMI in the fasting group was 19,81 while the average in the control group was 19,54. The highest BMI was in the fasting group, namely 31,2, while the lowest was in the control group, namely 15,8. The average WC in the fasting group was

55,55 while in the control group, it was 54,66. The largest WC was in the fasting group which was 76 while the smallest in the control group was 47. The mean WHR in the fasting group was 0.81 while in the control group, it was 0,80. The largest WHR was found in the fasting group, which was 0,95, while the smallest was found in the control group, which was 0,72.

Table 5. Results of descriptive analysis of before and after fasting treatment

Parameters	Descriptive analysis	Pre-Test		Post-test	
		Fasting Treatment Group	Control Group	Fasting Treatment Group	Control Group
Body Mass Index (BMI)	Mean	19,81	19,54	21,02	20,45
	Standard deviation	3,65	2,51	4	2,31
	Minimum	16,5	15,8	17	17,2
	Maximum	31,2	27,2	32,60	27,2
Waist circumference (WC)	Mean	55,55	54,66	68,22	68,77
	Standard deviation	7,62	5,29	9,26	5,88
	Minimum	49	47	49	63
	Maximum	76	66	97	87
Waist-hip ratio (WHR)	Mean	0,81	0,8	0,85	0,84
	Standard deviation	0,05	0,05	0,06	0,09
	Minimum	0,74	0,72	0,75	0,77
	Maximum	**0,95**	**0,93**	**0,95**	**1,17**

Data collected after Dawood's fasting treatment (post-test) showed that the average BMI of the fasting group was 21,02, while the control group's average was 20,45. The highest BMI in the fasting group was 32,60, and the lowest was 17. The average WC in the fasting group was 68,22, whereas in the control group, it was 68,77. The largest WC was found in the fasting group, measuring 97, while the smallest was also in the fasting group, measuring 49. The mean WHR was 0,85 in the fasting group and 0,84 in the control group. The control group had the highest WHR, which was 1,17, while the lowest was found in the fasting group, measuring 0,75.

Data normality test

Data were tested for normality using the Shapiro-Wilk test because the total sample was <50. The statistical normality test results for BMI, WC, and WHR are presented in the table 6.

Based on Table 6, it can be seen that before Dawood's fasting treatment BMI significance of the fasting group (p = 0,000) and the control group (p = 0,007) so that the data is not normally distributed. The significance of WC in the fasting group was p = 0,000 so the data were not normally distributed, while p > α was in the control group so the data were normally distributed. Significance of WHR in the fasting and control groups, p > α so that the data is normally distributed. After Dawood's fasting treatment, the significance of BMI in the fasting group (p = 0,002) and control group (p = 0,014) can be seen so that the data is not normally distributed. The

significance of WC in the fasting and control groups was p<α so the data were not normally distributed. The significance of WHR in the fasting group was p>α so the data was normally distributed, whereas in the control group, it was p < α so the data was not normally distributed.

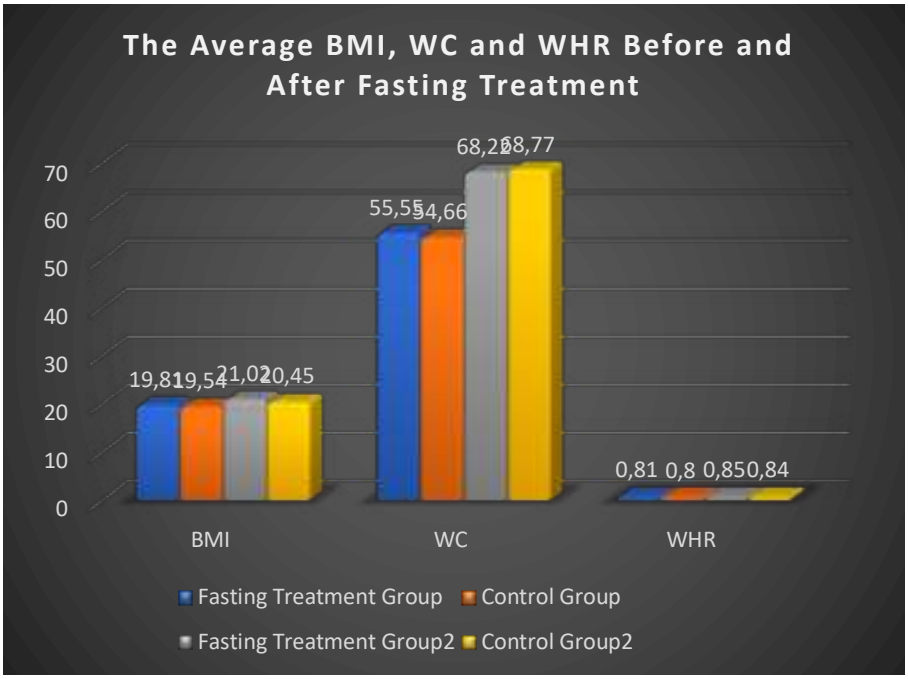

Figure 5. Figure comparison of mean pre-test and post-test WHR between groups

Table 6. Data normality test results before fasting

	Groups	Pre-Test			Post-Test		
		Statistic	Df	Sig.	Statistic	Df	Sig.
BMI	Fasting treatment group	.748	18	.000	.813	18	.002
	Control group	.843	18	.007	.863	18	.014
WC	Fasting treatment group	.696	18	.000	.801	18	.002
	Control group	.901	18	.060	.821	18	.003
WHR	Fasting treatment group	.920	18	.129	.950	18	.431
	Control group	.972	18	.832	.714	18	.000

Table 7. The BMI, WC, and WHR difference normality test results pre-test and post-test

Parameters	Groups	Shapiro-Wilk		
		Statistic	df	Sig.
BMI difference pre and post-test	Fasting treatment group	.891	18	.039
	Control group	.798	18	.001
WC difference pre and post-test	Fasting treatment group	.835	18	.005
	Control group	.959	18	.578
WHR difference pre and post-test	Fasting treatment group	.867	18	.016
	Control group	.924	18	.151

Based on the data above, it is known that the significance of the difference in BMI in the fasting and control groups is p <0.05 so the data is not normally distributed. Then the difference in WC in the fasting group was p < 0.05 so the data was not normally distributed. The significance of the difference in WC in the control group is p > 0.05 so the data is normally distributed. The significance of the WHR difference in the fasting group was p < 0.05 so the data was not normally distributed, while the difference in the WHR in the control group was p > 0.05 so the data was normally distributed.

Data homogeneity test (Levene's test)

The results of the data homogeneity test using Levene's test method are presented in the following table.

Table 8. WHR homogeneity test results before fasting

		Levene's Statistic	df2	df1	Sig.
WHR	Based on Mean	.034	1	34	.855

3.4. Differences in data before and after intervention

The data were not normally distributed so the Wilcoxon signed-rank test was used to test the differences in BMI, WC, and WHR values before and after treatment in both groups, while the Mann-Whitney test was used to determine differences in changes in BMI, WC, and WHR values in the two groups. The statistical test results are presented in the following table.

Table 9. Differences in BMI, WC, and WHR before and after intervention

Groups	BMI		Difference in BMI	P^a	P^b
	Pre-test	Post-test			
Fasting Treatment Group **(Mean ± SD)**	19,81 ± 3,65	21,02 ± 4	1,21 ± 1,20	0,001	0,601
Control Group **(Mean ± SD)**	19,54 ± 2,51	20,45 ± 2,31	0,90 ± 1,59	0,010	
	WC				
	Pre-test	**Post-test**	**Difference in WC**	**P^a**	**P^b**
Fasting Treatment Group **(Mean ± SD)**	55,55 ± 7,62	68,22 ± 9,26	12,66 ± 5,97	0,000	0,599
Control Group **(Mean ± SD)**	54,66 ± 5,29	68,77 ± 5,88	14,11 ± 3,46	0,000	
	WHR				
	Pre-test	**Post-test**	**Difference in WHR**	**P^a**	**P^b**
Fasting Treatment Group **(Mean ± SD)**	0,81 ± 0,05	0,85 ± 0,06	0,04 ± 0,05	0,025	0,861
Control Group **(Mean ± SD)**	0,8 ± 0,05	0,84 ± 0,09	0,04 ± 0,10	0,107	

Note: [a] = Wilcoxon signed-rank test, [b] = Mann-whitney test

Based on Table 9, it is known that the treatment group experienced a significant increase in BMI values (p = 0,001) after fasting Dawood for six consecutive weeks, namely 1,21 ± 1,20, while the control group also experienced a significant increase in BMI values (p = 0,010). namely 0,90 ± 1,59. Based on the Mann-Whitney test, there was no significant difference in changes in BMI values (p = 0,601) between the treatment and control groups. The WC variable shows that the treatment group experienced a significant increase in WC values (p=0,000) after fasting Dawood for six consecutive weeks, namely 12,66 ± 5,97, while the control group also experienced a significant increase in WC values (p = 0,000), namely of 14,11 ± 3,46. Based on the Mann-Whitney test, there was no significant difference in changes in WC values (p = 0,599) between the treatment and control groups. The WHR variable showed that the treatment group experienced a significant increase (p = 0,025) after Dawood's fast for six consecutive weeks, namely 0,04 ± 0,05, while the control group did not experience a significant difference in WHR values (p = 0,107). Based on the Mann-Whitney test, there was no significant difference in changes in WHR values (p = 0,861) between the treatment and control groups.

3.5. Discussion

Before treatment, most subjects in the fasting group were underweight and normal (44,4%) based on BMI values, normally based on WC values (100%), and WHR (88,9%) while in the control group, most were normal based on BMI values (61,1%), WC (100%), and WHR (94,4%). After Dawood's fast, there was a change in percentage with the subjects in the fasting group being mostly normal based on BMI values (61,1%), WC (100%), and WHR (66,7%), while in the control group, most were normal based on BMI values (88,9%), WC (100%), and WHR (83,3%). At first, the BMI of the fasting group was mostly in the underweight (44,4%) and normal (44.4%) categories of eight people each, but after Dawood's fast, the BMI category of research subjects was mostly normal (61,1%) for eleven people. Initially, the BMI of the control group was mostly in the normal category (61,1%) as many as 11 people. After Dawood's fast, the number of subjects in the normal category increased (88,9%) by 16 people. Before the study, WC was in the normal category in both groups (100%). After Dawood’s Fast, WC in both groups remained in the normal category (100%). Before treatment, there were two obese WHRs in the fasting group and one person in the control group. After treatment, the number of individuals with obese WHR increased in the fasting group by six people and in the control group by three people. This could be because although the frequency of meals decreased during Dawood's fast, the main meals, desserts and snacks may have been consumed more because the day before had fasted. The increase in the BMI and WHR categories which both occurred in the two groups could occur because even though the feeding

was uniform for all research subjects, the portion of each individual's meal varied depending on the physical activity undertaken, psychological, etc.

Descriptive analysis was carried out on each group to determine the average, standard deviation, minimum and maximum value. The results of the descriptive analysis before starting Dawood's fasting study showed that the average BMI in the fasting group was 19,81 while the average in the control group was 19,54. The highest BMI was in the fasting group, namely 31,2, while the lowest body mass index was in the control group, namely 15,8. The average WC in the fasting group was 55,55 while in the control group, it was 54,66. The largest WC was in the fasting group which was 76 while the smallest in the control group was 47. The average WHR in the fasting group was 0,81 while in the control group, it was 0,80. The largest WHR ratio was in the fasting group, namely 0.95, while the smallest was in the control group, namely 0,72. The results of the descriptive analysis after fasting of Dawood found that the average BMI in the fasting group was 21,02 while the average in the control group was 20,45. The highest BMI was 32.60 and the lowest was 15.8 in the fasting group. The average WC in the fasting group was 68,22 while in the control group, it was 68,77. The largest WC was in the fasting group, namely 97 and the smallest, namely 49, was in the fasting group. The average WHR in the fasting group was 0,85 while in the control group, it was 0,84. The largest WHR was found in the control group, which was 1,17, while the smallest was found in the fasting group, which was 0,75.

The results of the data normality test before starting Dawood's fast showed that BMI and WC in both groups had abnormal data distribution ($p < 0.05$) while WHR in both groups had normal data distribution ($p > 0.05$). The results of the normality test after Dawood's fast showed that BMI, WC, and WHR in both groups had abnormal data distribution ($p < 0.05$). The normality test results of the difference between BMI, WC, and WHR data in the two groups had an abnormal data distribution ($p < 0.05$). The results of the homogeneity statistical analysis showed that there was no significant difference in the variable WHR between the two groups, the difference in BMI, the difference in WC, and the difference in WHR ($p > 0.05$).

The data were not normally distributed so the Wilcoxon signed-rank test was used to test the differences in BMI, WC, and WHR values before and after treatment in both groups, while the Mann-Whitney test was used to determine differences in changes in BMI, WC and WHR values in the two groups. The results of the Wilcoxon signed-rank test showed that there was an increase in BMI and WC values between the two groups and the WHR variable in the fasting group ($p<0.05$). Then, a Mann-Whitney test was performed to determine the difference in BMI and WC increases in the fasting and control groups and found no significant differences between BMI, WC and WHR

in the fasting and control groups (p>0.05).

This is in line with research conducted by Yucel, et al., (2004) which confirmed that there were no significant differences in BMI, WC and WHR in research subjects (p > 0.05).[4] Changes in BMI, WC, and WHR which were not significant between the two groups could occur because even though the frequency of eating decreased during Dawood's fast, more main meals and desserts were consumed when breaking the fast because the day before the body did not receive any food intake at all so the incoming calories became uncontrollable.[5] Initially, the weight may drop at the beginning of Dawood's fast. This can happen because when a person is fasting, he no longer gets energy intake. The remaining blood glucose will be synthesized as an energy source. When it runs out, the process of glycogenolysis is initiated, namely the breakdown of glycogen into glucose as the body's energy source. Glycogen reserves in the liver and muscles are only sufficient to meet basal energy requirements for a few hours or less than a day. Therefore, the initiation of the glycogenolysis process coincides with the process of gluconeogenesis, namely the formation of glucose from raw materials other than carbohydrates such as fat, through the process of lipolysis in the liver. Another advantage of the liposis process is detoxification, namely fat-soluble toxic substances are degraded. However, when the fat reserves are depleted, protein will be metabolized as an energy source so that the body's physical activity will be slightly disturbed.[6] This can reduce the risk of obesity-related diseases such as heart disease, metabolic syndrome, hypertension, etc.

But in the future, if the excessive eating pattern when breaking the fast is not controlled, the weight will continue to increase to become uncontrollable. Many studies have been conducted on the effects of fasting on anthropometric indices, but the results still vary. This is caused by the characteristics of the respondents, such as age, gender, physical activity, and geographical differences that determine the duration of fasting in several countries.[7]

4. Conclusion

Statistically, there was no significant difference between the BMI of the group that did Dawood's fast compared to the control group that did not do Dawood's fast. However, there was increased BMI from the underweight group to become normal group either in the fasting treatment group or the control group.

Statistically, there was no significant difference between the waist circumference of the group that did Dawood's fast compared to the control group that didn't do Dawood's fast. However, there was increased WC from the underweight group to become normal group either in the fasting treatment group or the control group.

Statistically, there was no significant difference between the ratio of waist and hip circumference in the group that did Dawood's fast compared to the control group that did not do Dawood's fast. However, there was increased WHR from the underweight group to become a normal group in the fasting treatment group.

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Literature Review

Understanding the relationship between albumin levels and the frailty risk in elderly people

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Abstract: Frailty is an emerging public health priority recognized by the World Health Organization. It is a multifaceted geriatric syndrome characterized by decreased physiological reserve, increased susceptibility to stresses, and a higher likelihood of unfavorable health outcomes. It's been observed that the decline of albumin levels has been associated with the advancement of frailty. This review aims to understand the correlation between frailty and albumin levels in the geriatric population. The result of this review reveals that low albumin levels have a strong association with increased frailty risk in geriatric individuals. Inflammation, malnutrition, and impaired protein synthesis were observed as potential underlying conditions that might be the causes of this connection. Low albumin levels are also seen as a potential prognostic marker for frailty-related health outcomes. In conclusion, thisreview emphasizes the relevance of serum albumin levels in assessing frailty risk in the geriatric population. It also recognizes the role of serum albumin as a potential biomarker and therapeutic target for intervention to improve health outcomes and quality of life for older individuals. Further research is still needed to establish the causality and strategies to assuage frailty and its complications in the geriatric population.

Keywords: albumin; frailty; geriatric

1. Introduction

As a country, Indonesia had shown an increasing growth in ageing population. By, 2021, it is said that one out of ten individuals in Indonesia are of the geriatric age group. The data also suggests that although the country has a higher number of ageing individuals, they are also suffering from numerous health problems, with as many as one out of five elderly persons suffering from various illness each month.[1]

The most problematic downside of having an aging population is frailty. Frailty is a result of the cumulative decrease in numerous physiological systems throughout a lifetime. It is a condition of susceptibility to poor resolution of homeostasis following a stressor event.[2] In 2016, the WHO Consortium of Healthy Ageing in Geneva directed its attention towards the topic of frailty. It sheds a

demand toward well-executed research, especially in low- and middle-income nations, to comprehend the factors contributing to frailty.[3]

In the general population, and especially in older people, low blood albumin levels, or hypoalbuminemia, are associated with increased all-cause mortality. Increased risk in individuals with acute illnesses, frailty, cognitive decline, and a lack of trace elements are all connected with this syndrome.[4] Frail older people are more likely to be malnourished and thus having a lower blood level of albumin.[5] Many study has emphasizes the strong correlation between frailty and a spectrum of progressively detrimental health outcomes, encompassing worsening disability, increased falls, higher hospitalization rates, elevated mortality risk, and a heightened probability of requiring admission to long-term care.[2]

Given the significance of the impact frailty and albumin levels can have on an elderly person's health outcome. Understanding the complex relationship between albumin levels and frailty appears as a crucial step towards improving the health and well-being of Indonesia's senior population in the context of the country's expanding aging population. This review aims to explore the potential relationship between albumin levels and frailty risks.

2. Frailty

Frailty is a state of heightened susceptibility to problems regaining physiological equilibrium following a stressor event.[2] Due to its main characteristics of weakness, diminished endurance, and reduced performance, the condition of frailty may be a physiological precursor and etiologic component in disability.[6] Globally, frailty is a common problem among elderly persons. However, due to study concentration in high-income nations and discrepancies in how frailty is characterized, its precise global incidence is still unclear.[7]

A breakdown in several inter-related physiological systems is what defines frailty. It speeds up the normal reduction in physiological reserve that comes with age, which contributes to the breakdown of homeostatic processes. Frailty should be understood in the context of how the complex processes of aging cause cumulative degradation across several physiological systems, reducing the body's capacity to maintain stability and rendering people vulnerable to serious health changes brought on by modest stresses. These intricate aging processes are controlled by environmental and genetic variables, which together with epigenetic mechanisms that variably control gene expression play a critical part in the aging process.[2]

Recent years have seen the development of several frailty assessment techniques, including questionnaires, performance metrics, and routine data. However, there isn't a standardized global

frailty evaluation technique. The frailty phenotype or frailty indices built on the deficit accumulation method are the most often utilized tools.[7]

The most famous instrument being used to assessed frailty states are by assessing it using the Frailty Phenotype.[6] These instruments consist of five criteria of assessment explained in Table 1.

Table 1. Frailty phenotype as a main assessment instrument[6]

	Frailty phenotype
Unintentional Weight Loss	Decreased of ≥4.5 kg unintentionally within a year
Weakness	Hand grip strength are less than <20% quintile adjusted by sex and body mass index
Exhaustion	Self-reported exhaustion, identified by two items of Centre for Epidemiological Studies Depression Scale
Slowness	Standardized times to walk 4.57 m, adjusted by sex and standing height
Low Activity	Low energy usage, based on physical activity questioners
Frailty States	
Non-Frail/Robust=0 criteria present, Pre-frail= 1-2 criteria presents, Frail= > 2 criteria presents.	

The frailty index is another tool for measuring frailty.[8] The frailty index is thought to be a gauge of the general loss in health that comes with ageing due to the accumulation of health-related impairments. Diseases, aberrant test findings, functional evaluations, or self-reported health conditions are a few examples. A frailty score between 0 and 1 is produced by dividing a person's deficit count by the sum of all deficits that were assessed; a higher score denotes a higher degree of frailty.[9]

Frailty is a problem that affects older persons all around the world, and early detection and management are crucial. Primary care professionals are in a good position to identify and treat frailty in elderly patients, perhaps halting its development. Adults in their middle years with comorbidities at high risk might also need to be screened. The evidence for primary care-based treatments to prevent or treat frailty is patchy, although some studies indicate that encouraging physical exercise and healthy eating may slow the transition from pre-frailty to frailty.[7]

3. Albumin

Albumin is a protein consisting of a single peptide chain with around 580-585 amino acid residues, varying by species.[10] In healthy human plasma, albumin concentrations vary from 33 to 52 g per Liter. Only the liver produces albumin, and on average it does so at a rate of roughly 0.2 g per kilogram of body weight every day. Albumin production and metabolism are in equilibrium under normal circumstances. Each day, around 10% of the albumin in plasma is digested, and the plasma concentration determines how long it stays in the system. Longer half-lives are caused by lower albumin levels, whereas up to 50% more metabolism is accelerated by greater levels.[11]

Albumin serves two functions: it protects tissues and the microcirculation from damage brought on by inflammation, and it acts as a vehicle for the absorption and disposal of drugs.[12] The body needs human albumin to keep their balance. It controls colloid osmotic pressure, reducing oedema and promoting vascular health. Drugs, fatty acids, and hormones are all carried by albumin, which has an impact on how they work. Additionally, it acts as a pH buffer, protects cells from oxidative damage, and helps keep blood clots under control. Albumin's broad functions include potential drug roles and detoxification.[13]

Hypoalbuminemia, or low albumin levels, is primarily influenced by heightened vascular permeability and interstitial volume expansion. This phenomenon is observed in various situations such as trauma, critical illness, chronic diseases, and life events including pregnancy and cancer growth. Inflammation triggers capillary permeability increase, leading to oedema and positive fluid balance. The retention of plasma solutes like albumin, fibrinogen, and electrolytes contributes to this expansion.[14] Albumin's kinetics and function are changed in severe infections by systemic inflammation, which can lead to a deterioration of the clinical outcome.[12]

Reduced synthesis is associated with low serum albumin levels in stressed or undernourished people. In healthy individuals, nutrition supplementation can increase synthesis rates, but it is yet unclear how it would affect critically sick patients. Albumin degradation can be accelerated by elements including oxidation, glycation, and substance binding. Loss of serum albumin is a result of conditions including nephrotic syndrome and protein-losing enteropathy, which reduce bulk. Stress may reduce the half-life of albumin, accelerating breakdown and affecting overall mass. Similar patterns appear in strained muscle cells when production and breakdown both increases.[14]

Serum albumin concentrations below 35 g/L are indicative of hypoalbuminemia, and clinical relevance is frequently observed at concentrations below 25 g/L. Elderly individuals with severe chronic illnesses or malnutrition as well as those receiving care in nursing homes or hospitals are more susceptible to it.[13]

Poor outcomes in a wide range of disorders, from medical to surgical situations, are predicted by preexisting hypoalbuminemia. In operations such orthopaedic, cardiovascular, gynaecologic, and visceral procedures, albumin levels serve as prognostic markers. Low blood albumin levels independently predict mortality risks across all age groups and health conditions. Sepsis correlates with in-hospital mortality with diseases such liver cirrhosis, malnutrition, nephrotic syndrome, and hypoalbuminemia that are often treated in hospitals. Due to their reflection of the intensity of inflammation, changes in blood albumin levels without medication might signal improvement or worsening in critically sick individuals.[12]

Human albumin used intravenously to treat hypoalbuminemia is debatable; it frequently just addresses hypoalbuminemia as a 'symptom' rather than the underlying problem. Priority should be given to treating the underlying causes of hypoalbuminemia, such as those that make older individuals more likely to suffer from malnutrition (diabetes, thyroid problems, and other drugs). When other colloids are ineffective, albumin is used to treat hypovolemia and fluid depletion, mainly in acute instances or specific chronic conditions.[13]

4. Albumin and frailty risk

Study by Smit et al. in 2013.[5] discover reduced blood albumin levels in elderly US individuals with traits such as muscular weakness and sluggish walking. The study implies that decreased blood albumin levels may be associated with slower walking speeds and more muscle mass loss, potentially indicating a deterioration in muscular strength.[5] Slower walking speeds and mass loss being 2 of the phenotype criteria for frailty suggest that the study founding can be inferred as a connection between frailty and albumin level.

In 2020, a meta-analysis research revealed that frail people had lower plasmatic albumin levels. Even after considering factors like age, BMI, gender, and chronic diseases, low albumin levels at the time of hospital admission and discharge are associated with higher rates of short- and long-term mortality. Regardless of BMI, frail people tend to have the lowest daily calorie consumption. Reduced gait speed and handgrip strength, two characteristics of sarcopenia and frailty, are linked to lower albumin levels. Numerous studies demonstrate that the plasma albumin levels of frail individuals are lower than those of robust persons, highlighting the importance of malnutrition in the frailty syndrome.[15]

An intriguing correlation between growth hormone (GH) and albumin levels was found in a cross-sectional study done in Tanushimaru. The GH reduction that frequently happens as people get older, which may influence nutritional status, seems to be a factor in this connection. The serum levels of Insulin-like Growth Factor 1 (IGF-1) and albumin were revealed to be positively correlated by the study. This result is in line with earlier studies that found a correlation between decreased IGF-1 levels and decreased handgrip strength and physical performance. This relationship between IGF-1 and albumin levels may be mediated by aging-related declines in IGF-1 secretion.[4] Decrease handgrip being an indicator for weakness phenotype criteria on frailty might suggest that decline in IGF-1 secretion were related to lower albumin level in frail individuals.

Yanagita et all [16], in their 2018 study found that low levels of albumin were statistically significant and were a strong independent risk factor for frailty.[16] This result were then strengthened on their 2020 study, where they recommend serum albumin concentration for elderly person to be maintained

at >4.0 g/dL to prevent frailty. Their study believe that low protein intake might be the cause of Frailty in Japan's elderly population.[17]

In Belgium, a study aimed to assess the prevalence of frailty in older individuals found that those classified as frail had lower levels of albumin and prealbumin. This connection is attributed to serum albumin's role as a significant blood protein and a marker of nutritional status. Hypoalbuminemia, or low serum albumin levels, can indicate issues across various systems in the elderly. Given that frailty often involves dysfunction in multiple organs, it's plausible that the observed inverse relationship between albumin levels and frailty index in the study participants could be explained by these underlying factors. They also believe that since hypoalbuminemia has also been used as a marker of malnutrition, the observed correlation between frailty and albumin deficiency can reflect a poor nutritional status in their studied population. Suggesting that malnutrition is associated with higher frailty.[18] These combined results highlight the necessity for an all-encompassing strategy to address nutritional status as a key component in identifying and treating frailty in the senior population.

Albumin's connection to frailty involves muscle loss, inflammation, and oxidative stress. Overall, inflammation and oxidative stress are central mechanisms in frailty development across various nutritional indicators in aging individuals.[19] By considering the mechanism of protein breakdown and its effects on muscle mass loss, which is indicated by low serum albumin levels, the relationship between serum albumin and the risk of frailty may be clarified. The possibility that protein catabolism plays a role in frailty is highlighted by this phenomenon. Low albumin levels may also be a sign that systemic inflammation, which was previously discussed, is contributing to the development of frailty. This link supports the idea that inflammation is a key factor in the emergence of frailty. Additionally, the link between low serum albumin levels and an increased risk of frailty may be a sign of increased oxidative stress. This emphasizes the link between oxidative stress and frailty and the role that oxidative stress may play in aggravated frailty conditions.[20]

5. Clinical implication

Presence of low albumin level and frailty can cause some clinical implication. Due to albumin being one of the proteins that represents the body's total protein and energy status, low serum albumin levels are a sign of malnutrition. Malnutrition is an issue that affects frail people frequently and can hasten the physical deterioration that comes with frailty.[19]

Muscle wasting and weakening are indicators of frailty. As the body may break down muscle tissue to produce amino acids for other crucial processes when appropriate nutrition is not available, low serum albumin levels might be an indicative of muscle wasting.[16,17,19]

A robust immune system is maintained in part by albumin. Low albumin levels can impair the body's response to infections and other immunological challenges, which is especially worrisome for frail people who are already more susceptible to infections because of their compromised physical condition. Frail people are more likely to suffer fractures and injuries, and they could take longer to heal. Low albumin levels can make it more difficult for the body to recover from operations and accidents, which further delays the process of restoring function.[12–14]

Low albumin levels in the frail may make them less responsive to medical interventions and therapies. This may affect the effectiveness of interventions meant to improve frailty or manage related medical issues.[14] Low blood albumin levels are linked to worsening physical impairment and decreased physical function. Low albumin levels in frail people can make it harder for them to maintain their independence and carry out everyday tasks.[7,12]

Frail people are more likely to suffer fractures and injuries, and they could take longer to heal. Low albumin levels can make it more difficult for the body to recover from operations and accidents, which further delays the process of restoring function. Low serum albumin levels have been linked to higher mortality risk in a number of demographics, including the elderly. Low albumin levels might further increase an individual's susceptibility in the context of frailty and raise their likelihood of suffering negative health effects.[21]

6. Conclusion

In the context of an aging population, the complex link between albumin levels and frailty has significant consequences for the health and wellbeing of elderly people. Understanding and addressing the connection between these two issues is urgent because of the aging population that is both expanding in Indonesia and around the world. Frailty is a complicated state that is impacted by the breakdown of numerous physiological systems and is characterized by a susceptibility to physiological imbalance after stressors. The risk of negative health outcomes, such as disability, falls, hospitalizations, and mortality, is increased because of this.

Albumin levels are a potential indicator of frailty risk and should be taken into consideration by clinicians and other healthcare professionals. To prevent or lessen frailty in older people, it may be beneficial to address malnutrition and encourage interventions to raise albumin levels. Additionally, a comprehensive strategy incorporating nutritional assistance, physical activity, and medical interventions may help the elderly population achieve greater functional independence and overall quality of life.

It is critical to carry out additional research and put strategies into action with the goal of elucidating the underlying mechanisms relating albumin levels and frailty as well as coming up with efficient

interventions to reduce these risks. Healthcare systems can significantly improve the health and longevity of the elderly population by addressing albumin deficiencies and focusing on factors that contribute to frailty.

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Literature Review

High dose vitamin D as adjuvant therapy in diabetic foot ulcer

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Abstract: Diabetic Foot Ulcer (DFU) is a combination of neuropathy and ischemic complication of DM disease that occurs in the feet with characteristics of sensory, motor, autonomic neuropathy as well as macrovascular and microvascular disorders. The method used in writing this literature review is a systematic review using the keywords "vitamin D", "vitaminD3", "cholecalciferol", "cholecalciferol-D3", "calcitriol", "1,25-dihydroxycholecalciferol", "diabetic foot ulcer", "diabetic ulcer", "diabetes*", "wound", "ulcer", "healing", "DFU". There were 10 journals studied about Vitamin D and Diabetic Foot Ulcer; Eight study randomized controlled trial (Halschou-Jensen PM et al., 2021, Tatiana Karonova, et al., 2020, El Hajj C, et al., 2020, Gupta B, et al., 2017, Razzaghi et al, 2017, Alam U et al.,2017, Hassan Mozzavari, 2016, Masood, et al., 2015), two clinical trials (Rahman, et al,. 2014, Khosravi, et al.,2016). High doses of Vitamin D can be used as an additional therapy for DFU patients to help wound healing. In the process of ulcer healing, vitamin D is able to repair factors that interfere with diabetic wound repair and stimulate immune system.

Keywords: diabetic foot ulcers; vitamin D

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disease associated with an abnormal increase in blood glucose levels, this can cause many complications, such as retinopathy, hypertension, neuropathy, ulcers, amputations, and others. Diabetic Foot Ulcer (DFU) is a combination of neuropathy and ischemic complication of DM disease that occurs in the feet with characteristics of sensory, motor, autonomic neuropathy as well as macrovascular and microvascular disorders.[1–7]

Diabetic Foot Ulcer (DFU) or diabetic foot ulcer is a serious complication in patients with diabetes mellitus (DM). The World Health Organization (WHO) in 2016 stated that DM sufferers reached 422 million worldwide, and estimated the number of DM sufferers in Indonesia would increase from 8.4 million people in 2000 to around 21.3 million people in 2030. DFU sufferers have a higher mortality rate than diabetics without foot ulcers. DFU patients are reported to have a 2-fold higher risk of death compared to DM patients without DFU. The proportion of DM with

complications in Indonesia is 6.9%, reaching 8.7% in the form of DFU. The worsening prognosis of DFU is closely related to hyperglycemia, which can stimulate oxidative stress, resulting in immune dysfunction, persistent inflammatory conditions, microvascular complications, impairing wound healing.[8,9]

Vitamin D is a steroid prehormone and plays an important role in the regulation of calcium and phosphorus. Recently, it has been shown that the vitamin D receptor (VDR) is found in almost all tissues of the human body and that vitamin D has several pleiotropic effects far beyond regulation of bone metabolism. Low vitamin D levels have been reported to impair the differentiation and proliferation of skin keratinocytes and fibroblasts and ultimately delay DFU healing.[10–13]

In recent years, many studies have addressed the beneficial role of vitamin D in DM. Vitamin D can affect the wound healing process because it is associated with its ability to increase proliferation and remodeling of wound tissue. Vitamin D is reported to act as an immunomodulator for proinflammatory cytokines among many other roles. This leads to the potential of vitamin D as an adjuvant therapy for DFU cases.[13–15]

Vitamin D has been suggested to play an important role in many chronic diseases, such as diabetes. Low serum vitamin D levels are associated with insulin resistance, impaired β-cell function, and the development of DM. Vitamin D has an immunomodulatory effect. Studies show that vitamin D receptors are expressed in several types of immune cells and vitamin D acts as an anti-inflammatory stimulus by increasing the amount of anti-inflammatory cytokine (IL-10) and decreasing pro-inflammatory cytokine (IL-1, IL-6, TNF), therefore, biologically vitamin D supplements can play a role in the wound healing process. In addition, severe vitamin D deficiency is known to increase the risk of DFU. 16–19 There are no guidelines for vitamin D therapy for DFU cases, so there is no standardized dosage regimen, route of administration, and timing of administration. It is hoped that this systematic review will gather existing literature regarding the use of Vitamin D from a therapeutic point of view for DFU cases.[16–18]

2. Methods

This literature review using keywords "vitamin D", "vitaminD3", "cholecalciferol", "cholecalciferol-D3", "calcitriol", "1,25-dihydroxycholecalciferol", "diabetic foot ulcer", "diabetic ulcer", "diabetes*", "wound", "ulcer", "healing", "DFU". A literature search was carried out using the Pubmed NCBI database, ScienceDirect, Google scholar. The inclusion criteria in this literature search are evidence-based medicine research literature at levels 1, 2, 3, randomized controlled trial (RCT) research, clinical trial, which is research with human objects; as well as publication requirements with a minimum of the last 10 years starting in 2013. The exclusion criteria used were

non-reputable literary sources; which are difficult or fail to translate; those who are not in the medical or biological field; which discusses more about aspects of vitamin D deficiency, genetic factors, receptors, multivitamins, honey, or herbs; topical vitamin D administration, the literature generally does not address vitamin D administration.

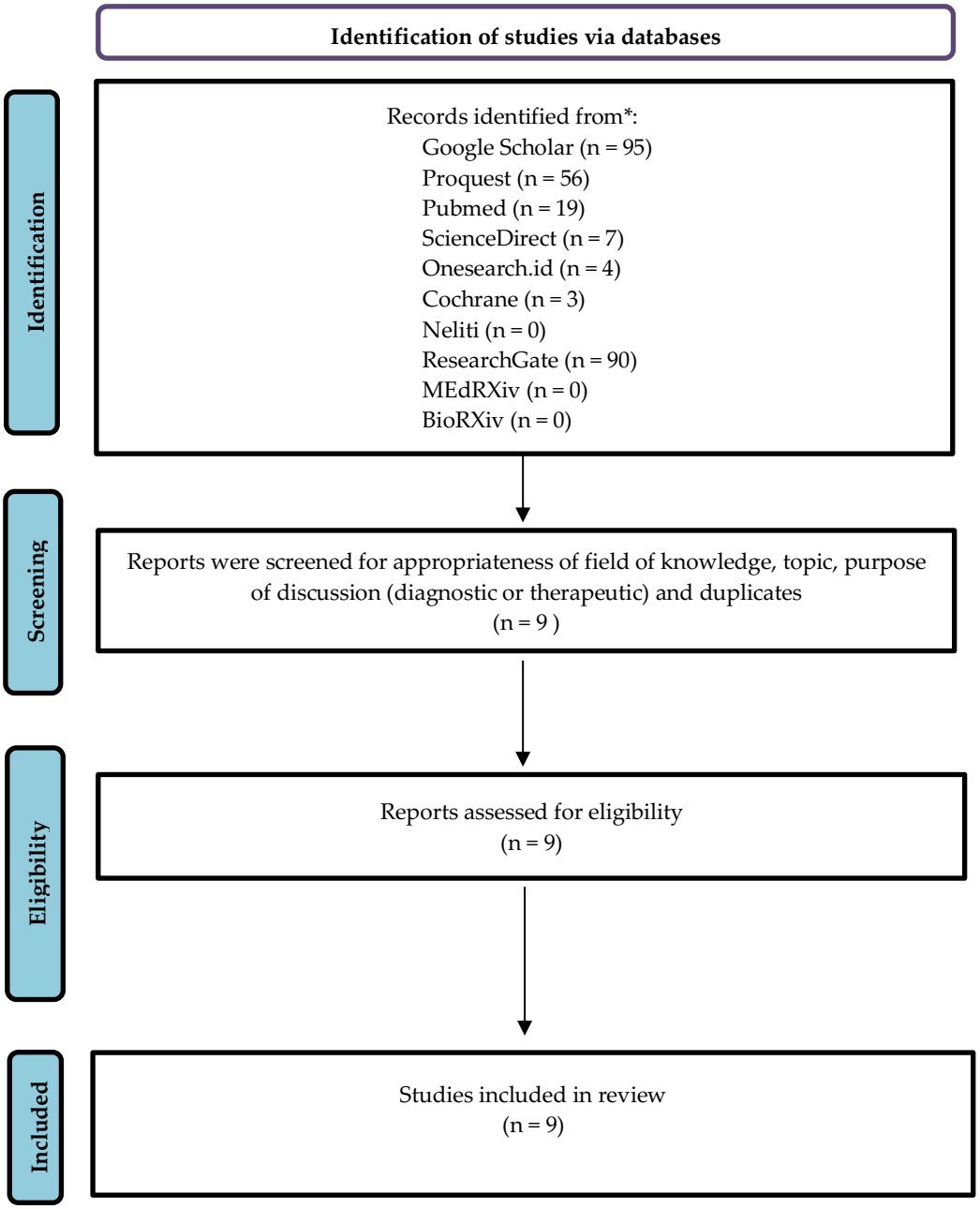

Figure 1. Data search flow using the preferred reporting items for systematic reviews and meta-analysis PRISMA method for articles spanning the period 2012 to June 2023.

From the results of a literature search, evaluation of inclusion and exclusion criteria was carried out by assessing the title and abstract first for the initial step by considering critical appraisal tools for systematic reviews from CEBM Oxford, the search was presented with a protocol diagram preferred reporting items for systematic reviews and meta-analysis (PRISMA). Then the full text is reviewed if there is a relationship or correlation of keywords with one another in the literature so that it can support writing a description or analysis in this systematic review. From the results of a literature search according to the inclusion and exclusion criteria, 9 articles were found that were used to write this systematic review.

3. Results and Discussion

Based on search results using Google scholar: found 96 articles, Proquest: 56, Pubmed: 19, ScienceDirect: 7, Onesearch.id: 4, Cochrane: 3, Neliti: 0; other sources, namely ResearchGate: 90 articles, MedRXiv: 0, and BioRXiv: 0 search, obtained 9 journals in accordance with the research objectives regarding the dosage method of Vitamin D in DFU that met the inclusion criteria. There were 10 journals studied about Vitamin D and Diabetic Foot Ulcer; Eight studies randomized controlled trial (Halschou-Jensen PM et al., 2021[19] , Tatiana Karonova, et al., 2020[20], El Hajj C, et al., 2020[21], Gupta B, et al., 2017[22], Razzaghi et al, 2017[23], Alam U et al., 2017[24] , Hassan Mozzavari, 2016[16], Masood MQ, et al., 2015[25]), two clinical trials (Rahman, et al,. 2014[26], Khosravi, et al., 2016[16]. As explained in table 1.

The results of this study indicate that vitamin D can improve Diabetic Foot Ulcers (DFU). The relationship between vitamin D levels and DFU has been described in other studies. There were 10 journals studied about Vitamin D and Diabetic Foot Ulcer; Eight study randomized controlled trial (Halschou-Jensen PM et al., 2021[19] , Tatiana Karonova, et al., 2020[20], El Hajj C, et al., 2020[21], Gupta B, et al., 2017[22], Razzaghi et al, 2017[23], Alam U et al.,2017[24] , Hassan Mozzavari, 2016[16]), two clinical trials (Rahman, et al,. 2014[26], Khosravi, et al.,2016[16].

Seven study randomized controlled trial (RCT); Halschou-Jensen PM et al., 2021[19] , Tatiana Karonova, et al., 2020[20], El Hajj C, et al., 2020[21], Gupta B, et al., 2017[22], Razzaghi et al, 2017[23], Alam U et al.,2017[24] , Hassan Mozzavari, 2016. [16]

According to RCT conducted by Halschou-Jensen PM et al., 2021[19]; Improved Healing of Diabetic Foot Ulcers After High-dose Vitamin D: A Randomized Double-blinded Clinical Trial, 48 patients (24 in each group), total 64 ulcers, daily oral intake of high-dose (170 μg) or low-dose (20 μg) vitamin D3. Follow up; 4, 12, 24, 36, and 48 weeks, The intention-to-treat analysis showed a significantly higher rate of ulcer healing in the high-dose group with 21 of 30 (70%) healed ulcers compared to 12 of 34 (35%) in

the low-dose group (P=.012). Median ulcer reduction at final follow-up was 100% (interquartile range [IQR]: 72-100) in the high-dose group and 57% (IQR: −28 to 100) in the low-dose group. Furthermore, we found a significant effect of high-dose vitamin D on ulcer reduction in the repeated measures analysis of variance. High-dose vitamin D3 to be efficient, compared to low-dose vitamin D3, in promoting healing in chronic diabetic foot ulcers.

Tatiana Karonova, et al., 2020[20] in Rusia performed RCT to 67 patients 5000 IU and 40,000 IU once/week orally for 24 weeks, Assessed the effect of different doses of vitamin D supplementation on microcirculation, signs and symptoms of peripheral neuropathy and inflammatory markers in patients with type 2 diabetes. significant decrease in neuropathy severity (NSS, $p = 0.001$; NDS, $p = 0.001$; VAS, $p = 0.001$) and improvement of cutaneous MC were observed ($p < 0.05$). Also, we found a decrease in IL-6 level (2.5 pg/mL vs. 0.6 pg/mL, $p < 0.001$) and an increase in IL-10 level (2.5 pg/mL vs. 4.5 pg/mL, $p < 0.001$). they conclude that high-dose cholecalciferol supplementation of 40,000 IU/week for 24 weeks was associated with improvement in clinical manifestation, cutaneous microcirculation and inflammatory markers in patients with T2DM and peripheral neuropathy.

El Hajj C[21], Effect of Vitamin D Supplementation on Inflammatory Markers in Non-Obese Lebanese Patients with Type 2 Diabetes: A Randomized Controlled Trial, Evaluate the effect of vitamin D treatment on inflammatory markers in non-obese Lebanese patients with T2DM. There were 88 patients with DFU, 30.000 IU cholecalciferol every week for a period of 6 months. The vitamin D group showed higher blood levels of (25(OH) D) ($p < 0.0001$), and a significant reduction in hs-CRP and TNF-α concentrations ($p < 0.0001$) compared to placebo. The decrease perceived in IL-6 concentrations was not significant ($p = 0.1$). No significant changes were seen in FBG ($p = 0.9$) and HbA1c levels ($p = 0.85$).

Gupta B, et al., 2017[22] in they RCT to Evaluate Effect of vitamin D supplementation on cytokines expression in patients with diabetic foot infection. There were 120 patients, 30.0000 IU per oral vitamin D in 5 divided doses in 4 weeks at the enrollment mean values of serum concentrations of 25-OH vitamin D3 at baseline and after 4 weeks were significantly different in both controls (group I, $p = 0.0122$) and cases (group II, p < 0.0001). Difference in mean levels of circulating serum inflammatory cytokines TNF-α and IL-6 at baseline and follow up was not significant in group I but significant in group II ($p = 0.0001$ for TNF-α, $p = 0.0026$ for IL-6). High dose vitamin D supplementation reduced inflammatory cytokines in diabetic foot patients having heightened response of these cytokines.

Razzaghi et al, 2017[23] in Iran conducted Randomized Controlled Trial to Evaluation of the effect of vitamin D supplementation on wound healing and metabolic status in patients with diabetic foot ulcers. 60 samples; 50.000 IU vitamin D every 2 weeks for 12 weeks;12 weeks. Reductions in ulcer length (−2.1 ± 1.1 vs. −1.1 ± 1.1 cm, P = 0.001), wound width (−2.0 ± 1.2 vs. −1.1 ± 1.0 cm, P = 0.02) and

wound depth (−1.0 ± 0.5 vs. −0.5 ± 0.5 cm, P b 0.001), erythema (100% vs. 80%, P = 0.01), Serum insulin concentrations (−3.4 ± 9.2 vs. +2.8 ± 9.3 μIU/mL, P = 0.01), Insulin resistance assessment (−1.5 ±4.1 vs. +1.7 ± 5.1, P = 0.01) , HbA1c (−0.6 ± 0.6 vs. −0.1 ± 0.5%, P = 0.004), Cholesterol level, LDL (−17.2 ± 19.8 vs. +2.2 ± 28.6 mg/dL, P = 0.003), HDL cholesterol, ESR (−34.7 ± 32.4 vs. −18.0 ± 26.6 mm/h, P = 0.03. Vitamin D supplementation 50.000 IU had beneficial effects on Wound healing and metabolic status in DFU.

Alam U, Asher Fawwad, Fariha Shaheen, Bilal Tahir, Abdul Basit, Rayaz A. Malik[24] Assess the effect of treatment with a single intramuscular injection of high-dose vitamin D on the quality of life of patients with painful diabetic neuropathy menggunakan kuesioner NeuroQoL143 samples; 600.000 IU IM; 10 months. High dose vitamin D 600.000 IU resulted in a significant increase in 25(OH)D (P < 0.0001) and effective in improving the quality of life in patients with diabetic neuropathy (DN) with DFU (n=143).

Hassan Mozaffari-Khosravi[16] in his study conducted to compare the effect of 150,000 and 300,000 IU of vitamin D on the healing status of diabetic foot ulcer among the patients with diabetes. 47 patients 150.000 IU 300.000 IU, intra muscular injection, 4 weeks follow up. Serum vitamin D level in both groups was significantly increased compared to the baseline (P<0.01). The mean of serum vitamin D changes were 12.6±5.0 and 18.4±6.4 ng/ml (P=0.001) in G150 and in G300, respectively. The ulcer area was significantly reduced in both groups compared to the baseline (P<0.01).WBC, ESR, FBS and CRP were significantly declined compared to the baseline in both groups. However, the mean changes of serum FBS and CRP levels were found to be significantly different between groups. Administration of 150,000 and 300,000 IU of vitamin D improved the ulcer and vitamin D status and reduced ESR, CRP, WBC and FBS in the patients with diabetic foot ulcer. In addition, the 300,000 IU of vitamin D was significantly more effective than 150,000 IU.

Two clinical trials (Rahman, et al,. 2014.[15], Khosravi, et al.,2016.[13]. Rahman NMA, et, al, 2013.[15] in Iraq; 30 DFU patients Participants were divided into two groups of vitamin D and placebo. Vitamin D tablets 1000 IU orally after food daily for 4 weeks. Percentage of ulcer area reduction between the vitamin D group and the control group = (71.86 ± 4.79% vs 32.06 ± 4.28%; p<0.01). There was a significant decrease in fructosamine levels in the treatment group after 4 weeks of treatment. There was no significant change in High density lipoprotein (HDL) and Low Density Lipoprotein (LDL). There was a decrease in ulcer area in the vitamin D group.

Khosravi, et al.,2016.[13], Iran, to compare the effect of Vitamin D of 150,000 and 300,000 IU doses on the healing status of diabetic foot ulcers among patients with diabetes. Single dose intramuscular injection, patients (47 DFU) were divided into two groups; 150,000 IU of vitamin D and 300,000 IU. Serum vitamin D levels in both groups were significantly increased compared to baseline (P<0.01).

Changes in serum vitamin D were 12.6±5.0 and 18.4±6.4 ng/ml (P=0.001) at G150 and at G300, respectively. Ulcer area significantly reduced in both groups (P<0.01). Leukocytes, ESR, FBS and CRP decreased significantly in both groups. Vitamin D, especially 300,000 IU promotes ulcer healing and vitamin D. Therefore, vitamin D status is recommended for assessment in clinical care of patients with diabetic foot.

Table 1. There were 10 journals studied about Vitamin D and Diabetic Foot Ulcer; Seven study randomized controlled trial (Halschou-Jensen PM et al., 2021[8] , Tatiana Karonova, et al., 2020[20], El Hajj C, et al., 2020[21], Gupta B, et al., 2017[22], Razzaghi et al, 2017[23], Alam U et al.,2017[24] , Hassan Mozzavari, 2016[16], Masood MQ, et al., 2015[25] two clinical trials (Rahman, et al,. 2014[26], Khosravi, et al.,2016[16].

No.	Author	Year, country and design	Aim of study	Number of sample, dose and duration of study	Result	Conclusion
1.	Halschou-Jensen PM, Sauer J, Bouchelouche P, Fabrin J, Brorson S, Ohrt-Nissen S[19]	2021 *Randomized Controlled Trial*	Improved Healing of Diabetic Foot Ulcers After High-dose Vitamin D: A Randomized Double-blinded Clinical Trial	48 patients (24 in each group), total 64 ulcers, daily oral intake of high-dose (170 μg) or low-dose (20 μg) vitamin D3. Follow up; 4, 12, 24, 36, and 48 weeks	The intention-to-treat analysis showed a significantly higher rate of ulcer healing in the high-dose group with 21 of 30 (70%) healed ulcers compared to 12 of 34 (35%) in the low-dose group (P=.012). Median ulcer reduction at final follow-up was 100% (interquartile range [IQR]: 72-100) in the high-dose group and 57% (IQR: −28 to 100) in the low-dose group. Furthermore, we found a significant effect of high-dose vitamin D on ulcer reduction in the repeated measures	High-dose vitamin D3 to be efficient, compared to low-dose vitamin D3, in promoting healing in chronic diabetic foot ulcers.

					analysis of variance.	
2.	Tatiana Karonova, Anna Stepanova, Anna Bystrova and Edward B. Jude[20]	2020, Rusia, *Randomized Controlled Trial*	Assessed the effect of different doses of vitamin D supplementation on microcirculation, signs and symptoms of peripheral neuropathy and inflammatory markers in patients with type 2 diabetes	67 patients 5000 IU and 40,000 IU once/week orally for 24 weeks	significant decrease in neuropathy severity (NSS, $p = 0.001$; NDS, $p = 0.001$; VAS, $p = 0.001$) and improvement of cutaneous MC were observed ($p < 0.05$). Also, we found a decrease in IL-6 level (2.5 pg/mL vs. 0.6 pg/mL, $p < 0.001$) and an increase in IL-10 level (2.5 pg/mL vs. 4.5 pg/mL, $p < 0.001$)	High-dose cholecalciferol supplementation of 40,000 IU/week for 24 weeks was associated with improvement in clinical manifestation, cutaneous microcirculation and inflammatory markers in patients with T2DM and peripheral neuropathy.
3.	El Hajj C, Walrand S, Helou M, Yammine K[21]	2020, Lebonon, *Randomized Controlled Trial*	Evaluate the effect of vitamin D treatment on inflammatory markers in non-obese Lebanese patients with T2DM	N=88 30.000 IU cholecalciferol/ week for a period of 6 months	The vitamin D group showed higher blood levels of (25(OH) D) ($p < 0.0001$), and a significant reduction in hs-CRP and TNF-α concentrations ($p < 0.0001$) compared to placebo. The decrease perceived in IL-6 concentrations was not significant ($p = 0.1$). No significant changes were seen in FBG ($p = 0.9$) and HbA1c levels ($p = 0.85$).	Vitamin D supplementation led to a decrease in some inflammatory markers in patients with T2DM.
4.	Gupta, B., Dwivedi, A., Singh, S. K.[22]	2017, India, *Randomized Controlled Trial*	Evaluate Effect of vitamin D supplementation on cytokines expression in patients with	120 patients, 30.0000 IU per oral in 5 divided doses at the enrollment	Mean values of serum concentrations of 25-OH vitamin D3 at baseline and	Vitamin D supplementation reduced inflammatory cytokines in diabetic foot

			diabetic foot infection.		after 4 weeks were significantly different in both controls (group I, p = 0.0122) and cases (group II, p < 0.0001). Difference in mean levels of circulating serum inflammatory cytokines TNF-α and IL-6 at baseline and follow up was not significant in group I but significant in group II (p = 0.0001 for TNF-α, p = 0.0026 for IL-6)	patients having heightened response of these cytokines
5.	Razzaghi R, Pourbagheri H, Momen-Heravi M, Bahmani F, Shadi J, Soleimani Z[23]	2017, Iran *Randomized Controlled Trial*	Evaluation of the effect of vitamin D supplementation on wound healing and metabolic status in patients with diabetic foot ulcers.	60 samples; 50.000 IU vitamin D every 2 weeks for 12 weeks;12 weeks	Reductions in ulcer length (−2.1 ± 1.1 vs. −1.1 ± 1.1 cm, P = 0.001), wound width (−2.0 ± 1.2 vs. −1.1 ± 1.0 cm, P = 0.02) and wound depth (−1.0 ± 0.5 vs. −0.5 ± 0.5 cm, P b 0.001), erythema (100% vs. 80%, P = 0.01), Serum insulin concentrations (−3.4 ± 9.2 vs. +2.8 ± 9.3 μIU/mL, P = 0.01), Insulin resistance assessment (−1.5 ±4.1 vs. +1.7 ± 5.1, P = 0.01) , HbA1c (−0.6 ± 0.6 vs. −0.1 ± 0.5%, P = 0.004), Cholesterol	Vitamin D supplementation 50.000 IU had beneficial effects on Wound healing and metabolic status in DFU

					level, LDL (−17.2 ± 19.8 vs. +2.2 ± 28.6 mg/dL, P = 0.003), HDL cholesterol, ESR (−34.7 ± 32.4 vs. −18.0 ± 26.6 mm/h, P = 0.03	
6.	Alam U, Asher Fawwad, Fariha Shaheen, Bilal Tahir, Abdul Basit, Rayaz A. Malik[24]	2017, Pakistan, *Randomized Controlled Trial*	Assess the effect of treatment with a single intramuscular injection of high-dose vitamin D on the quality of life of patients with painful diabetic neuropathy menggunakan kuesioner NeuroQoL	143 samples; 600.000 IU IM; 10 months	41.3% participants were deficient in vitamin D (vitamin D < 20 ng/ml). Treatment with vitamin D resulted in a significant increase in 25(OH)D (P < 0.0001) and a significant increase in the Neuro QoL subscale score for emotional distress (P = 0.04), without significant changes in the other Neuro QoL domains of pain and paresthesia symptoms; loss of temperature and touch sensation, instability, limitations in daily activities, and interpersonal problems.	High dose vitamin D 600.000 IU resulted in a significant increase in 25(OH)D (P < 0.0001) and effective in improving the quality of life in patients with diabetic neuropathy (DN) with DFU (n=143).
7.	Hassan Mozaffari-Khosravi[16]	2016, Iran, *Randomized Controlled Trial*	Comparative Effect of Two The current study was conducted to compare the effect of 150,000 and 300,000 IU of vitamin D on the healing	47 patients 150.000 IU 300.000 IU, intra muscular injection, 4 weeks follow up	Serum vitamin D level in both groups was significantly increased compared to the baseline (P<0.01). The mean of serum vitamin D	Administration of 150,000 and 300,000 IU of vitamin D improved the ulcer and vitamin D status and reduced ESR, CRP, WBC and FBS in the

			status of diabetic foot ulcer among the patients with diabetes.		changes were 12.6±5.0 and 18.4±6.4 ng/ml (P=0.001) in G150 and in G300, respectively. The ulcer area was significantly reduced in both groups compared to the baseline (P<0.01).WBC, ESR, FBS and CRP were significantly declined compared to the baseline in both groups. However, the mean changes of serum FBS and CRP levels were found to be significantly different between groups.	patients with diabetic foot ulcer. In addition, the 300,000 IU of vitamin D was significantly more effective than 150,000 IU.
8.	Masood MQ, Khan A, Awan S, Dar F, Naz S, Naureen G, Saghir S, Jabbar.[25]	2015	To ascertain the frequency of correction of vitamin D deficiency (VDD) with single or multiple doses of oral (PO) and intramuscular (IM) administration of 2 high-dose preparations of vitamin D3 (VD3).	This was a prospective 100 participants with VDD (25-hydroxy vitamin D [25-OHD] <20 ng/mL) were randomized to receive a dose of 600,000 or 200,000 IU of VD3 via a PO or IM route. The main outcome measure was serum 25-OHD levels at 2, 4, and 6 months after the	At 2 months, VDD was corrected in 93.8% of participants in Group 1 (600,000 IU IM); 83.3% in Group 2 (600,000 IU PO), 87.5% in Group 3 (200,000 IU IM), and 70.6% in Group 4 (200,000 IU PO). The mean changes from baseline in vitamin D levels at 2 months were 29.6 ± 13.7, 19.8 ± 12.3, 18.3 ± 10.6, and 13.7 ±	Two months after the intervention, VDD was corrected in more than 70% of participants with a single dose of either 600,000 or 200,000 IU given PO or IM.

				intervention. The same dose was repeated in participants if 25-OHD remained <30 ng/mL at 2 and 4 months.	7.8 ng/mL in Groups 1, 2, 3, and 4, respectively. The mean levels remained significantly higher from baseline in all groups at all time points during the 6 months of observation. The mean 25-OHD level achieved in Group 1 was significantly higher than all other groups at 6 months.	
9.	Rahman NMA, et.[26]	2013, Iraq, Clinical Trial	Comparing the effects of zinc and vit.D3 on fructosamine levels, percentage of healing, and lipid profiles in diabetic patients with DFU	30 DFU patients Participants were divided into two groups of vitamin D and placebo. Vitamin D tablets 1000 IU orally after food daily for 4 weeks	Percentage of ulcer area reduction between the vitamin D group and the control group = (71.86 ± 4.79% vs 32.06 ± 4.28%; p<0.01) There was a significant decrease in fructosamine levels in the treatment group after 4 weeks of treatment. There was no significant change in High density lipoprotein and Low Density Lipoprotein.	There was a decrease in ulcer area in the vitamin D 1.000 IUgroup
10.	Khosravi, et. al[16]	2016, Iran, Clinical Trial	To compare the effect of Vitamin D of 150,000 and 300,000 IU doses on the healing status of diabetic foot ulcers	Single dose intramuscular injection, patients (47 DFU) were divided into two groups;	Serum vitamin D levels in both groups were significantly increased compared to baseline	Vitamin D, especially 300,000 IU promotes ulcer healing and vitamin D. Therefore,

	among patients with diabetes.	150,000 IU of vitamin D and 300,000 IU	(P<0.01). Changes in serum vitamin D were 12.6±5.0 and 18.4±6.4 ng/ml (P=0.001) at G150 and at G300, respectively. Ulcer area significantly reduced in both groups (P<0.01). Leukocytes, ESR, FBS and CRP decreased significantly in both groups.	vitamin D status is recommended for assessment in clinical care of patients with diabetic foot.

4. Conclusion

High doses of Vitamin D can be used as an additional therapy for DFU patients to help wound healing. In the process of ulcer healing, vitamin D is able to repair factors that interfere with diabetic wound repair and stimulate the immune system.

5. Acknowledgments

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Literature Review

Understanding the potential of stem cells in endometriosis

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Abstract: Endometriosis is a disorder in which tissue comparable to the uterine lining grows outside the uterus, causing pain and infertility. It affects approximately 5-10% of women of reproductive age globally, and roughly 10% (190 million) of reproductive-age women and girls globally. There is evidence supporting evidence of a stem cell population in the endometrium that provides a source of regenerated endometrial cells, and there is also new evidence that stem cells play a role in the pathogenesis of endometriosis. The review was then carried out using electronic databases according to PRISMA flowchart utilizing specific search terms, as well as systematic selection criteria to exclude any potential data inaccuracy. Furthermore, understanding in order to provide an update on the most recent evidence for the role of stem cells in both endometrial function and endometriosis is important. The review discusses the stem cell population in the endometrium that serves as a source of regenerative endometrial cells, as well as the emerging evidence of the involvement of stem cells in the pathogenesis of endometriosis.

Keywords: endometriosis; endometrial stem cells; eMSCs; ESCs; MSCs; pathogenesis

1. Introduction

Endometriosis is a disorder in which tissue comparable to the uterine lining grows outside the uterus, causing pain and infertility.[1] It affects approximately 5-10% of reproductive-age women worldwide, and approximately 10% (190 million) of reproductive-age women and girls worldwide. Endometriosis is generally diagnosed years later, misdiagnosis is widespread, and effective treatment is delayed. Endometriosis must be identified and treated as soon as possible, which is made easier by an accurate clinical diagnosis.[2,3] Endometriosis is believed to result from endometrial stem cell retrograde menstruation, hematogenous/lymphatic dispersion of endometrial or extrauterine stem cells, and/or stem cells in persisting Müllerian structures.[2]

There is evidence that there is a stem cell population in the endometrium that provides a source of regenerated endometrial cells, and there is also new evidence that stem cells play a role in the pathogenesis of endometriosis.[3,4] Endometrial stem cells have been identified in the eutopic endometrium, menstrual blood, and ectopic lesions of women with endometriosis, which not only gives new study objects in the context of endometriosis but also promotes and improves our understanding of its pathophysiology.[5,6] A recent review study discusses the many stem cell types implicated in endometriosis pathogenesis, as well as their genetic underpinnings. The three types of endometrial stem/progenitor cells identified in periodically regenerated female endometrium are endometrial epithelial stem/progenitor cells, CD140b+CD146+ or SUSD2+ endometrial mesenchymal stem cells (eMSCs), and side population cells (SPs).[7]

The aims of the literature review described in the search result is to offer an update on the existing evidence for the role of stem cells in endometrial function and endometriosis. The review examines the endometrium's stem cell population as a source of regenerating endometrial cells, as well as new evidence of stem cell participation in endometriosis pathogenesis. The authors also highlight stem cell therapy's potential therapeutic applications in the treatment of endometrial illnesses such as endometriosis. Overall, the role of stem cells in endometriosis pathogenesis has received considerable attention, and recent material focuses on prospective therapeutic options for infertility caused by various reproductive problems. More research in this area could lead to new insights and treatment options for women. with endometriosis.

Endometrial stem/progenitor cells are essential for the regeneration of the uterine endometrial lining in both humans and animals. Endometrial epithelial stem/progenitor cells, CD140b+CD146+ or SUSD2+ endometrial mesenchymal stem cells (eMSCs), and side population cells (SPs) are the three types. Endometrial stem/progenitor cells are found in almost all postnatal tissues and organs, where they help to maintain cellular homeostasis. Stem/progenitor cells are originally distinguished by functional characteristics that distinguish them from the majority of the cells that comprise the tissue or organ. Self-renewal, strong proliferative potential, and the ability to develop into one or more lineages are identifying markers for stem/progenitor cells. Functional experiments that evaluate important characteristics of stem/progenitor cells are used to characterize them. Clonogenicity, defined as a single cell's ability to initiate a colony of cells when planted as single cells at extremely low seeding densities, is the most often used method for detecting stem/progenitor cell activity. Self-renewal is a distinguishing property of stem/progenitor cells that can be measured by lineage tracing.

2. Methods

This review was carried out using electronic databases according to PRISMA flowchart. Authors have separately searched journals and articles based on inclusion and exclusion criteria. Several sources, such as PubMed [MEDLINE], Sage Journal, and Science direct, were utilized to find relevant references with following search terms: "Stem cell", and "Endometriosis". Optimal search results could be thoroughly done via usage of Boolean operators "AND" and "OR". Additionally, inclusion criteria comprise of studies involving eligible full-text articles and least published in past 10 years. The exclusion criteria are unavailable or inconsistent data, and studies with potential confounding factors. Systematic selection of the sources would exclude any potential inaccuracy for further analysis.

The main findings of the study will be presented in tabular form (table 2). The review and extraction of the article's contents will be presented in the discussion section as a narrative. The papers in this review have been checked for quality. Using Scimago Journal Rankings, each paper was confirmed and reviewed in the journal. The NIH Quality Assessment Tool is then used to evaluate the validity of each paper. The researcher avoided bias caused by author, affiliation, sponsor, area, and journal when searching for and evaluating publications, focusing on journal quality.

Table 1. Search queries of this literature review

Databases	Search queries	Hits
PubMed [MEDLINE]	("Stem Cell") AND ("endometriosis")	138
SageJournal	(Stem cell AND endometriosis)	550
Sciencedirect	Stem cell, Endometriosis	1.534
Wiley Online Library	(Stem cell AND endometriosis)	504

3. Results and Discussion

From the search conducted, a total of 2,762 articles were included in this review. After that, deduplication was carried out, and 218 duplicate articles were obtained. After further screening, 23 articles were found that matched the research inclusion criteria. After reading the full text to see if the contents of the articles obtained are appropriate and included in the review. There were 11 articles that matched the topic of the review, so 12 articles were excluded. The reasons for article exclusion were: it was not in accordance with the purpose of this review (n = 6, and one was an editorial article). The article search flowchart for this review is shown in Figure 1. Of the 14 studies obtained, consisting of 10 review articles, one experimental study, and one systematic review.

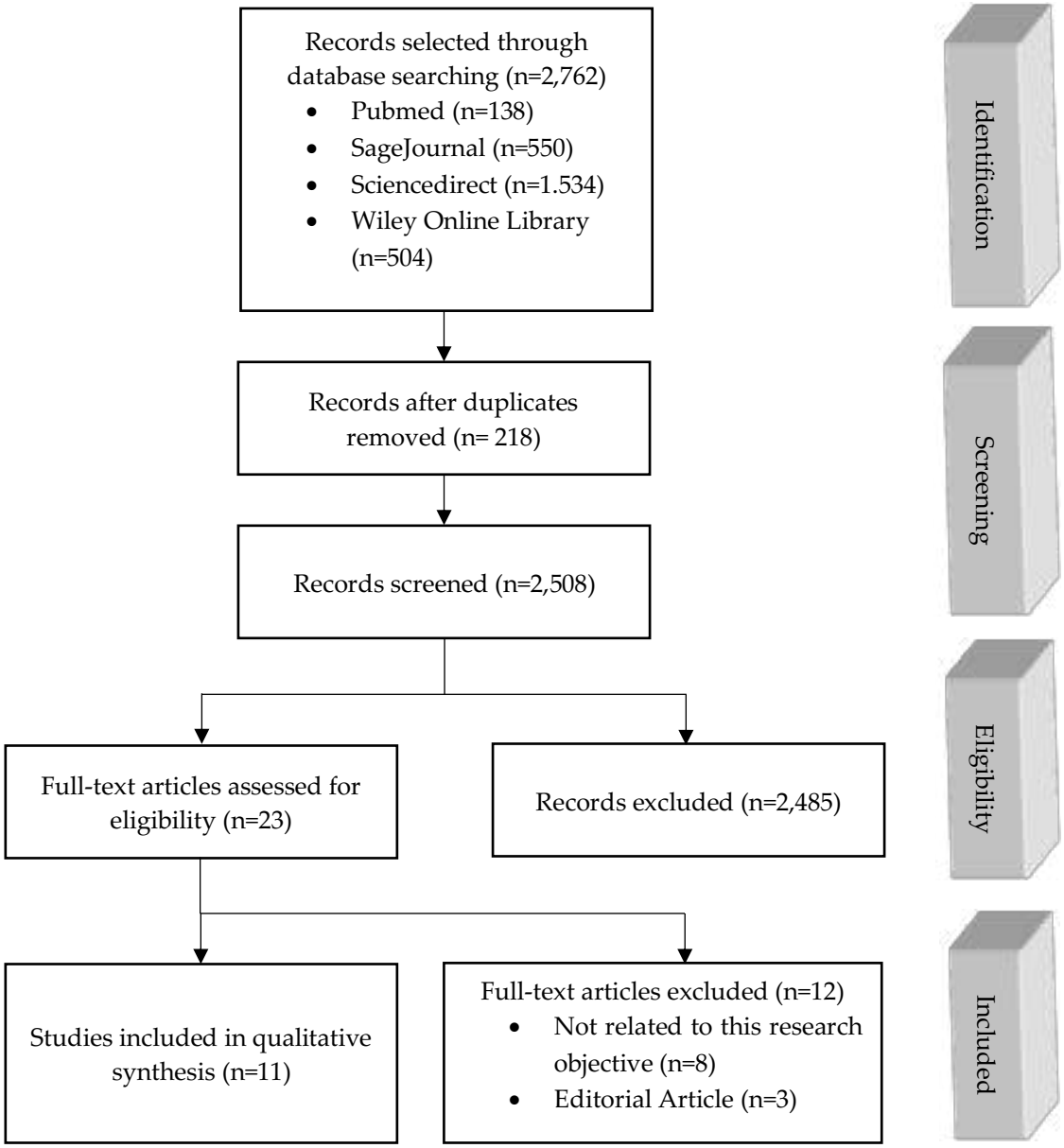

Figure 1. Flow diagram of article selection

Table 2. Study characteristics and findings

First author (year)	Title	Type	Findings
Kong Y (2021)[8]	Endometrial stem/progenitor cells and their roles in immunity, clinical application, and endometriosis	Review article	• The complex mechanisms involved in the development and progression of endometriosis. • The various factors contribute to the pathogenesis of endometriosis • The current diagnostic and treatment strategies for endometriosis
Hong I (2022)[9]	Endometrial stem/progenitor cells: Properties, origins, and functions	Review article	• How the endometrium is the inner mucosal lining of the uterus • Undergoes extensive cyclic growth, regeneration, differentiation, and shedding
Abuwala N (2021)[10]	Endometrial stem cells: Origin, biological function, and therapeutic applications for reproductive disorders	Review article	• An overview of the origin, biological function, and potential therapeutic applications of ESCs for reproductive disorders. • The potential of ESCs in regenerative medicine, particularly for conditions such as endometrial injury and infertility
Hufnagel D (2015)[11]	The Role of Stem Cells in the Etiology and Pathophysiology of Endometriosis	Review article	• The role of stem cells in both the physiological and pathological processes of the human endometrium.
Liu Y (2020)[12]	The role of endometrial stem cells in the pathogenesis of endometriosis and their application to its early diagnosis	Review article	• Pathogenesis of endometriosis and provide support for the application of EnSCs as therapeutic and early diagnostic targets in endometriosis treatment.
Pluchino et al. (2016)[13]	Endometriosis and Stem Cell Trafficking	Review article	• Stem cells's aberrant trafficking and implantation in ectopic sites may contribute to the development of endometriotic lesions. • Mesenchymal stem cells (MSCs), in particular, are involved in the initiation and maintenance of endometriosis. • Inflammation, angiogenesis, and hormonal changes in the menstrual cycle may influence stem cell migration and implantation in endometriotic lesions.

Gopalakrishnan et al. (2017)[14]	Mesenchymal stem cells for restoring endometrial function: An infertility perspective	Review article	• Stem cells derived from endometriotic lesions exhibited characteristics of mesenchymal stem cells (MSCs), including self-renewal and differentiation potential into various cell types. • The expression of certain stem cell markers, such as CD146 and CD105, was significantly increased in endometriotic lesions compared to healthy endometrium. • The number of stem cells was significantly higher in endometriotic lesions compared to healthy endometrial tissue, suggesting a potential role for these cells in the development and progression of endometriosis.
Kalohoei et al. (2023)[15]	Therapeutic effects of L-arginine, L-carnitine, and mesenchymal stem cell conditioned medium on endometriosis-induced oocyte poor quality in an experimental mouse model	Experimental	• L-arginine, L-carnitine, and bone marrow mesenchymal stem cell-conditioned medium have therapeutic effects on endometriosis-induced oocyte poor quality. • Offer potential alternatives to conventional therapies during assisted reproductive technologies for patients with EMS-associated sub/infertility.
Gao et al. (2022)[16]	Mesenchymal stem cells therapy: A promising method for the treatment of uterine scars and premature ovarian failure	Review article	• Mesenchymal stem cell therapy is a promising method for the treatment of uterine scars and premature ovarian failure. • Extracellular vesicles secreted by MSCs may be a promising tool to restore normal reproductive function.
Wilczyński J et al. (2022)[17]	Endometriosis Stem Cells as a Possible Main Target for Carcinogenesis of Endometriosis-Associated Ovarian Cancer (EAOC)	Systematic review	• EMSCs were present in endometriotic lesions and exhibited characteristics of mesenchymal stem cells, including their ability to differentiate into various cell types, self-renew, and migrate
Levent M (2015)[18]	The Endometrium as a Source of Mesenchymal Stem Cells for Regenerative Medicine	Review article	• MSCs can be obtained through non-invasive procedures such as endometrial biopsy or menstrual blood collection. • eMSCs exhibit superior regenerative potential compared to other sources of MSCs, making them attractive candidates for various regenerative medicine

	applications, including tissue engineering, wound healing, and immunotherapy.

Endometriosis is a complex disease characterized by the growth of endometrial-like tissue outside the uterus, leading to a variety of symptoms and complications.[19,20] The complex mechanisms involved in the development and progression of endometriosis, factors contributing to its pathogenesis, current diagnostic and therapeutic strategies, the importance of the endometrium as the endometrium of the uterus, and potential consider treatment.[21–24]

A number of complex mechanisms are involved in the development of endometriosis. The theory of retrograde menstruation suggests that menstrual blood containing endometrial cells flows back into the pelvic cavity and implants and grows in ectopic sites.[25,26] Other mechanisms, such as coelomic metaplasia and stem cell transformation, may also contribute to the development of endometriosis.[8,27]

The factors also have been identified that contribute to the pathogenesis of endometriosis. These factors include hormonal imbalance, genetic predisposition, immunodeficiency, inflammation, angiogenesis, etc. Each factor creates a favorable microenvironment for the survival and growth of ectopic endometrial tissue.[8] Accurate diagnosis of endometriosis is essential for effective treatment.[28] Diagnostic strategies often include a combination of imaging studies to assess history, pelvic examinations (such as ultrasound imaging and magnetic resonance imaging), and laparoscopic surgery for definitive diagnosis and staging. Treatment options depend on the severity of symptoms, age, and reproductive goals. This may include pain management, hormone therapy, surgery, and assisted reproductive technology (ART) for related infertility.[9,29]

The endometrium serves as the lining of the uterus and undergoes dynamic changes during the menstrual cycle.[30] Cyclic growth regeneration, differentiation, and excretion occur under the influence of hormonal fluctuations, particularly estrogen and progesterone. To understand the pathophysiology of endometriosis, it is important to understand the role of the endometrium.[9,31,32] ESCs are of interest for their potential therapeutic applications in reproductive disorders. They are derived from the endometrium and have the ability to self-renew and differentiate. [10,33]ESCs play an important role in endometrial repair and regeneration, contributing to the development of new therapeutics. ESCs show promise in diseases such as endometrial damage and infertility, offering potential benefits in regenerative medicine.[10,32]

Stem cells play an important role in the regeneration and maintenance of the human endometrium. During each menstrual cycle, these cells contribute to the cyclical growth regeneration and differentiation of the endometrium. In the pathological setting of endometriosis, stem cells may be

involved in the development and progression of endometriotic lesions.[11,34] The pathogenesis of endometriosis involves the ectopic implantation and proliferation of endometrial-like tissue outside the uterus.[35] Research results suggest that EnSCs may have therapeutic potential in the treatment of endometriosis due to their ability to repair and regenerate the endometrium.[35,36] Furthermore, EnSCs are promising early diagnostic targets for detecting endometriosis.[12] Aberrant stem cell trafficking and engraftment at ectopic sites is associated with the development of endometriotic lesions. This process involves the migration of stem cells from the endometrium to ectopic sites, where they contribute to the formation and maintenance of endometrial tissue.[13,37]

Mesenchymal Stem Cells (MSCs) have been found to be involved in the development and maintenance of endometriosis. MSCs have the ability to differentiate into various cell types, including cells found in endometriotic lesions. Due to their presence in the endometrium and their role in angiogenesis due to inflammation and hormonal changes, they play an important role in the pathogenesis of endometriosis.[13] Inflammatory angiogenesis and hormonal changes during the menstrual cycle affect stem cell migration and implantation in endometriotic lesions.[21,38,39] It has been shown to give. These factors create a favorable microenvironment for stem cell survival and differentiation and contribute to the progression of endometriosis.[13]

Stem Cells from Endometriotic Lesions exhibit characteristics of MSCs, including self-renewal and potential to differentiate into different cell types[40]. Stem cells play an important role in both physiological and pathological processes in the human endometrium. The lining of the uterus, the endometrium, undergoes periodic changes during the menstrual cycle and is controlled by hormonal interactions and the presence of stem cells. Stem cells are undifferentiated cells with the ability to divide and generate progeny cells, and can either persist as stem cells or differentiate into specialized cell types.[41,42] In the case of the endometrium, stem cells are involved in tissue regeneration and maintenance.[42,43]

Studies have shown that stem cells derived from endometriotic lesions, which are abnormal proliferations of endometrial tissue outside the uterus, exhibit mesenchymal stem cell (MSC) characteristics.[8,44] MSCs are a type of stem cell that can self-renew and differentiate into various cell types.[33,45] Expression of certain stem cell markers such as CD146 and CD105 was found to be significantly increased in endometriotic lesions compared to healthy endometrium, indicating the presence of stem cells in these lesions.[8,46] Furthermore, the number of stem cells is significantly higher in endometriotic lesions than in healthy endometrial tissue, suggesting a possible role for stem cells in the development and progression of endometriosis.

Endometriosis is a disease characterized by the growth of endometrial tissue outside the uterus, causing a variety of symptoms and complications.[42,43] Although the pathogenesis of endometriosis is not fully understood, it is believed that the presence of stem cells and their abnormal behavior are related to the development of endometriotic lesions. Aberrant trafficking and engraftment of stem cells at ectopic sites may contribute to the formation of endometriotic lesions. These stem cells, especially MSCs, are involved in the initiation and maintenance of endometriosis.[47]

Inflammatory angiogenesis (the formation of new blood vessels) and hormonal changes during the menstrual cycle are important factors that can influence stem cell migration and implantation into endometriotic lesions. These processes create a microenvironment conducive to stem cell survival and proliferation.[33,37] The self-renewal and differentiation potential of stem cells derived from endometriotic lesions contributes to the heterogeneity of lesions and the presence of different cell types within lesions.[8,29]

The therapeutic potential of stem cells, especially MSCs, in the treatment of endometriosis is an area of active research. Conditioned medium containing L-arginine, L-carnitine, and bone marrow mesenchymal stem cells has shown therapeutic efficacy against poor egg quality caused by endometriosis and has been shown to reduce endometriosis-associated infertility. It has the potential to replace conventional treatment in assisted reproductive technology for patients. In addition, mesenchymal stem cell therapy has shown promise in the treatment of uterine scarring and premature ovarian failure. [15]

Extracellular vesicles (EVs) have emerged as essential components of cell-to-cell communication). These small vesicles are released by various cell types, including mesenchymal stem cells (MSCs), to facilitate intercellular biological interactions. Plays an important role in signalling and molecular cargo transduction.[48] In the context of reproductive medicine, electric vehicles derived from mesenchymal stem cells may be used to restore normal reproductive function and treat conditions such as endometriosis. can be expected.[49] MSCs, including endometrial MSCs (eMSCs), exhibit unique properties, such as the potential for self-renewal differentiation into different cell types and the ability to migrate. These properties make MSCs a valuable resource for regenerative medicine applications. EVs produced by MSCs inherit the regenerative capacity of their parental cells and act as intercellular communication mediators.[50] In the field of reproductive medicine, MSC-equipped electric vehicles are a potential tool for restoring normal reproductive function and treating conditions such as endometriosis.

Endometriosis is a complex gynecological condition characterized by the presence of endometrium-like tissue outside the uterus, causing pain, inflammation and fertility problems. Recent studies suggest

that mesenchymal stem cell-derived EVs may have therapeutic potential in the treatment of reproductive disorders associated with endometriosis.[45,51,52] These electric vehicles can transport a cargo of bioactive molecules such as proteins, lipids, nucleic acids, and growth factors that can modulate the cellular microenvironment and promote tissue regeneration and repair.[44,46]

In the case of endometriosis, MSC-derived EVs may influence multiple aspects of the disease.[42,43] For example, electric vehicles can modulate immune responses, reduce inflammation, and promote tissue regeneration. EV charging may contain anti-inflammatory factors such as cytokines and chemokines that modulate immune cell function and may alleviate the inflammatory milieu associated with endometriosis. By delivering these bioactive molecules, EVs restore balance to the endometrial microenvironment and help normalize reproductive function.[34]

One advantage of using MSC-derived EVs is that the MSCs themselves can be obtained non-invasively.[9,45] MSCs can be obtained through procedures such as endometrial biopsy and menstrual blood sampling, making them readily accessible as a source for research and potential therapeutic use. The use of endometrial or menstrual-derived MSCs, so-called eMSCs, has particular advantages over other MSC sources. eMSCs have been shown to have superior regenerative capacity compared to other sources, making them attractive candidates for a variety of regenerative medicine applications, including tissue engineering, wound healing, and immunotherapy.[34]

4. Conclusion

Endometriosis is a complex disease affected by different mechanisms. and factors. To effectively manage this condition, it is important to understand etiologic diagnostic strategies and therapeutic options. Furthermore, exploring the role of the endometrium and the potential of ESCs in regenerative medicine will provide insight into innovative therapeutics for reproductive disorders. Ongoing research and advances in this field promise to improve the lives of those affected by endometriosis.

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Research Article

Evaluation of erythrocyte index as metabolic indicator in stunted children

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Abstract. Stunting is characterized by impaired blood profiles in stunted children. This study aims to identify changes in the red blood cell parameters in children as a metabolic parameter in stunting. This study used laboratory observational analytic methods with a cross-sectional approach. The study included a total of 74 participants aged 6-10 years. Primary data was collected by collecting sociodemographic data from the subjects, measuring anthropometric variables, and taking blood samples to analyze the erythrocyte index based on the hematological parameters of Erythrocytes, Hb and Ht Data analysis was performed using One Way Anova test for erythrocytes, Hb, Ht at 95% confidence level. Participants were 74 children, consisting of 33 boys and 42 girls. Based on the WHO classification of anthropometric measurements, it was found that 46 people were normal and 29 people were classified as stunting. Erythrocyte index analysis based on hematological parameters showed significantly different values in the boy group compared girl group on the parameters Erythrocytes, Hb, and Ht. There was a significant decrease in the erythrocyte index parameter in the boy and girl groups, where the stunting condition further exacerbated the decline in the erythrocyte parameters, Hb, and Ht in the boys group and in girls group.

Keywords: eritrocyte index; metabolic indicator; stunted children

1. Introduction

The most common form of malnutrition worldwide is stunting or linear growth deficit, which is generally recognized as an important public health indicator for monitoring the health of the population.[1] In 2020, it is estimated that around 149.2 million children under the age of 5 are classified as stunted, and this number constitutes 22% of the total global population of children under 5 years.[2] Most cases of stunting occur in low and middle income countries (LMICs) where there are inadequate sources of nutrition for development which causes chronic malnutrition.[3] Stunting is still one of the unresolved nutritional problems in Indonesia.[4] Indonesia ranks third in the world with the highest number of stunting cases.[5] According to the 2018 Basic Health Research (Riskesdas), there are 30.8 percent of toddlers who are stunted in Indonesia, down from 37.2 percent in 2013.[6,7]

Stunting is assessed based on the height-for-age index (H/A) with a threshold (z-score) < -2 Standard Deviation (SD) for child growth and development.[8] The underlying cause of stunting is a gradual and continuous process that can be started from the nutritional factors experienced by the mother before and during pregnancy. These factors impact children's growth both during fetal development, during childhood, and throughout their lives.[9] Barker and colleagues were the first to discover that malnutrition throughout fetal, infancy, and early childhood can permanently alter body structure and function through a phenomenon known as 'programming'.[10] Stunted children are observed to have a higher likelihood of experiencing recurrent infectious diseases and an increased risk of metabolic disorders. This condition arises from disturbed energy utilization in the affected child's body.[11]

Stunting is associated with anthropometric and hematological abnormalities in children, which are common in many developing countries, especially among economically disadvantaged families.[12] The hematological condition of children plays an important role in reflecting their metabolic and nutritional status, as well as showing various hematological disorders such as anemia, infection and vitamin deficiency.[13] Erythrocyte indices/parameters are an integral part of the complete blood count (CBC), which is a collection of analytical tests commonly used to differentiate between different forms of anemia.[14] These parameters include red blood cell count (RBC), hematocrit (HCT), and hemoglobin (Hb).[15] As the main transport of oxygen from the lungs to the peripheral tissues, Erythrocytes and their components play an important role in the tissues as well as metabolism throughout the human body.[16] Changes in the RBC index are associated with metabolic disorders such as high blood pressure, type 2 diabetes, and metabolic syndrome.[17]

In developing countries, about 53% of school-aged children and 42% of preschool-age children suffer from anemia. The occurrence of malnutrition or stunting in these children is not solely related to a lack of intake of carbohydrates, protein and other macronutrients, but also due to unmet mineral needs, such as calcium and iron.[13] Therefore, it is very important to implement blood laboratory-based screening assessments to facilitate the early identification of cases of stunting and associated comorbidities. This approach plays an important role in early detection of stunting in children by utilizing indicators derived from the erythrocyte index. Thus, it is hoped that appropriate management strategies can be developed to deal with stunting cases effectively, thereby reducing the prevalence of stunting in children based on blood laboratory profiles.

2. Methods

2.1. Study design

This research is a laboratory analytic observational study with a cross-sectional approach that aims to analyze and describe the erythrocyte index variable as a metabolic parameter and its potential for early detection of stunting in children. The data collection process included collecting sociodemographic and anthropometric information from participants in public elementary schools located in South Lampung Regency. Subjects were selected using cluster random-sampling. Furthermore, blood samples were taken from the subjects, and the erythrocyte index was analyzed at the Laboratory of Biomolecular, Biochemistry, and Physiology, Faculty of Medicine, University of Lampung. The study was conducted from May to October , 202 1 . The research proposal has received ethical approval with reference number 3898/UN26.18/PP.05.02.00/2022 from the Ethics Committee for Health Research, Faculty of Medicine, University of Lampung

2.2. Research participants, inclusion and exclusion criteria

Study participants were selected from the accessible population based on specific inclusion criteria, exclusion criteria and their willingness to give informed consent. Inclusion criteria included children aged 6-10 years, both boys and girls, with stunting and non-stunting cases. In addition, the parents of these children cooperated in being asked for the required information and expressed their willingness to participate in the study by signing a consent form. Exclusion criteria included subjects or parents who did not complete the treatment according to the procedure. Following the study design, sample size was determined using Slovin's formula, resulting in a total of 70 participants. The target population of this study were elementary school children grades 1 to grade 4 regardless of stunting status, aged 6-10 years, in a defined geographical area. A sample of 74 participants was selected using cluster random sampling technique.

2.3. Data collection and analysis of blood samples

Prior to the data and sample collection process, each parent/guardian of the study participants received a detailed explanation of the purpose, sequence of activities, potential benefits, and possible adverse effects associated with the research. Parents were asked to sign a consent form if they agreed to participate in the entire research process. The participants in charge (parents/guardians) were given pre-research counseling regarding the procedures and stages involved in the research as well as shared views on the questionnaire questions. This was followed by interviews about the social and demographic profiles of parents and study participants.

Anthropometric measurements, including the use of a microtoise to measure height and digital scales to measure body weight, were carried out on study participants. This measurement is carried out to identify the characteristics associated with stunting. Stunting cases were determined based on WHO criteria, with a z-score for height for age more than 2 standard deviations below the World Health Organization (WHO) Child Growth Standards median.[18]

After interviews and anthropometric measurements, a 3 mL venous blood sample was collected from each eligible participant sterilely using a sterile 3 cc syringe. Blood is drawn using aseptic technique. The collected blood is then channeled into a labeled test tube containing ethylene-diamine-tetra acetic acid (EDTA) as an anticoagulant for the analysis of the red cell index. To maintain the integrity of the sample, the test tube containing the blood specimen was immediately brought to the Laboratory of Biomolecular, Biochemistry and Physiology, Faculty of Medicine, University of Lampung. Erythrocyte parameter analysis was carried out on the same day as specimen collection to avoid hemolysis of whole blood. Erythrocyte inedx parameters were analyzed using an automated hematology analyzer.

2.4. Statistic analysis

Data were checked for completeness and entered into the Statistical Package for Social Science (SPSS) software for statistical analysis. All variables are cleaned through missing data analysis to avoid missing values. One way ANOVA was used to determine the strength of the relationship between stunting status and each erythrocyte index, a P value of less than 0.05 was considered statistically significant.

2.5. Data quality management

To ensure data quality, comprehensive training and orientation is provided to all data collectors. Principal researchers diligently review and check data for accuracy, clarity, and completeness on a daily basis. Every step of sample collection, handling, processing and analysis is carried out with care to address potential quality issues and to ensure proper test results. In addition, thorough checks were carried out to verify the expiry dates of all reagents used, and both instruments were calibrated daily according to the manufacturer's recommendations prior to testing the actual samples. Strict adherence to these quality control measures is in place to maintain data integrity and reliability.

3. Result and Discussion

3.1. Characteristic of study participants

The sample involved in this study was 74 children aged 6-10 years. Most of the samples were girls (56%), with an average age of 7.89 years. The majority of the sample, both male and female, have a nutritional status that is in the normal category with an average BMI of 15.17. In this study, there were 24.3% of children diagnosed with stunting and 13.5% of children diagnosed with very stunting. The characteristics of the sample are presented in table 1 below. The sample involved in this study was 74 children aged 6-10 years. Most of the samples were women (55.4 %), with an average age of 7.95 years . The majority of the samples had normal nutritional status (81.1 %) with an average BMI of 15.17 . In this study, there were 28 children (37.8 %) who were stunted, while the rest were not stunted. The characteristics of the sample are presented in table 1 below.

Table 1. Sample characteristics

Parameter	Total (n = 74)	Male (n = 33)	Female (n = 41)
Age (Mean±SD)	7.89±0.94	7.82±0.92	7.95±0.97
Nutritional status			
- Normal	60 (81.1%)	25 (75.8%)	35 (85.4%)
- Underweight	11 (14.9%)	7 (21.2%)	4 (9.8%)
- Overweight	3 (4.1%)	1 (3.0%)	2 (4.9%)
BMI (Mean±SD)	15.17±1.59	15.11±1.59	15.22±1.61
Stunting Status			
- Normal	46 (62.2%)	20 (60.6%)	26 (63.4%)
- Stunt	18 (24.3%)	9 (27.3%)	9 (22.0%)
- Very Stunting	10 (13.5%)	4 (12.1%)	6 (14.6%)

In this study, out of 74 study participants, 28 children (37.8%) were stunted. This percentage is higher than the stunting rate in Indonesia, which is 30.7%.[7] This is because South Lampung Regency is included in the stunting priority area because the stunting rate in that area is quite high.[19] Meanwhile, based on their nutritional status, most of the study participants were in the normal nutrition category (81.1%), then underweight (14.9%) and overweight (4.1%). Research by Voanesch et al also found that the majority of stunting sufferers had good nutritional status, namely as many as 86%, followed by 10% fat BMI, and the remaining 4% thin BMI.[20] Nutritional status describes the adequacy of calories and nutrition in the acute phase. Primary acute malnutrition is caused by food intake due to economic (poverty), social, political and environmental factors which are usually found

in lower middle-income countries, but stunting is often associated with chronic malnutrition, especially in the first 1000 days of life.[21] On the other hand, abnormal nutritional losses, increased energy expenditure, or inadequate nutrient intake due to underlying disease, such as chronic renal failure, chronic liver failure, malignancy in children, cystic fibrosis, or tuberculosis lead to secondary acute malnutrition.[22] In contrast to acute malnutrition, stunting is caused by a long-term lack of protein intake, even looking further back, several studies have found a relationship between maternal nutritional status during pregnancy and maternal protein intake and stunting.[23]

3.2. Correlation between erythrocyte index and stunting status

The average sample in this study had erythrocyte parameter values that were still within normal limits. This is reflected in the average Hb, Ht and total erythrocyte counts of all samples which were still within normal limits (Table 2).

Table 2. Study participant erythrocyte index

Parameter	N	Average±SD (Min – Max)	
		Male	Female
Erythrocytes (million/µl)	74	4.4±0.3	4.4±0.4
Hb (g/dL)	74	12.3±1.3	12.1±1.1
Ht (%)	74	35.9±2.7	35.6±2.2

In this study, it was found that children who suffered from severe stunting, both in the male and female groups, had significantly lower levels of Hb, Ht, and the number of erythrocyte cells when compared to normal children (Table 3).

Table 3. The levels of hematological parameters in children with different nutritional status

Parameter	Male				*p*	Female				*p*
	Total (n=33)	Normal (n=20)	Stunted (n=9)	Severe Stunting (n=4)		Total (n=41)	Normal (n=26)	Stunted (n=9)	Severe Stunting (n=6)	
Erythrocyte (Mill/µl)	4.4±0.3	4.5±0.4	4.3±0.2	4.2±0.2	0.031*	4.4±0.4	4.5±0.3	4.2±0.3	4.0±0.2	0.003*
Hb (g/dL)	12.3±1.3	12.6±1.1	12.4±1.2	10.2±0.4	0.001*	12.1±1.1	12.5±0.8	11.8±0.8	10.5±0.7	0.000*
Ht (%)	35.9±2.7	36.7±2.5	35.8±2.5	32.5±1.9	0.015*	35.6±2.2	36.6±1.8	34.7±1.9	32.6±1.3	0.000*

Note: * significantly different based on the One Way Anova and Kruskall Wallis tests at the 95% confidence level.

In general, the results of examining the parameters of the erythrocytes in this study were still within normal limits. Based on statistical tests, in boys found significant differences in the parameters of

erythrocytes (p= 0.001), Hb (p= 0.031), Ht (p=0,015) in the normal, stunting and severe stunting groups. Whereas in women found significant differences in almost all parameters. Research by Gaston et al showed that there was a positive correlation between the incidence of stunting and anemia, which indicated that there was a high probability that stunting would be followed by stunting or vice versa, resulting in a reciprocal effect between the two.[24] One of the possible reasons for this problem is that children's hematological and immune systems are still developing and develop developmental defects, requiring more nutrients to maintain rapid body growth, chronic nutritional deficiencies in stunted children trigger a decrease in various metabolic variables that can reduce various parameters of the erythrocyte index. In addition, many children in the first 1000 days of life are not optimally breastfed either from early initiation of breastfeeding or lack of exclusive breastfeeding or cessation of breastfeeding/weaning too early which affects their hematological and immune status making them susceptible to various diseases. Some of these immune disorders can also cause hemoglobin levels in the blood to drop, which can cause anemia and stunting. In addition, as children grow and are introduced to new foods and are able to digest a variety of nutrients, they will be less likely to develop anemia or stunting.[25] As an adaptation to a more limited oxygen supply, poor convective O_2 transport by blood and decreased O_2 uptake in cardiac myocytes are projected to shift metabolism towards a glycolytic phenotype in anemia.[26] Elevated glycolytic levels will also result in increased lactic acid formation, potentially lowering intracellular pH, a significant regulator of cardiac function[29,30]. Iron deficiency anemia can alter cardiac gene expression via iron and oxygen sensitive enzymes such as prolyl hydroxylases (PHDs) and lysine demethylases (KDMs), which regulate the transcription factor hypoxia inducible factor (HIF) and histone methylation markers.[27]

4. Conclusion

It was found that there was a significant decrease in the erythrocyte index from the parameters of erythrocytes, hemoglobin, hematocrit which were significant with different distribution of parameters in the group of boys and girls. Laboratory results showed that the more severe stunting conditions would trigger an decrease in the parameters of Erythrocytes, Hb, Ht, MCH in the boys group and the the girls group.

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Literature Review

Modality interventions for neurogenerative individuals: a literature review

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Abstract: Neurodegenerative disorders are terms used for the progressive loss of neuronal cell structure or function, including neuronal cell death. Neurocognitive disorders are a group of conditions that often lead to impaired mental function. Neurocognitive disorders are also called organic brain syndromes. Neurocognitive disorders are often found in the elderly, but neurocognitive disorders can also occur in younger people. Alzheimer's, Parkinson's and ALS are some of the many neurodegenerative diseases that are triggered by the damage and death of brain cells, leading to various disorders of body functions. These disorders are commonly experienced by senior citizens over the age of 60. So it is necessary to take action to ward off damage that worsens a disease in neurodegenerative disorders by means of modality interventions that can be adjusted to the current situation. The purpose of this study was to determine the Intervention Modalities for Neurodegenerative Individuals. The method used is a literature review by conducting a structured literature review using academic database sources from ScienceDirect, proquest and Google Scholar. The search results found that Modality Interventions for Neurodegenerative Individuals are very varied so that we can adjust to their respective circumstances.

Keywords: modality intervention; neurodegenerative

1. Introduction

Neurodegenerative disorders are pathological conditions in nerve cells where the nerve cells experience a progressive loss of their actual structure or function. Neurodegenerative disorder is a term used for the progressive loss of neuronal cell structure or function, including neuronal cell death. The number of elderly people in Indonesia is increasing rapidly compared to other countries in the world. Neurodegenerative disease refers to an umbrella term for a variety of health conditions that primarily impact neurons in the brain. The word neurodegenerative can be divided into two parts; neuro and degenerative. Neuro refers to the brain, while degenerative means breaking down or loss of function of organs and tissues. It is also considered a slow progressive failure of nerve cells. Neurons are important brain cells as they are responsible for effective brain function. This includes remembering certain tasks, movements, conversing with friends or thinking about school work. Brain disorders due to miscommunication in one region disrupt brain activity. Identification of disturbances in resting state functional connectivity between neurocognitive networks shows conflicting connectivity patterns.[1]

This results in various diseases and illnesses of which the most complicated is neurodegeneration disease. Increase in the number of elderlies in Indonesia BPS noted that Indonesia has entered an old population structure since 2021. This is because the percentage of the elderly population has reached more than 10 percent. This figure has increased by 3 percent for more than a decade, reaching 10.82 percent. The increasing number of elderly people is the result of successful development, especially in the health sector. Risk factors that influence the occurrence of mistreatment of the elderly include low social support, stress burden from caregivers, cognitive impairment of the elderly, low economic levels and physical function dependence such as the elderly needing assistance in daily activities. So with a significant increase, if not accompanied by a health management process, it becomes a risk. Neurodegenerative disorders are terms used for the progressive loss of neuronal cell structure or function, including neuronal cell death. Neurodegenerative disorders are a group of conditions that often lead to impaired mental function. Especially degenerative diseases. This disease is mainly suffered by the elderly. The identification of disturbances in the functional connectivity of resting states between neurocognitive networks shows conflicting patterns of connectivity neurodegenerative diseases seem to have in common some common multifactorial degenerative processes that contribute to neuron death, leading to functional impairment Due to multifactorial and complex aspects.[2] The elderly are prone to disease, neurocognitive disorders are also called organic brain syndromes. Neurocognitive disorders are often found in the elderly, but neurocognitive disorders can also occur in younger people Alzheimer's, Parkinson's and ALS are some of the many neurodegenerative diseases that are triggered by the damage and death of brain cells, leading to various disorders of body functions. Moreover, the elderly are less active and do not carry out routine health checks. So the need for interventions that can be carried out by the elderly, one of which is with modality therapy that can be done by the elderly to reduce the risk of neurodegenerative disorders. The results of research by Memon et al, 2020 state that exercise therapy is the best way to monitor the response to the effect of exercise on sleep dysfunction in Huntington's disease and amyotrophic lateral sclerosis; and the mechanisms underlying exercise-induced sleep modification. These disorders are commonly experienced by senior citizens over the age of 60. So it is necessary to take action to ward off damage that worsens a disease in neurodegenerative disorders by means of modality interventions that can be adjusted to the current situation. The purpose of this study was to determine the intervention modalities for neurodegenerative individuals.

2. Methods

Writing this systematic review is designed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines which discuss modality interventions for neurodegenerative individuals. The period of searching for articles was from 10 - 15 August 2023. The journals selected in this study were obtained based on accredited databases ScienceDirect, proquest and Google Scholar. Article searches use words by looking at the match of keyword writing, results and discussion of articles. The literature was searched using the keywords Modality Intervention, and Neurodegenerative which were made based on Boolean operators (AND, OR NOT or AND NOT) so that it could be used to expand or specialize the search. In the ScienceDirect database search, the keywords "Modality Intervention AND Neurodegenerative" were typed in, then specifically selected only "Reasearch Articles" and "Open Access & Open Archieve". The search time limit starts from 2013 to 2023 with specific criteria, namely open access in manuscript form and in English and Indonesian. The literature search begins with the title screening stage and then thoroughly reviews articles to obtain articles that are in accordance with the theme and research objectives. Literature searches in systematic reviews are only limited to research articles with quantitative research types. In this literature review, the inclusion criteria used are: 1) The year of publication of the article starts from 2013 - 2023, 2) Open access and full text, 3) Category of research articles related to the modality interventions for neurodegenerative individuals, 4) Articles in English and Indonesian. English and Indonesian. While the exclusion criteria used, namely: 1) Publication articles that cannot be accessed in full. The stages of searching for articles are as shown in Figure 1.

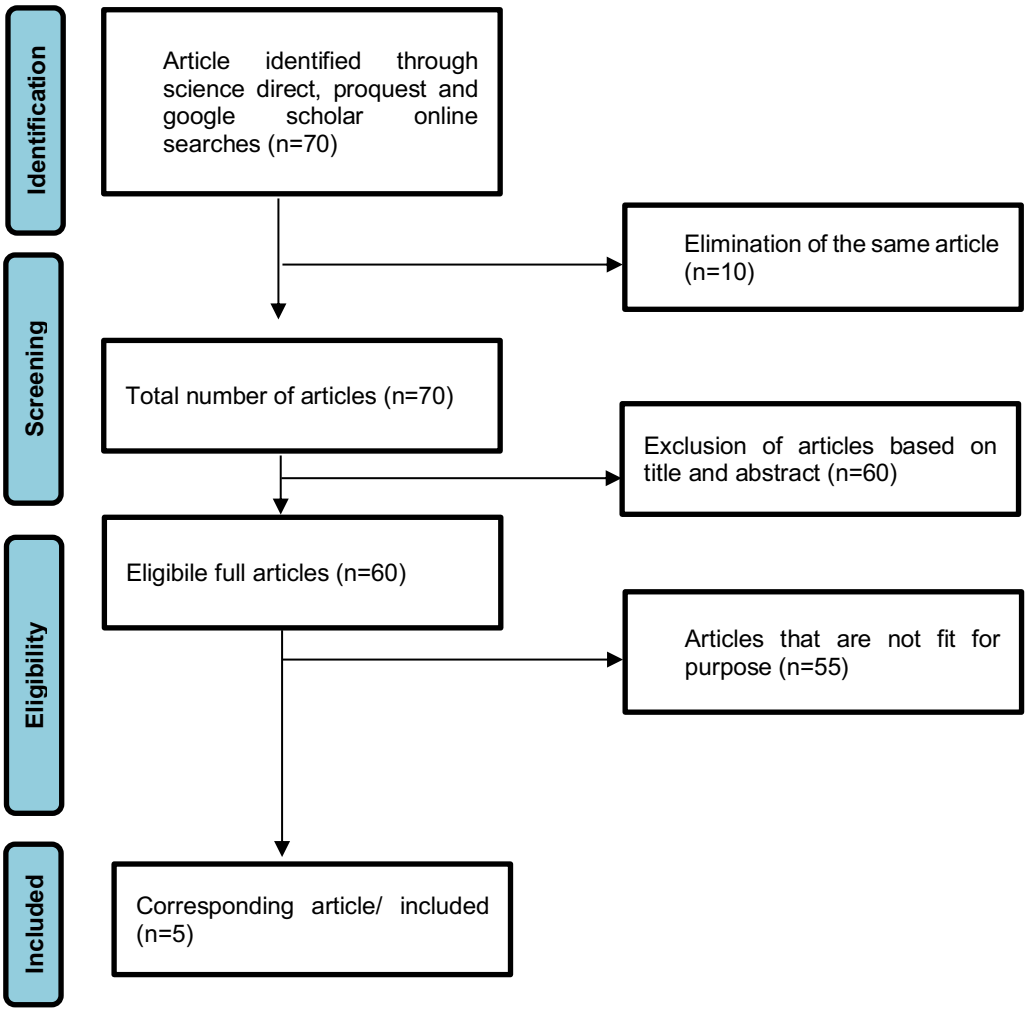

Figure 1. Prisma diagram

3. Results and Discussion

The first stage carried out by the author is to select appropriate research results. After that, look at the year of preparation based on the selection set.

Number	Author	Research methods	Research results
1	Adeel A. Memon, Juliana J. Coleman Amy W. Amara	Review article	Additional research is required in order to understand the most effective exercise therapy for these indications; the best way to monitor the response to interventions; the influence of exercise on sleep dysfunction in Huntington's disease and amyotrophic lateral sclerosis; and the mechanisms underlying exercise-induced sleep modifications.

2	Douglas A. Wajda, Anat Mirelman, Jeffrey M. Hausdorff & Jacob J. Sosnoff	To be included in the review the following inclusion criteria had to be met: 1) experimental design (randomized controlled trials (RCT), non-RCT or pre-post study); 2) clinical intervention; 3) included individuals with a NDD; 4) incorporated at least one outcome measure of cognitive-motor dual tasking both prior to and following training; 5) be peer reviewed; and 6) published in English. Exclusion criteria consisted of: (1) Non- English language publications, (2) non-experimental design study (e.g. review paper), 3) not including individuals with NDD; and (4) non-peer reviewed articles (dissertation or conference proceedings). It is important to note that interventions focusing on CMI in stroke where not included. Although neurodegeneration can occur after a stroke and that some do include stroke among neurodegenerative diseases. It is commonly thought that neurodegeneration in stroke is a secondary process and not the primary pathology. Included articles were analyzed for methodological rigor. This was accomplished utilizing an eleven-item system, with each item rated on a 0-2 scale. The customized checklist was created based on the recommendation of the Cochrane Methodological Quality Assessment Tool [41]. Scores of 0 indicate the article did not meet the criterion, 1 indicated partial fulfillment and/or inadequate explanation and a score of 2 indicated completely meeting the criterion. The eleven categories encompassed study design (e.g. RCT vs. Cross	Overall, the relevant literature contained samples from 4 different neurological diseases and one NDD precursor: Parkinson's disease, multiple sclerosis, Alzheimer's disease (AD), dementia other than AD, and mild cognitive impairment. Of these, 14 studies focused on PD, 3 on MS, 2 on AD, and 1 each on dementia and mild cognitive impairment (MCI). Additionally, three predominant intervention strategies were identified including virtual reality/interactive training (n=6), multimodal gait and balance training (n=11) and cueing training (n=4). The findings from the included literature are expanded upon below in relation to the main training methods. The methodological quality assessment resulted in scores ranging from 3-20 out of a possible 22. The primary driver of scores was study design, with RCTs receiving higher scores based on the inclusion of control groups, blinded randomization and generally the ability to have assessors and trainers blinded to group assignment. Table 4 outlines the scores across each of the eleven items as well as the overall total for each of the included studies. In general, the multimodal exercise studies had greater quality scores compared to virtual reality/interactive training and cueing training.

		Sectional), randomization and blinding procedures (e.g. blinded assessors and trainers), statistical methods (e.g. Loss to follow up examination) and group comparisons at baseline.	
3	Jose A. Santiago and Judith A. Potashkin	Review article	We propose an evidence-based multidomain approach that includes physical activity, diet, cognitive training, and sleep hygiene to treat and prevent neurodegenerative diseases.
4	Ruiz-González David Hernández-Martínez Alba Valenzuela Pedro L. Morales Javier S. Soriano-Maldonado Alberto	A systematic review was performed on the following electronic databases: PubMed, Scopus, Web of Science, PsycINFO and Cochrane (with no restriction on the starting date and up to November 26th, 2020). The full search strategy is presented in Supplementary File 1. We also manually scanned reference lists, Google Scholar, and grey literature (TESEO and OpenGrey). The literature search results were first screened independently by two reviewers (A.H-M., D.R-G.) by title and abstract. Articles eligible for full-text screening were independently assessed by the same two reviewers. Disagreements were resolved through discussion with a third reviewer (J.S.M.).	Physical exercise interventions increase plasma BDNF levels in individuals with neurodegenerative disorders.
5	Raafi Haidar Arrasyid , Endah Sari Ratna Kumala , Nisrina Hanifah Afnan	Analyzed related sources, the majority of which came from journals retrieved from PubMed, Science Direct, and Spinger. Memantine is a drug that has been used in Alzheimer's treatment by blocking NMDA receptors thereby protecting nerve cells from inflammatory reactions and inhibiting the formation of β-Amylod aggregates	To improve the bioavailability, therapeutic precision, and the the ability to penetrate the Blood-Brain Barrier (BBB) of the two ingredients, nano-chelators are used. Through the effect of inhibiting the formation of β-amyloid aggregation and antioxidants from the combination of memantine and melatonin with nano-

	chelator encapsulation, it is expected that Alzheimer's therapy can be more effective and efficient.

In the first research results stated that the best way to monitor the response to interventions; the influence of exercise on sleep dysfunction in Huntington's disease and amyotrophic lateral sclerosis; and the mechanisms underlying exercise-induced sleep modifications. this suggests that modal therapy in degenerative diseases can be done and has a good effect on neural changes.[3] In both research studies, multimodal exercise had greater quality value than virtual reality/interactive. In the third study, it was stated that physical activity, diet, cognitive training, and sleep hygiene to treat and prevent neurodegenerative diseases.[4] So it is necessary for families who care for individuals who experience neurodegenerative disorders to pay attention to the surrounding environment for a better healing or treatment process. The fourth research result states that physical exercise interventions increase plasma BDNF levels in individuals with neurodegenerative disorders.[5] Regular physical activity has a good impact on the management of neurodegenerative diseases. So that the modality therapy carried out will be more effective.[6] The fifth research result states that β-amyloid aggregation and antioxidants from the combination of memantine and melatonin with nanocellator encapsulation are expected to make Alzheimer's therapy more effective and efficient.[7] This shows that the interventions carried out can have a good impact on neurodegenerative (Alzheimer's). Intervention and the evaluation of multiple task functions require the selection of cognitive therapy.[8] Resting-state functional MRI can be used to show aberrant functional connectivity within the basal ganglia of patients with neurodegeneration (early Parkinson's disease and not seen in Alzheimers disease.[9]

4. Conclusion

Modality therapy has different effects with neurodegenerative diseases, so that the therapy carried out must be adjusted, seen from the results of research stating that from the five studies stated that modality therapy has a good effect on neurodegenerative diseases.

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Research Article

The association between the duration of the semester and vitamin D status among anesthesia resident doctors

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Abstract: Vitamin D is one of the essential micronutrients needed by the body. Vitamin D deficiency can increase the risk of various degenerative diseases. One of the populations who are at risk of experiencing vitamin D deficiency are health workers who work in closed rooms or are not exposed to sunlight, including doctors who are undergoing specialist medical education programs in operating room education facilities. The aim of this study is to analyze the vitamin D status of anesthesia residents working in the operating room and the risk factors that influence it. 61 Anesthesia Residents of the Faculty of Medicine, Universitas Sriwijaya. The study used is cross-sectional study. Sampling technique isusing total sampling. Serological test used is the ELISA test. Statistical test is using Fisher-Exact Test. 42 (68.85%) of the anesthesia residents are experiencing vitamin D deficiency (<12ng/ml), while 19 (31.15%) are experiencing vitamin D insufficiency (12<=20 ng/ml). There is a significant association between duration of the semester and vitamin D status among anesthesia resident doctors with p=0.014 (p<0.05). Duration of the semester associated with vitamin D status among anesthesia resident doctors.

Keywords: anesthesia resident doctors; duration of semester; vitamin D status

1. Introduction

Vitamin D is one of the essential micronutrients needed by the body. Vitamin D plays a role in calcium and phosphate metabolism, calcium hemostasis, vascular health, cell differentiation and proliferation. In addition, vitamin D is also often associated with several diseases, ranging from degenerative diseases to malignancies. Vitamin D deficiency can cause a decrease in the efficiency of absorption of calcium and phosphorus resulting in an increase in parathyroid hormone (PTH) levels. In addition, vitamin D deficiency is associated with insulin resistance, diabetes mellitus, ß-cell dysfunction, autoimmune disease, arthritis, multiple sclerosis, colon cancer, breast cancer, prostate cancer, hypertension, and cardiovascular disease.[1,2]

One of the populations who are at risk of experiencing vitamin D deficiency are health workers who work in closed rooms or are not exposed to sunlight, including doctors who are undergoing specialist medical education programs in operating room education facilities. Based on research conducted by Rimahardika, et al in 2017, it can be seen that even though people who work indoors get frequent sunlight exposure, the intensity of sunlight at these exposure times is relatively low. This is different

from people who work outdoors who get more frequent exposure to sunlight with optimal sunlight intensity.[3]

There are several risk factors for vitamin D deficiency (deficiency or insufficiency): age, sex, melanin levels, use of sunscreen, weather or season where you live, as well as the length and time of exposure to sunlight. Meanwhile, the main cause of vitamin D deficiency is lack of sun exposure.[2]

Many studies have shown the incidence of vitamin D deficiency in health workers who work in closed spaces, namely operating rooms, including doctors who are undergoing specialist medical education programs in operating room education facilities. However, in RSUP. Dr. Mohammad Hoesin Palembang, research like this has never been done. Therefore, this research is needed to determine the vitamin D status in anesthesia resident doctors and the risk factors that influence it.

2. Methods

The study used in this research is cross-sectional study. The sampling technique is total sampling, 61 Anesthesia Residents of the Faculty of Medicine, Universitas Sriwijaya. Independent variables studied are: age, gender, weight, height, BMI, duration of the semester, sunlight exposure, workout frequency, and vitamin D supplement consumption. Dependent variable studied is vitamin D concentration. Serological test used is the ELISA test. Statistical test used to analyze the data obtained is the Fisher-Exact Test.

3. Results and Discussion

The study's primary aim is to analyze the vitamin D status of anesthesia residents working in the operating room and the risk factors that influence it. In this study, 61 anesthesia resident doctors were screened. Respondents who participated in this study had a mean age of 32.2 ± 3.06. The subject's mean ± SD vitamin D level was 10.75 ± 2.89. The distribution of respondents are shown in Table 1. The result showed that 42 (68.85%) of the anesthesia residents are experiencing vitamin D deficiency (<12 ng/ml), while 19 (31.15%) are experiencing vitamin D insufficiency (12<=20 mg/ml). Independent variable studied showed different results in the association to vitamin D status. The significance association between variables are shown in Table 2. There is no significant association between age (p=0.6), gender (p=0.746), weight (p=0.237), height (p=0.942), BMI (0.366), sunlight exposure (0.469), outdoor workout frequency (0.753), vitamin D supplement consumption (1.000) and vitamin D status among anesthesia resident doctor (p>0.05). Meanwhile, there is a significant association between duration of the semester and vitamin D status among anesthesia resident doctor with p= 0.019 (p<0.05) with OR=7.143.

Table 1. Distribution of respondents

Variable	Results
Age, years (mean ± SD)	32.2 ± 3.06
Gender, n(%)	
Male	45 (73.78%)
Female	16 (26.22%)
Weight, (mean ± SD)	73.87 ± 17.15
Height, (mean ± SD)	166.9 ± 7.43
BMI, n(%)	
Normal	28 (459%)
Fat	9 (14.8%)
Obese	24 (39.3%)
Duration of the semester, n(%)	
Phase 1 (1-4)	24 (39.34%)
Phase 2 (5-6)	15 (24.59%)
Phase 3 (>7)	22 (36.07%)
Sunlight exposure, n(%)	
<5 hours/week	42 (68,9%)
5-10 hours/week	17 (27,9%)
Others	2 (3,28%)
Outdoor workout frequency, n(%)	
>3 times/week	1 (1.6%)
2-3 times/week	3 (4.9%)
1 times/week	36 (59%)
Never	21 (34.4%)
Vitamin D supplement consumption in the last 14 days, n(%)	
Yes	1 (1.6%)
No	60 (98.4%)
The dose of vitamin D consumed	
<1.500 unit/day	1 (1.6%)
1.500-2.000 unit/day	0 (0%)
Others	0 (0%)
Duration of vitamin D consumption	
3-6 months ago	0 (0%)
<3 months ago	0 (0%)
<14 days ago	1 (1.6%)
Vitamin D concentration, (mean ± SD)	10,75 ± 2,89
Vitamin D status	
Deficiency	42 (68.9%)
Insufficiency	19 (31.1%)
Sufficiency	0 (0%)

Currently, vitamin D has been the main focus of public health problems around the world. Besides causing bone disorders, vitamin D deficiency is also responsible for increasing various non-communicable diseases, such as obesity, hypertension, cardiovascular disease, diabetes mellitus, metabolic syndrome, cancer, neurological disorders, and others. Until recently, it is estimated that 1 billion people in the world are experiencing vitamin D deficiency. Using a cut-off of <20 ng/mL, the prevalence of vitamin D deficiency in Southeast Asia varies between 60-70%.[4] This fact in line with the result of this study which shown that 68.9% of the respondents fall in the category of deficient and the other 31.1% in the category of insufficient.

Table 2. Risk factors of vitamin D status

Variable	Deficiency (N=42)	Insufficiency (N=19)	P
Age, years (mean ± SD)	32.5 ± 2.99	31.6 ± 3.20	0.6*
Gender, n(%)			0.746+
Male	34 (80.95%)	11 (57.89%)	
Female	8 (19.05%)	8 (42.11%)	
Weight, (mean ± SD)	74.0 ± 15.,5	73.6 ± 20.9	0.237*
Height, (mean ± SD)	166.8 ± 7.5	167.2 ± 7.5	0.942*
BMI, n(%)			0.366+
Normal	18 (42.86%)	10 (52.63%)	
Fat	8 (19.05%)	1 (5.26%)	
Obese	16 (38.10%)	8 (42.11%)	
Duration of the semester, n(%)			0.019+
Phase **1 (1-4)**	14 (33.33%)	10 (52.63%)	
Phase **2 (5-6)**	8 (19.05%)	7 (36.84%)	
Phase **3 (>7)**	20 (47.62%)	2 (10.53%)	
Sunlight exposure, n(%)			0.469+
<5 hours/week	28 (66.67%)	14 (73.68%)	
5-10 hours/week	13 (30.95%)	4 (21.05%)	
Others	1 (2.38%)	1 (5.26%)	
Outdoor workout frequency, n(%)			0.753+
>3 times/week	1 (2.38%)	0 (0%)	
2-3 times/week	1 (2.38%)	2 (10.53%)	
1 times/week	25 (59.52%)	11 (57.89%)	
Never	15 (35.71%)	6 (31.58%)	
Vitamin D supplement consumption in the last 14 days, n(%)			1.000+
Yes	0 (0%)	1 (5.26%)	
No	42 (100%)	18 (94.74%)	

*Independent Samples Test
+Chi-Square Test

In terms of risk factors, the study conducted by Gowda, et al in 2016 showed that age and gender had a significant association to vitamin D status among general practitioners. The study indicates that advancing age and female gender were associated with vitamin D status.[5] While this study found that there is no significant association between either age or gender and vitamin D status.

A previous study has a different approach in variable with this study. The study conducted by Tantri, et al in 2023 didn't include weight, height, and BMI as the independent variable.[6] While this study included those variables and was found that there was no significant link to the vitamin D status.

Both of the studies have also found that there was no significant association between sun exposure and vitamin D levels among anesthesiologist residents, although there was a difference in the average span of sun exposure between the previous study and this study. The previous study showed that most of the residents got >10 hours of sun exposure a week, while in this study, the residents are lacking sun exposure with a duration of <5 hours a week. Another similarity in both results was the fact that outdoor exercise proven to have no significant association to vitamin D levels.[6]

On the other hand, the previous study stated that there was a link between dietary intake (vitamin D supplement and high calcium food) and vitamin D levels.[6] Meanwhile, in this study, there was no significant link found between vitamin D supplement consumption and vitamin D status.

Another variable that was studied in a number of studies but had not been included in this study was history of COVID-19 infection. There were different results shown for this variable. There was a study by Luo, et al in 2020 that stated that there was a significant association between vitamin D deficiency and COVID-19 severity in Chinese population. It was contradictory with the study conducted by Tantri, et al in 2023 that have found no significant association between the two variables.[7]

Despite all of the above, this study included one variable that had not been studied a lot, which was the duration of the semester. The reason was that the duration of the semester caused a difference in the term of resident's work. Long-term residents that work in operation room or ICU could develop vitamin D deficiency, and this study have found that there was a significant association between the duration of the semester and vitamin D status. Anesthesia resident doctors in phase 2 is 7.143 times more at risk of vitamin D deficiency compared to residents in phase 1. While, resident doctors in phase 3 is 8.750 times more at risk of vitamin D deficiency compared to residents in phase 1.

The limitation of this study that needs to be improved is that this study does not have a control group for comparison, because the goal was to analyze the vitamin D status of anesthesia residents working in the operating room and the risk factors that influence it with all of anesthesia resident doctors of Faculty of Medicine Universitas Sriwijaya. The overall result of vitamin D status in this study are low. Therefore, further thorough research is needed in term of determining the risk factors of vitamin D deficiency in anesthesia resident doctors.

4. Conclusions

All of anesthesia resident doctors of Faculty of Medicine Universitas Sriwijaya has vitamin D status under the designated cut off. There is an association between duration of the semester and vitamin D status among anesthesia resident doctors. Intervention in the form of providing vitamin D supplements, providing education, and further research on risk factors that affect vitamin D status in anesthesia residents of the Faculty of Medicine, Unsri is urgently needed.

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Research article

Physical activity and body fat are related to the physical fitness level of medical students

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Abstract: Physical fitness is an essential component for a person to be able to carry out physical activities every day effectively and efficiently without experiencing excessive fatigue. Physical fitness is essential in determining health status, morbidity, and mortality, both in children and adults. Physical fitness level is influenced by various factors such as age, gender, race, genetics, physical activity, lifestyle, nutritional status, and health status. The aim of this study was to analyze the relationship between physical activity level and body fat with physical fitness. This study was an observational analytic with a cross-sectional approach. Sixty-eight respondents were candidates for the student organization of the medical assistance team at the faculty of medicine, Universitas Sriwijaya. Data were taken primarily through filling out physical activity questionnaires using IPAQ-SF, measuring body fat levels using BIA, and measuring physical fitness levels using the Cooper Test. The results of the examination are then analyzed using the 26th edition of Statistical Package for Social Science (SPSS) software. This study found that most respondents had moderate physical activity levels (60.3%), and high body fat (60.3%). Most students have poor fitness levels (85.3%). There is a relationship between physical activity levels and body fat levels with student fitness levels (all $p<0.05$). Physical activity and body fat are related to the fitness level of medical students.

Keywords: body fat; physical activity; physical fitness

1. Introduction

Physical fitness is a prerequisite for someone to engage in daily physical activity in an effective and efficient manner without experiencing excessive fatigue.[1] Physical fitness is essential in determining in determining health status, morbidity, and mortality in children and adults.[2] Physical fitness is determined by a variety of factors, including age, sex, race, genetics, activity physique, lifestyle, dietary state, and health status.[2,3]

Nutritional status is one of the intervening factors associated with physical fitness status.[2] The nutritional status is the combination of a person's health status, nutrition intake, and nutrition use for the body's various biological processes.[4] Body composition is one of the methods for determining nutritional status.[5] Body composition is proportionally composed of fat mass and fat-free mass.[6] Body composition measurement is meant to evaluate nutritional status by determining fat mass, fat-free mass, bone mineral content, and body water (intracellular and extracellular).[7] n comparison to further anthropometric measurements, body composition measurements can reveal the ratio of fat mass and the likelihood of catching metabolic syndrome.[6]

Poor nutritional status can be subdivided into two categories: insufficient nutrition and excess nutrition.[4] Globally, 1,9 billion individuals are overweight or obese, and 462 million adults are underweight, due to the prevalence of poor nutritional status.[8] The average prevalence of malnutrition among the adult population in Asia is 7.5% for men and 10.3% for women.[9] Based on data from the Indonesian Ministry of Health's Basic Health Research for 2018, Indonesia's population over 18 is 9.3% lean, 13.6% overweight, and 21.8% obese.[10]

Physical activity is one of the most influential factors in healthy fitness and nutritional status.[1] The public's daily activities were altered during a pandemic.[11] A number of studies indicate that momentary pandemic-related decreases in students' level of physics-related activity include a 32.5% fall in light activity and a 52.8% decrease in heavy activity.[12] The decline of this activity will also result in a decline in physical fitness and nutritional status.[1,6]

Physical fitness is essential for individuals of productive age.[13] The productive age range is 15 to 64 years old.[14] College students are included in the productive age demographic. A college student is an individual who is formally registered to participate in educational activities at a university.[15] In universities, medical education is one of the majors.[16] This educational program is a prerequisite for earning a doctorate degree.[16] In truth, a majority of medical students are physically unfit. it happens Due to a packed lecture schedule; students ignore their physical fitness. [17] Inadequate physical fitness and nutritional status will have a negative effect on health status, work productivity, and the risk of cardiovascular and metabolic disorders.[1]

Medical students gain information and understanding not only through classroom lecture activities but also through organizational activities.[18] Sriwijaya-Medical Assistance Team (Tim Bantuan Medis-Sriwijaya/ TBM-S) is one of the organizations affiliated with the Faculty of Medicine.[19] This organization requires students who have a fit body to become candidates. TBM-S candidates are expected to represent the best fitness quality among other medical students. This study analyzes the relationship between physical activity level and body fat with the physical fitness of the candidates.

2. Methods

This study is an analytical investigation employing a cross-sectional observational methodology. The study population was a candidate for TBM-S batch 2022. Participants who had no physical impairment and were prepared to sign an informed consent form met the study's inclusion criteria. The study was carried out at the Madang campus of the Faculty of Medicine, Universitas Sriwijaya, and the Jakabaring Sport City Palembang Water Ski Lake Venue over a three-month beginning in August 2022 and ending in October 2022.

The level of physical activity was determined using the International Physical Activity Questionnaire (IPAQ). IPAQ measures MET performance. Walking equals 3.3 MET, moderate activity equals 4.0 MET, and strenuous exercise equals 8.0 MET; these values are multiplied by the intensity in minutes and days and then combined to obtain the overall physical activity score. Physical activity is considered low if it is 600 MET-minutes per week, moderate if it is 600–2999 MET-minutes per week or 1500–3000 MET-minutes per week with 1 or 2 days of strenuous exercise intensity, and high if it is 3000 MET-minutes per week or 1500 MET-minutes per week with at least 3 days of vigorous-intensity exercise.

Bioelectric Impedance Analysis (BIA)-based measurement of body composition. The instrument utilized in the investigation was a TANITA BC 601 model. Measurements were made early in the day. Before being measured, the participants in the study fasted in the morning, removed their shoes and jackets, and emptied their heavy bags of items such as keys, wallets, and jewelry. The outcomes will then be divided into groups based on gender and age.

Those who fail the initial screening of The Physical Activity Readiness Questionnaire for Everyone (PAR-Q) will be disqualified. The Cooper Test was used to evaluate physical fitness. The Cooper test is a timed running test in which participants are required to run as far as they can in 12 minutes. Prior to taking the Cooper Test, the participants will warm up together. The respondent's running distance was determined using the smartphone application STRAVA. The obtained distance-based results will be grouped according to age and gender.

An ethical eligibility letter for this research bearing the number 086/KEPKKFKUNSRI/2022 has been issued by the UNSRI FK Ethics Committee. The results were analyzed using SPSS 26.0 and chi-square for bivariate analysis.

3. Results and Discussion

3.1. Respondent characteristic

The total of candidates was 71. Three candidates declined to continue, therefore failing to meet the

inclusion requirements. Consequently, 68 participants participated in this survey as respondents (Table 1).

Table 1. Characteristics of the students

Characteristics	n	%
Sex		
Male	25	36.8
Female	43	63.2
Age (years)		
18	26	38.2
19	28	41.2
20	13	19.1
21	1	1.5

3.2. Level of activity, body fat, and physical fitness

Table 2 shows that there are as many as 41 respondents with moderate levels of physical activity (60.3%). It was determined that respondents tended to have greater body fat, specifically 41 individuals (60.3%). The results revealed that the majority of respondents had poor physical fitness (85.3%).

Table 2. Level of activity, body fat, and physical fitness of the students

Variable	n	%
Physical Activity Level		
Low	21	30.9
Moderate	41	60.3
High	6	8.8
Body Fat Level		
Low	0	0
Ideal	4	5.9
Moderate	23	33.8
High	41	60.3
Physical fitness Level		
Excellent	1	1.5
Above average	0	0
Average	5	7.4
Below average	4	5.9
Poor	58	85.3

3.3. The relationship between physical activity level and body fat level with student fitness level

The association between respondents' physical activity and body fat with their physical fitness is presented in Table 3. The Chi-Square analysis yielded a p-value of 0.001, indicating that there was a substantial association between the level of physical activity and the level of physical fitness.

A strong association was aso between body fat level and fitness level (p=0.007).

Table 3. The relationship between physical activity level and body fat level with student fitness level

Variable	Student Fitness level										P
	Excellent		Above average		Average		Below average		Poor		
	n	%	n	%	n	%	n	%	n	%	
Physical Activity Level											
Low	0	0.0	0	0.0	0	0.0	0	0.0	21	100	
Moderate	0	0.0	0	0.0	2	4,9	3	7,3	36	87.8	0.001
High	1	16,7	0	0.0	3	50.0	1	16,7	1	16,7	
Body Fat Level											
Low	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	
Ideal	0	0.0	0	0.0	2	50.0	1	25.0	1	25.0	0.007
Moderate	1	4,3	0	0.0	3	13.0	1	4,3	18	78.3	
High	0	0.0	0	0.0	0	0.0	2	4,9	39	95,1	

WHO recommends 150 minutes of moderate-intensity physical activity or 75 minutes of vigorous-intensity physical activity per week for adolescents and adults, equivalent to 600 MET minutes per week.[20] With a compliance rate of 60.3%, this survey revealed that more than half of all respondents had complied with WHO physical activity recommendations. These results are matched by a similar survey conducted on students at the Faculty of Medicine Mataram University, which found that 53.5% of respondents participate in moderate physical exercise each week.[21] However, the findings of this study reveal that more than 30 percent of respondents do not fulfill the weekly physical activity recommendations because they engage in light physical activity or less than 600 MET minutes each week. This is due to the consequences of the COVID-19 pandemic, which has led to a decline in students' physical activity.[22] This result is supported by another research study which revealed a 44.1% weekly decline in physical activity during the COVID-19 pandemic, compared to the period before the pandemic.[22] This may be a result of online learning activities in which students interact more with electronic devices than with physical activities, resulting in a sedentary lifestyle.[22]

The optimum body fat according to age and gender is 8–14% (for males aged 20 years) and 18–23% (for females aged 20 years); hence, it is considered extra fat if it exceeds these percentages; therefore, more than half of the respondents did not reach the specified ideal body fat. This finding is consistent with another study that indicated the average student has extra body fat. [23] That study, which evaluated 271 students in the city of Zagreb, Croatia, at random and with an average age of 19 to 20 years old, discovered that the average percentage of fat mass in females is 31.78% and in males, is 22.29%; when combined, the two percentages equal a total average of 27.86%. Psychological stress is one of several variables that contribute to this.[24] Psychological stress in first-year students is linked to the strain of academic activities that must adjust from the transition of learning activities from high

school to college life and is regarded as one of the factors related to excess weight in students.[25] According to the findings of earlier studies, medical students are more academically pressured than students in other disciplines. Because of lots of lecturers, numerous tests, and the strain of dealing with patients, medical students are under increased pressure.[26] Stress can cause a rise in cortisol production, which results in the redistribution of white adipose tissue to the abdomen and an increase in hunger.[27] Additionally, during the COVID-19 pandemic, students spent twice as much time as they did before the pandemic in front of computers or other electronic devices, which reduced outdoor activities and lengthened sitting periods.[22]

The physical fitness level of the students in this study was similar to a study performed on 40 Sports Education students at Yogyakarta State University, 20 of whom were male and 20 of whom were female. According to the VO2max score, the data indicate that 55% of males and 85% of females have poor physical fitness.[28] In addition, it is well-known that medical students face increased academic pressure due to the busy curriculum schedule and monthly exams, causing them to spend more time studying to catch up. It turns out that the amount of time students spend studying each day affects their physical condition.[29] It turns out that the duration of daily study affects the students' physical health.[30] This condition comes as a result of a sedentary lifestyle brought on by prolonged and extensive study, which causes a person to be in a seated position indefinitely and reduces physical activity.[30] This condition comes as a result of a sedentary lifestyle brought on by prolonged and extensive study, which causes a person to be in a seated position indefinitely and reduces physical activity. In addition to a sedentary lifestyle, this condition is aggravated by COVID-19's online lectures, which diminish one's fitness level. [25] This will result in an increase in oxidative stress, resulting in a decrease in muscle metabolism and heart function if it is carried out under prolonged settings.[30]

The significance of the relationship between activities and physical fitness is supported by previous research in India. Physical fitness is better in medical students who are active in basketball (athletes) than medical students who have a sedentary lifestyle (non-athletes). Someone who often does basketball sports has optimal aerobic and anaerobic capacities because the game requires agility and repeated jumps.[31]

The results of the analysis of the relationship between body fat and physical fitness are in line with a study conducted by Firdaus et al.[32] Similar populations were also obtained in TBM FKIK students of Warmadewa University, it was found that there is a relationship between body composition and fitness level.[33]

One of the other studies looked at the body fat percentage with aerobic capacity in 68 physical education students from Universitas Jendral Soedirman, and the results show a significant relationship.[34] Increase in body fat mass is related to the fitness index of a person's body. [35]

4. Conclusion

Physical activity and body fat are related to the fitness level of medical students. Regular physical exercise is required, both for the candidates of the Sriwijaya-Medical Assistance Team in particular, as well as medical school students in general.

5. Acknowledgments

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Research Article

Comparative analysis of lung functions in young adults following COVID-19 infection: A case-control study

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Abstract: There is a growing concern regarding the long-term respiratory effects of the coronavirus disease of 2019 (COVID-19), yet research on this matter is still limited, especially in young adults. Our objective was to compare the lung function parameters between young adults with a history of COVID-19 at least three months prior and those without. We conducted a case-control study that recruited individuals between 18 and 35 y/o and obtained their informed consent. The NDD Easy On-PC Spirometry was utilized to measure lung function parameters, including FEV1 (Liter), FVC (Liter), FEV1/FVC ratio, FEF25-75% (Liter/second), and PEF (Liter/second). The Shapiro-Wilk test assessed data normality, and all normally distributed data were presented as Mean±SD(95%CI), while all skewed data were presented as Median(Range). The comparison was performed with t-tests or Mann-Whitney tests, with p-value<0.05, meaning a significant difference. We included 64 participants (35(54.69%) females, 29(45.31%) males, aged 22.55±3.61 years), with equal numbers in the COVID-19 group (with COVID-19 history) and control group (without COVID-19 history). We found significant differences between the COVID-19 group and the control group in FEV1 (2.77±0.61(2.55–2.99) vs 3.16±0.54(2.97–3.36), p-value=0.008), FEF25-75% (3.20±1.06(2.82–3.58) vs 3.95±0.90(3.62–4.27), p-value=0.003), and PEF (6.09±1.79(5.45–6.74) vs 7.07±1.85(6.40–7.74), p-value=0.036). Meanwhile, no differences were found in FVC (3.23±0.75(2.96– 3.50) vs (3.54±0.64(3.31–3.78), p-value=0.08) and FEV1/FVC (0.87(0.62–0.98) vs 0.88(0.80–1.00), p- value=0.20). Young adults who recovered from COVID-19 may experience decreased lung function. Thus, it is advisable to maintain monitoring of the potential long-term respiratory effects of COVID-19.

Keywords: covid-19 long-term effect; lung physiology; sars-cov-2; spirometry; young adults

1. Introduction

The COVID-19 pandemic has profoundly impacted global health, affecting millions with respiratory symptoms.[1] As the COVID-19 pandemic evolves, there is growing concern about potential long-term respiratory consequences in survivors.[2] In particular, it is crucial to understand the degree of pulmonary dysfunction that may persist in the post-acute phase of the disease. This concern regarding the impact of COVID-19 on lung function is not limited to older adults or those with pre-existing comorbidities but also extends to the younger demographic, which has been relatively underexplored.

Research on the long-term respiratory effects of COVID-19 in young adults is lacking.[3] Emerging

evidence suggests that COVID-19 can have long-term effects on lung function, regardless of age or initial disease severity. Therefore, it is critical to investigate this phenomenon in young adults.[4] Young adults have a longer life expectancy and robust lung function, allowing for extended COVID-19 aftermath assessment.

This study aims to compare lung function parameters in young adults with a history of COVID-19 infection at least three months prior to those without. This study aims to investigate the long-term effects of COVID-19 on the respiratory health of young adults, with a specific focus on lung function parameters such as forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and their ratio (FEV1/FVC). The study intends to shed light on whether COVID-19 has a lasting impact on these parameters. Our aim is to gain insights on potential functional impairments and early indications of chronic respiratory issues that may persist beyond the acute phase of the disease.[5]

This investigation is crucial to understand the long-term health effects of COVID-19 and to develop tailored public health strategies and clinical management guidelines for young adults. Our research could contribute to the ongoing discussion surrounding the post-COVID-19 syndrome. This would provide valuable guidance for healthcare providers, policymakers, and individuals working to mitigate the long-term health effects of this unprecedented global pandemic.

2. Methods

2.1 Study design

We conducted a comparative analysis study to assess the long-term pulmonary effects of COVID-19 in young adults aged between 18 and 35 years. Ethical approval was obtained from the Research Ethics Committee of the Faculty of Medicine, Universitas Andalas (No: 474/UN.16.2/KEP-FK/2021), and all participants provided informed written consent before participating in the study.

2.2 Participant recruitment

Participants were recruited in the Faculty of Medicine, Universitas Andalas. Inclusion criteria comprised individuals aged 18 to 35 years with a documented history of COVID-19 infection at least three months prior to enrollment. Exclusion criteria included individuals with pre-existing chronic respiratory conditions, current smokers, and those with incomplete medical records.

2.3 Lung function assessment

Lung function parameters were measured using the NDD Easy On-PC Spirometry system, a validated and widely accepted tool for assessing pulmonary function. The following parameters were

recorded:

- Forced Expiratory Volume in one second (FEV1): Expressed in liters (L).
- Forced Vital Capacity (FVC): Expressed in liters (L).
- FEV1/FVC ratio: Calculated as a percentage.
- Forced Expiratory Flow between 25% and 75% of FVC (FEF25-75%): Expressed in liters per second (L/s).
- Peak Expiratory Flow (PEF): Expressed in liters per second (L/s).

2.4 Statistical analysis

Data normality was assessed using the Shapiro-Wilk test. Normally distributed data were presented as mean ± standard deviation (SD) with a 95% confidence interval (CI), while skewed data were presented as median (range). Comparative analysis between individuals with a history of COVID-19 and those without was performed using the appropriate statistical tests, depending on the data distribution. Specifically, independent t-tests were employed for normally distributed data, and Mann-Whitney U tests were used for skewed data. A significance threshold of $p < 0.05$ was established to determine statistically significant differences between the two groups.

2.5 Data collection

Data collection was carried out by trained personnel who followed standardized procedures for spirometry testing, ensuring accuracy and reliability. The data collected included demographic information, COVID-19 history, and the aforementioned lung function parameters.

3. Results and Discussion

3.1 Participant characteristics

A total of 64 participants (54.69% females, 45.31% males) with an average age of 22.55±3.61 years were included in this comparative analysis study. The study cohort was evenly divided into two groups: the COVID-19 group, consisting of participants with a history of COVID-19 (n=32), and the control group, comprised of participants without a COVID-19 history (n=32). Participant characteristics are summarized in the Table 1.

Table 1. Participant characteristics

Characteristic	COVID-19 group (n=32)	Control group (n=32)
Age (years)	24.68±4.00	20.41±0.98
Sex (Female/Male)	18 (54.25%)/ 14 (43.75%)	17 (53.12%)/ 15 (46.88%)

3.2 Comparison of lung function parameters

The comparative analysis revealed significant differences in several lung function parameters between the COVID-19 group and the control group, as summarized in Table 2.

Table 2. Comparison of lung function parameters between COVID-19 and control groups

Characteristic	COVID-19 group (n=32)	Control group (n=32)	p-value
FEV1 (L)	2.77±0.61 (2.55–2.99)	3.16±0.54 (2.97–3.36)	0.008
FEF25-75% (L/s)	3.20±1.06 (2.82–3.58)	3.95±0.90 (3.62–4.27)	0.003
PEF (L/s)	6.09±1.79 (5.45–6.74)	7.07±1.85 (6.40–7.74)	0.036
FVC (L)	3.23±0.75 (2.96–3.50)	3.54±0.64 (3.31–3.78)	0.080
FEV1/FVC	0.87 (0.62–0.98)	0.88 (0.80–1.00)	0.200

FEV1 (Forced Expiratory Volume in one second)

The mean FEV1 was significantly lower in the COVID-19 group compared to the control group (2.77±0.61 L vs. 3.16±0.54 L, p-value=0.008), indicating reduced expiratory volume in one second among individuals with a history of COVID-19.

FEF25-75% (Forced Expiratory Flow between 25% and 75% of FVC)

Participants in the COVID-19 group exhibited a significantly lower FEF25-75% compared to those in the control group (3.20±1.06 L/s vs. 3.95±0.90 L/s, p-value=0.003), indicating decreased mid-expiratory flow rates.

PEF (Peak Expiratory Flow)

The COVID-19 group demonstrated a significantly lower PEF compared to the control group (6.09±1.79 L/s vs. 7.07±1.85 L/s, p-value=0.036), indicating reduced peak flow rates during forced expiration.

FVC (Forced Vital Capacity) and FEV1/FVC Ratio

There were no significant differences observed between the two groups in terms of FVC (3.23±0.75 L vs. 3.54±0.64 L, p-value=0.080) and FEV1/FVC ratio (0.87 vs. 0.88, p-value=0.200), suggesting that these parameters were comparable between individuals with and without a history of COVID-19.

3.3 Discussion

The study provides valuable insight into COVID-19's long-term pulmonary effects on young adults. Our analysis showed significant lung function differences between those with and without COVID-19 history, highlighting the need for respiratory health monitoring and care in this group.[6]

FEV1 (Forced Expiratory Volume in one second)

The decrement in Forced Expiratory Volume in one second (FEV1) among individuals who have a history of COVID-19 infection is of notable significance. A lower FEV1 indicates a reduced ability to

forcefully expel air during the first second of expiration, which suggests a decline in lung function. This finding aligns with previous studies reporting pulmonary impairment in COVID-19 survivors.[7] The decreased FEV1 suggests that even in young adults, the virus may leave lasting damage to the airways and lung parenchyma, potentially contributing to long-term respiratory complications.

FEF25-75% (Forced Expiratory Flow between 25% and 75% of FVC) and PEF (Peak Expiratory Flow)

Similarly, the diminished FEF25-75% and PEF values in the COVID-19 group emphasize the potential for compromised mid-expiratory flow rates and peak expiratory flow during forced expiration. These parameters are critical for assessing the functioning of smaller airways and peak expiratory effort. The observed deficits suggest that COVID-19 may lead to persistent bronchial and airway abnormalities, possibly contributing to chronic respiratory symptoms and decreased exercise tolerance.[8]

FVC (Forced Vital Capacity) and FEV1/FVC Ratio

While there were no statistically significant differences in FVC and the FEV1/FVC ratio between the COVID-19 and control groups, it is essential to note that the trends leaned toward a reduction in FVC and a slight decrease in the FEV1/FVC ratio in the COVID-19 group. Although these trends did not reach statistical significance in our study, they could still indicate potential early-stage pulmonary abnormalities that may develop into clinically significant impairments over time. Nevertheless, previous studies were in accordance with the previous study by Yan, et al. (2021) assessing pulmonary function in people who had recovered from COVID-19 1 year previously.[9]

The findings from this study reinforce the growing body of evidence suggesting that COVID-19 can have lasting consequences on lung function, even in young adults. The observed differences in lung function parameters highlight the need for comprehensive post-COVID-19 care and monitoring, extending beyond the acute phase of the disease.[4] Young adults are often considered to have robust respiratory systems, making it all the more remarkable that they exhibit deficits in lung function following COVID-19 infection.

Further investigation is necessary to comprehend the mechanisms that are accountable for the persistent pulmonary effects that ensue after contracting COVID-19. The potential mechanisms include damage to lung tissue, inflammation, and fibrosis. In order to ascertain the trajectory and clinical significance of these impairments, it is imperative to conduct longitudinal studies that monitor the progression of lung function in young adults following COVID-19.

It is imperative to acknowledge that this study has certain limitations. Firstly, the cross-sectional design and relatively small sample size may impact the validity of the findings. Secondly, the severity of the initial COVID-19 infection was not considered, which could affect the extent of pulmonary

involvement. Finally, the lack of pre-COVID-19 baseline lung function measurements makes it challenging to accurately assess the level of post-infection impairments.

4. Conclusion

Our findings indicate that young adults who have previously contracted COVID-19 may experience persistent deficits in certain lung function parameters, which could indicate potential long-term pulmonary consequences. As such, it is imperative to provide ongoing monitoring and follow-up care for individuals who are in the process of recovering from COVID-19, even if their initial symptoms were not severe. To gain a more comprehensive understanding of the mechanisms and clinical implications of these respiratory impairments, it is necessary to conduct longitudinal studies and further research.

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Literature Review

Comparison of potato satiety index between white bread and white rice as reference food

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Abstract : Satiation is a person's feeling of fullness during the digestive process after eating meanwhile satiety is how long a person's fullness can be maintained between meals. Satiety index is the ratio of the satiety produced by the test food to the satiety produced by the reference food multiplied by 100%. This study aims to analyze the satiety index comparison between two reference foods (white bread and white rice) and the correlation between satiety scores and macronutrients (protein, fat, carbohydrates, and moisture content). This study involved 13 respondents with a crossover study design method. The satiety score for the scale of hunger, fullness, desire to eat and quantity of food were calculated based on the calculation of the area under the curve (AUC) using the trapezoidal method. The satiety index is calculated based on the comparison of the AUC of potato with white bread and also white rice multiplied by 100%. The satiety index for potatoes with white bread and white rice as reference food were 166.04% and 192,14% respectively. Moisture content and food weight are related to hunger (p= 0.00; r= -0.49), fullness (p= 0.00; r= 0.49), desire to eat (p= 0.00; r = -0.56), and food quantity (p=0.00; r= -0.61). There is no significant difference between two reference foods (p>0.05).

Keywords*:* macronutrients; satiety index; satiety score, staple foods

1. Introduction

Nowadays, a growing number of people are overweight and even obese. WHO in 2016 estimated that around 1.9 billion adults worldwide were overweight and around 650 million of them were obese. The WHO also estimated in the same year that at least 340 million children and adolescents aged 9-15 years were overweight [1]. A study conducted by Sudikno *et al*. [2] shows that obesity develops faster in women than in men. In 2018, the proportion of overweight and obesity in Indonesia was 13.6% and 21.8%, respectively. This figure increased from the previous year, which in 2013 amounted to 11.5% and 14.8%, respectively [3]. Based on research conducted by Septiyanti and Seniwati [4], the prevalence of obesity in urban areas in Indonesia is 28.4%.

A person who is overweight and obese generally has an excessive body fat composition [5]. Excess body fat composition can be a risk factor for several non-communicable diseases such as hypertension, heart disease, blood clotting, metabolic syndrome and other dangerous diseases [6]. Overweight and

obesity can be prevented by maintaining diet and regular physical activity. People with overweight and obesity tend to consume food frequently and consume large portions of food [7]. Different types of staple foods cause them to have different feelings of satiety [8]. The difference in satiety levels can affect the intake of food consumed [9] and thus can be utilized as a dietary strategy for overweight and obese people.

People who are overweight and obese generally consume excessive amounts of food. They do this to relieve the hunger they feel. The feeling of hunger can disappear when the feeling of satiety arises. Terminologically, the fullness is classified into 2, namely satiation and satiety. According to Camilleri [10] satiation is a person's feeling of fullness during the digestive process after eating and satiety is how long a person's feeling of fullness can be maintained between meals. Satiety can be influenced by various factors such as the macronutrient content, food forms, also physiological effects of the body when eating a food [11]. Satiety can be influenced by social and cultural perspectives [12]

Satiety can be calculated in several ways. It is commonly tested by measuring the level of perceived satiety recorded for varying durations after a meal. Satiety measurements reflect changes in subjective motivation to eat between two-time points and can be measured using a fixed portion, preload, or ad libitum test meal [13]. One of the types of measurement that can be done is the satiety scale score and index. Satiety scale scores are values generated by each individual which indicates the level of each scale. Satiety index is the ratio of the satiety produced by the test food compared to the satiety produced by the reference food multiplied by 100% [13]. Reference food is defined as food that is used as a standard food in calculating the satiety index. The common reference food used in the calculation of the satiety index is white bread [14]. Rice is a common staple food for Indonesian people, but there have been no studies comparing the difference in reference foods between bread and rice. Therefore, This study aims to analyse the satiety index of potatoes in comparison between two reference foods (white bread and white rice).

2. Methods

2.1. Design, location, and time

The design used in this study was a crossover study, which gave all types of 240 kcal (1000 KJ) isocaloric food to each subject. The test food was given 3 times on 3 different days with the subject given the same type of food each day. The time between administration of the test food was given a 3-day gap so that the subjects could return to their physiological conditions as usual days [15]. White bread was given as a reference food to control for differences in the satiety index of each subject [16]. This research was approved by the Ethics Committee of IPB University on April 25, 2022, with number 668/IT3.KEPMSM-IPB/SK/2022.

This study consisted of three stages, namely determining the macronutrient content (protein, fat, carbohydrate and water) through proximate analysis, measuring the height and weight of respondents, and assessing satiety and satiety index. Proximate analysis was conducted at the Nutrient Content Analysis Laboratory, Department of Community Nutrition, Bogor Agricultural University. Height and weight measurements were conducted at the IPB Nutrition Clinic. Satiety assessment was conducted at Teaching Cafeteria, IPB University. This study was conducted between August–November 2022.

2.2. Sampling

The population of this study were undergraduate students of IPB University. Subjects were taken by purposive sampling with inclusion criteria aged 21-23 years, male and female, normal body mass index (BMI), not on a special diet, medication and exercise [13]. Exclusion criteria: unable to finish the food. The subjects obtained based on the specified criteria were 15 people. In this study, 2 subjects were excluded so the total subjects analyzed further were 13 subjects. This was caused by subjects who could only finish the test food about 50% of the portion given.

2.3. Procedures

The types of data collected consisted of primary data consisting of the results of height and weight measurements, the results of the proximate test of macronutrient content of food samples, and satiety. The procedure for collecting satiety data was carried out with several stages as in Holt *et al*. [16] and Zhang *et al*. [8] with minor changes.

The first step was to determine the macronutrient content of the test food by proximate analysis. Protein content was tested using the Kjeldahl method (AOAC 2005: 955.04). Fat content analysis was tested using the Soxhlet method (AOAC 2005:920.39c). Moisture content was tested using the gravimetric method (AOAC 2005:925.10). Carbohydrate analysis was calculated by difference. The nutrient content is then calculated so that each test food has the same energy content of around 240 kcal.

The second step is screening by measuring the height using a microtoise, weight using a weight scale and calculating the subject's BMI. Subjects with a BMI range from 18.5 to 25.0 were included. The third step is clinical intervention, the subject is asked to fill out an informed consent, and subjects who agree will be asked to fast for 10-12 hours [17] and allowed to drink only water until the satiety test is carried out. This aims to equalize the physiological condition of the subject.

The next step was the satiety test, in the morning at around 08:00, subjects who had been asked to fast for 10-12 hours and gathered in the teaching cafeteria were given the equivalent of 240 calories of food (white rice, potatoes and white bread) which had to be finished within 10-15 minutes. Subjects were also allowed to drink a maximum of 240 ml of water. After eating, subjects were asked to fill out

the satiety questionnaire scale. Questionnaires are filled every hour with intervals of 0 hours (after eating), 1 hour, 2 hours, 3 hours, and 4 hours so that a total of 5 satiety data were obtained. The questionnaire used was developed by Blundell *et al*. [7] and consisted of 4 questions i.e 'How hungry are you?', 'How full are you?', How strong is your desire to eat?', and 'How much do you think you could eat right now?'. The questionnaire is then generated in the form of a visual analogue scale (VAS) as a line with two ends along 100 mm. Subjects were asked to fill in the questionnaire by marking a perpendicular line on a scale between not at all to very large/extreme. Respondents were asked to fill in the scale every hour for 4 hours.

Figure 1. Visual analogue scale (VAS) developed by Blundell *et al*. [7]

2.4. Data analysis

The filled VAS satiety questionnaire scale was measured using a ruler with an accuracy of 1 mm and then calculated using the area under the curve value for each subject. The AUC calculation uses the trapezoid method with the assessment time as the x-axis and the length of each subject's satiety scale score (mm) as the y-axis. Satiety scale scores are values generated by each individual which indicate the level of scale i.e. hunger, fullness, and desire to eat produced by a food after consuming it [14]. The scores were then averaged for analysis of the relationship test and the difference test [16].

The calculation of the satiety index is based on the calculation of Holt *et al* [16]. Satiety index was obtained based on the questionnaire question "How full are you now". The food satiety index measured the area under curve for each subject and then compared it with the area under curve for white bread.

Data were processed in several stages, namely entry, coding, editing, cleaning, and analyzing. Data processing was carried out using Microsoft Excel 2016 and SPSS version 21.0 for Windows. Microsoft Excel is used to fill in the data obtained for further processing. SPSS is used as a data-analyzing application. Data from each subject will be expressed using a code so that data confidentiality is guaranteed.

Subject characteristics in the form of BMI and age are expressed as mean (plus-minus) standard deviation. Data analysis in this study used the SPSS 26 for Windows application. Data analysis in this study began with analyzing data normality using the Shapiro-Wilk test. This step aims to see the distribution of data which will affect the test used for subsequent analysis. A comparison of satiety score and satiety index was tested using an independent t-test. The next step was to test the relationship using the Spearman test of each individual's satiety score with the macronutrient content of each test food.

3. Results and Discussion

3.1. Subject characteristic.

Table 1. Distribution of subject characteristics

Sex	N	%
Male	4	30,77
Female	9	69,23
Total	13	100
BMI (kg/m^2)		
18,5–20	3	23,08
20–25	10	76,92
Total	13	100
Mean ± SD (kg/m^2)	21,72 ± 1,78	

There were 23.08% respondents aged 21 years, 61.54% aged 22 years, and 15.38% aged 23 years. The average age of the subject is at the age of 22 years. Subjects are mostly female (69.23%). Most subjects have a body mass index (BMI) in the range of 20-25 (kg/m^2). The average BMI of the subjects was 21.72 (kg/m^2). The age and BMI of the almost homogeneous subjects can produce satiety measurement data that can describe the satiety ability of the food [13].

Test food composition. Macronutrients are nutrients that are needed in large quantities in the body. The functions of these macronutrients include being a source of energy, growth, replacing damaged cells, food reserves and others [18]. Macronutrients can also play a role in satiety when consuming food. The composition of the test food in this study includes protein, fat, carbohydrates, and moisture. The nutrient composition was obtained based on the results of the proximate test and using the nutrition label. The nutrients obtained were then converted to contribute 240 kcal of energy. The macronutrient composition of each test food is presented in Table 2.

Table 2 shows that the highest protein content of the test foods was found in potatoes. The high protein content of potatoes is due to the large number of potatoes used among the other test foods. The large number of potatoes given is due to the high moisture content of potatoes. The highest

carbohydrate content was found in rice. White bread was used as a comparison food which was later used to calculate the satiety index of other foods.

Table 2. Test food macronutrient composition

No	Test foods	Weight (g)	Composition				
			Energy (kcal)	Protein (g)	Fat (g)	Carbohydrate (g)	Moisture (ml)
1	White rice	143	240	6.0	0.1	53.7	83.2
2	Steamed potato	376	240	8.3	0.2	51.4	313.3
3	White bread	240	240	8.4	3.6	44.4	96.0

3.2. Comparison of satiety scale score and satiety index of test foods

Satiety score is a number used to see how strong the test food is in generating satiety. There are 4 satiety scores used following Zhang *et al*. [8]. These scores relate to hunger, satiety, food quantity, and food quantity. The satiety score is calculated based on the calculation of the area under curve (AUC) graph between the satiety question and time. The average satiety score of the four scores is presented in Table 3.

Table 3. AUC for satiety scale score

Scale	Mean AUC ± SD (mm. hour)		
	White rice	Steamed potato	White bread
Hunger	63.48 ± 9.27[a]	44.82 ± 16.26[b]	63.48 ± 9.27[a]
Fullness	28.80 ± 11.90[a]	50.90 ± 17.11[b]	28.80 ± 11.90[a]
Eating desire	69.82 ± 8.26[a]	48.69 ± 13.62[b]	69.82 ± 8.26[a]
Food quantity	66.69 ± 10.53[a]	47.20 ± 8.52[b]	66.69 ± 10.53[a]

Table 3 shows data related to satiety scores in the form of hunger, satiety, eating desire and food quantity. The highest hunger score is found in rice while potatoes have the lowest score. The highest satiety scale score is for potatoes and the lowest is for rice. The highest food quantity score is rice and the lowest is steamed potato. The highest food quantity score was found in rice while potatoes had the lowest score. In this study, it was found that potatoes can cause the lowest hunger, the longest fullness, the smallest quantity, and the smallest quantity of food consumed.

Satiety index was calculated based on the average satiety score of the test food against the average satiety score of white bread multiplied by 100%. The satiety index values of the test foods are presented in Table 4. Bread was not included in the comparison because white bread itself was used as a reference food in the calculation of satiety index.

Based on Table 4, The satiety index for potatoes with white bread and white rice as reference food were 166% and 192% respectively with no significant difference. This result is likely because both reference foods have similar macronutrient compositions. In addition to these two reference foods, it is

a type of carbohydrate that is quickly digested and absorbed by the body. Rice and bread also have similar glycemic index levels [19,20] so the two reference foods may produce satiety index values of potatoes that are not significantly different.

Table 4. Test foods satiety index

Satiety index based on standardized food types		p-value
Satiety index value[a] (%)		
Rice	Bread	
199 ± 89	166 ± 85	0.182

a) Calculated based on the comparison of the mean Area Under Curve satiety of the test food with white bread, b) significance value p<0,05

These results can be the basis that satiety index testing with reference foods from bread can be compared with satiety index testing with reference foods from rice with no significant difference. Therefore, subsequent studies in testing the food satiety index can use bread or rice according to the preferences of researchers. However, studies with larger sample numbers and wider age variations need to be done to confirm this result.

4. Conclusion

There was no significant no significant difference on satiety index of potatoes between two reference foods.

5. Acknowledgements

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Literature Review

A review of exercise-flavonoid combinations in the treatmentof hypertension

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Abstract: Hypertension, also known as high blood pressure, is a global health issue that affects millionsof individuals worldwide. It is crucial to manage high blood pressure because it can lead to serious complications such as heart disease and stroke. Exercise has a positive impact on cardiovascular disease and can help lower blood pressure in individuals with hypertension. Different types of exercise, such as moderate-intensity, high-intensity, and high-intensity interval training, are known to decrease blood pressure, improve blood vessel elasticity, and enhance overall cardiovascular function. Flavonoids, natural compounds found in many plants, have also gained attention for their potential role in controlling hypertension. Flavonoids possess anti-inflammatory, antioxidant, and vasodilator properties, which can help lower blood pressure by reducing inflammation and improving blood vessel function. Recent research suggests that there is a synergistic effect between exercise and the diet of flavonoids in the treatment of hypertension. This review adopts a mechanism that approach to the effects of different physical exercises and flavonoids on reducing blood pressure, specifically focusing on the molecular level and the inflammatory process associated with hypertension. The conclusion of this review is that the combined use of exercise and flavonoids has a more significant positive effect on blood pressure reduction compared to using them separately.

Keywords: Exercise; Flavonoid; Hypertension

1. Introduction

Hypertension, a risk factor for cardiovascular disease, is responsible for 10.8 million deaths worldwide. The prevalence and overall burden of hypertension are increasing globally. According to data from the World Health Organization's global target for treating hypertension, the prevalence of hypertension is projected to decrease by 33% between 2010 and 2030.[1] To treat hypertension, various measures can be taken, such as regular exercise and a healthy diet. Natural supplements are commonly used in hypertension treatment due to their minimal side effects compared to chemical drugs.

Various types of antioxidants can be found in fruits, vegetables, green tea, coffee, and

chocolate.[2] These antioxidants have been proven to have a positive effect on improving metabolic syndrome, the cardiovascular system, and blood pressure. One group of compounds that is found in natural products and has been extensively researched is flavonoids. Flavonoids have been shown to possess antioxidant, anti-inflammatory, and vasodilatory effects that could be beneficial in reducing blood pressure. The ability of flavonoid to regulate blood pressure was achieved by improving the functioning of the endothelium, either by directly influencing nitric oxide levels or indirectly through alternative pathways.[3]

Regular physical activity is a highly effective non-medication approach to lowering blood pressure and enhancing cardiovascular well-being. Engaging in activities like aerobic exercises (such as brisk walking, jogging, swimming, cycling), resistance training (weight lifting), and flexibility exercises (like yoga) can lead to a decrease in resting blood pressure.[4] This positive impact is observed in both individuals with hypertension and those with normal blood pressure. Additionally, physical activity can reduce the activity of the sympathetic nervous system, which is involved in blood pressure regulation. By lowering sympathetic activity, heart rate is reduced and blood vessels are less constricted, resulting in lower blood pressure. Some studies even suggest that physical activity can enhance vascular endothelial functions, leading to improved blood vessel health.[5]

Based on the explanation above, this review will discuss the potential synergistic effect of combining flavonoids and physical exercise in the treatment of hypertension.

2. The role of exercise in the treatment of hypertension

Exercise has been found to have various physiological mechanisms that contribute to the reduction of blood pressure. Regular physical activity can have both immediate and long-term effects on the regulation of blood pressure. When engaging in exercise, the muscles require more oxygen and nutrients, which leads to an increase in blood flow to these tissues. This increased demand triggers the release of nitric oxide (NO) from the endothelial cells that line the blood vessels. Nitric oxide is a powerful vasodilator, which relaxes and widens the blood vessels. As a result, the resistance to blood flow decreases, leading to a decrease in blood pressure.[6] Additionally, exercise can also reduce peripheral vascular resistance, which refers to the resistance encountered by blood flow in the small arteries and arterioles. During exercise, the contracting muscles release substances that promote vasodilation and decrease resistance to blood flow, facilitating easier movement of blood through the circulatory system. Regular exercise also helps reduce overall sympathetic nervous system activity, resulting in lower resting heart rates and less constriction of blood vessels. This effect further contributes to a decrease in blood pressure, especially during periods of rest.[5]

However, it is important to note that the mechanisms underlying the reduction of blood pressure through exercise and its associated outcomes are still being investigated. Many studies in this area are limited by their small sample sizes and significant heterogeneity. It is also crucial to recognize that the effects of exercise on blood pressure may vary among individuals due to factors such as the type, intensity, and duration of exercise, as well as individual genetics and baseline health status.

3. Low intensity training

Low-intensity exercise is commonly regarded as a safer and more manageable alternative than high-intensity exercise. Lopes et al. conducted a study that suggests a single session of low-intensity aerobic exercise can acutely reduce blood pressure in active adults. These findings provide additional evidence supporting the use of physical activity as a non-pharmacological approach to controlling hypertension. Even a low-intensity aerobic exercise session, which may be more tolerable for older adults with hypertension, has immediate benefits and should be considered when aiming to control blood pressure.[7]

One example of low-intensity training is walking leisurely. The release of endogenous opioids, such as β-endorphin, during various intensities of exercise, including walking, is indicated by the chemical structure of β-endorphin enclosed in a circle. Plant-derived opioids, symbolized by the poppy image, have comparable effects of vasodilation and euphoria to exercise-induced β-endorphin. β-Endorphin, a neuropeptide found in various tissues and organs, including cardiomyocytes, has well-established actions on neurons through Gi/0 protein-coupled receptors. These actions include the closure of voltage-gated calcium channels on presynaptic nerve terminals, which reduces transmitter release. Additionally, they involve the opening of potassium channels, which hyperpolarizes and inhibits postsynaptic neurons, including norepinephrine. With its tranquilizing, mild vasodilating, heart rate inhibiting, and analgesic effects, β-endorphin may act as a buffer against exercise-induced sympathetic excitation and cardiovascular overload. Therefore, we propose that the elevation of β-endorphin may also contribute to the blood pressure-lowering and heart rate-reducing effects of low-intensity walking. These effects help promote adherence to walking and may enhance compliance with exercise and medication in hypertensive individuals, while reducing the risk of drug dependence. The benefits of walking in the outdoors represent a form of gentle exercise that can be easily incorporated into daily life for the general population.[8]

Engaging in low-metabolic equivalent (MET) walking for 50-60 minutes resulted in a temporary decrease in blood pressure and heart rate, as well as increased levels of β-endorphins, in young volunteers. This slight, transient hypotensive response, along with a negative chronotropic response, was observed in all subgroups (hypertensive, prehypertensive, and rapid heart rate groups). When

low-intensity exercise is sustained for a sufficient duration, it may lead to a decrease in blood pressure through mechanisms similar to those of moderate-intensity exercise, which are widely accepted.[9,10] Lu et al.[8] demonstrated that two volunteers who engaged in slow walking for two consecutive years consistently experienced a transient elevation of urine endorphin levels after walking, along with a transient reduction in blood pressure. Other mechanisms, such as exercise-induced nitric oxide and carbon monoxide, can decrease sympathetic activity, improve baroreflex function, and modulate the renin-angiotensin system. These mechanisms may also contribute to the pressure-lowering effects of long-term exercise training, regardless of intensity. In addition to vasodilation, the reduction of heart rate is also a crucial factor in decreasing cardiac output and regulating blood pressure, especially during long-term regular low-intensity walking.

4. Moderate intensity training

Light, moderate, and vigorous-intensity aerobic exercises have been found to effectively manage blood pressure. However, research suggests that moderate-intensity aerobic exercise is more effective in managing blood pressure compared to both high-intensity and low aerobic exercise.[5] Furthermore, studies conducted by Huldani et al.[5] have demonstrated that engaging in intense exercise for at least 30 minutes, more than three times a week, can be beneficial in treating systemic hypertension. One of the mechanisms behind the reduction in systolic blood pressure during exercise is associated with an increase in functional sympatholysis. This refers to the attenuation of sympathetic vasoconstriction in contracting muscles, which is facilitated by vascular endothelial-derived factors such as nitric oxide (NO). However, it is important to note that vascular endothelial function tends to decline with age, leading to a decrease in functional sympatholysis among older individuals who lead a sedentary lifestyle.[11]

A study conducted by Perdomo et al.[12] demonstrated a decrease in both systolic and diastolic blood pressure following moderate-intensity aerobic exercise on a treadmill for a duration of 30 minutes. This suggests that intermittent 10-minute bouts of exercise may not be as effective as a continuous 30-minute session in improving cardiovascular fitness. The shorter duration of shear stress-induced nitric oxide release during 10 minutes of exercise may not be sufficient to counteract sympathetically induced vasoconstriction, possibly because of variations in the timing of exercise initiation.[12]

The mechanism by which aerobic exercise affects blood pressure is similar to that of beta-blockers. Both interventions attenuate sympathetic nerve activity and reduce heart rate, resulting in a decrease in blood pressure. During exercise, vascular smooth muscle (myogenic tone) and muscle afferent fibers reset blood pressure to higher levels. When exercise is ceased, decreased sympathetic activity resets the

baroreflex to a lower level. Aerobic exercise helps restore decreased peripheral vascular resistance and improves vascular elasticity. Consequently, changes in the elasticity of blood vessels following aerobic exercise amplify the increase in vascular pressure and the expansion of blood vessels due to the higher blood flow rate experienced during aerobic exercise.[13]

5. High intensity interval training

The ability of High-Intensity Interval Training (HIIT) to reduce blood pressure is a complex process that involves various physiological and molecular mechanisms. Regular training with high-intensity interval training (HIIT) leads to improved cardiovascular health and lower blood pressure. The mechanism through which HIIT achieves this involves a combination of neural, hormonal, endothelial, and molecular processes. The intensity of exercise also plays a role in improving endothelial function in individuals with hypertension. For instance, high-intensity interval training (HIIT) has been found to be more effective than continuous moderate-intensity training (CMT) in improving endothelial function in individuals with metabolic syndrome. In addition, High-Intensity Interval Training (HIIT) has been shown to improve the availability of nitric oxide, normalize factors that affect nitric oxide bioavailability, and enhance arterial compliance.[14]

A study conducted by Guimarães et al. compared the effects of continuous and interval intensity exercise on arterial stiffness and blood pressure in hypertensive patients undergoing treatment. The results indicated that both continuous and interval intensity exercise training were beneficial for controlling blood pressure. However, only interval intensity training reduced arterial stiffness in these patients. This suggests that aerobic-based interval exercise may have distinct advantages over continuous aerobic exercise in improving peripheral and central arterial compliance post-exercise.[14,15]

Different modes of exercise can have different effects on arterial stiffness. Aerobic exercise, high-intensity interval training (HIIT), and progressive resistance exercise (RE) have been shown to promote physiological and cellular responses in endothelial cells. These responses enhance the production of angiogenic factors and improve nitric oxide bioavailability. These exercises also modify markers of endothelial senescence, which assist in mitigating the adverse changes in blood pressure and vascular function. This includes an increase in arterial compliance and a decrease in arterial stiffness. However, high-intensity acute resistance exercise (RE) that causes muscle damage can temporarily increase inflammation, endothelial senescence, and sympathetic activation. This can potentially result in a temporary elevation in blood pressure and arterial stiffness.[16]

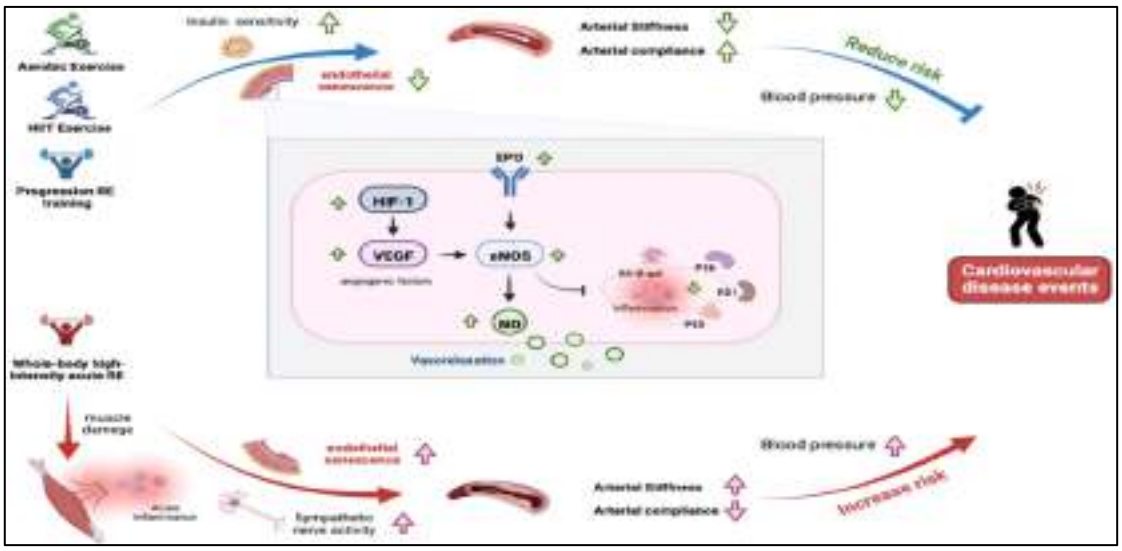

Figure 1. Arterial stiffness in cardiovascular events. Different types of exercise can have varying effects on arterial stiffness. Aerobic exercise, high-intensity interval training (HIIT), and progressive resistance exercise (re) can all promote the production of factors that support blood vessel growth (VEGF, HIF-1, EPO) and improve the availability of nitric oxide, which helps to relax blood vessels. These exercises can also reduce markers of endothelial senescence (p53, p21, p16, SA-β-gal), which is associated with aging of blood vessels. This leads to improvements in blood pressure and vascular function, including increased arterial compliance and decreased arterial stiffness. However, high-intensity resistance exercise that causes muscle damage can temporarily increase inflammation, endothelial senescence, and sympathetic activation, which may result in a temporary elevation in blood pressure and arterial stiffness

6. The role of flavonoid in the treatment of hypertension

Flavonoids are a class of compounds composed of 15 carbon atoms, characterized by a C6-C3-C6 arrangement where two benzene rings are bound to a propane chain. These phenolic compounds are naturally occurring and can be found in plants, imparting red, purple, blue, and yellow colors to various plant parts. Flavonoids are present in both the vegetative parts and flowers of larger plants. They play a crucial role in determining the color, taste, smell, and nutritional quality of food. Different plants produce specific types of flavonoids, which contribute to their survival by protecting against pests, diseases, competition, interactions with microbes, seed dormancy, UV radiation, and serving as signaling molecules in various biological processes such as transduction pathways, pollination, and male fertility.[17]

The mechanism by which flavonoids reduce blood pressure is associated with their ability to inhibit angiotensin-converting enzyme (ACE) and act as diuretics. ACE is known to play a significant role in hypertension by converting angiotensin I to angiotensin II, which causes blood vessels to constrict and elevate blood pressure. Flavonoids act as ACE inhibitors, promoting vasodilation and increasing blood

flow to the heart, thereby reducing blood pressure.[18] The renin-angiotensin-aldosterone system (RAAS) plays a major role in regulating blood pressure. Extensive research has demonstrated that chronic hyperactivity of this system is associated with the development of hypertension and other cardiovascular diseases (CVDs). Interventions targeting the renin-angiotensin-aldosterone system (RAAS), such as the administration of ACE inhibitors or subtype-specific angiotensin receptor blockers, have been shown to disrupt its functioning and result in a reduction in both blood pressure levels and the occurrence of cardiovascular events, particularly in populations at high risk.[19] Research by Larson et al.[20] showed that quercetin, a derivative of flavonoid, can reduce ACE activity by 31% compared to baseline. This indicates that quercetin can function as an ACE inhibitor. Furthermore, decrease in blood pressure was accompanied by a decrease in the angiotensin-I receptor in the kidney, an increase in urine volume, and an increase in urinary sodium excretion. These findings suggest that quercetin may lower blood pressure by affecting multiple points in the renin-angiotensin-aldosterone system. Since ACE inhibitors have been proven effective in lowering blood pressure in humans, and there is evidence suggesting that quercetin may act as an ACE inhibitor, further clinical trials are needed to determine if quercetin supplementation can decrease ACE activity in hypertensive individuals.[20,21]

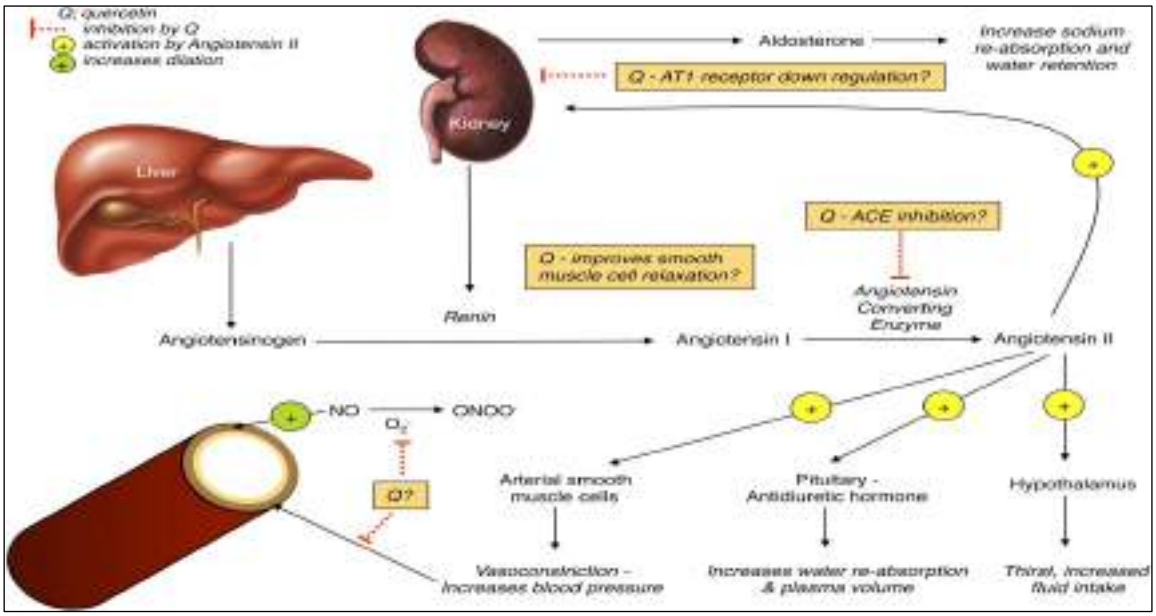

Figure 2. Potential mechanisms through which quercetin (Q) may lower blood pressure by interacting with the renin-angiotensin-aldosterone system

Furthermore, flavonoids, specifically polyphenols, can enhance the activity of Nitric Oxide Synthase (NOS) in vascular endothelial cells. They can also synthesize Nitric Oxide (NO) in these cells. NO acts as a signaling molecule, diffusing and stimulating guanylate cyclase to produce cyclic Guanosine Monophosphate (cGMP), leading to vasodilation. Flavonoids can also activate the Endothelium

Derived Relaxing Factor (EDRF), causing vasodilation and further reducing blood pressure. The endothelium's release of nitric oxide (NO) is well-known for its role in regulating vascular tone and blood pressure. NO activates the cGMP-protein kinase G cascade in smooth muscle cells, leading to vasodilation through potassium channel stimulation and inhibition of calcium influx. The action of protein kinase G is based on the phosphorylation of myosin light chains, a process by which the vasoconstriction of the smooth muscles in the vessels decreases.[22]

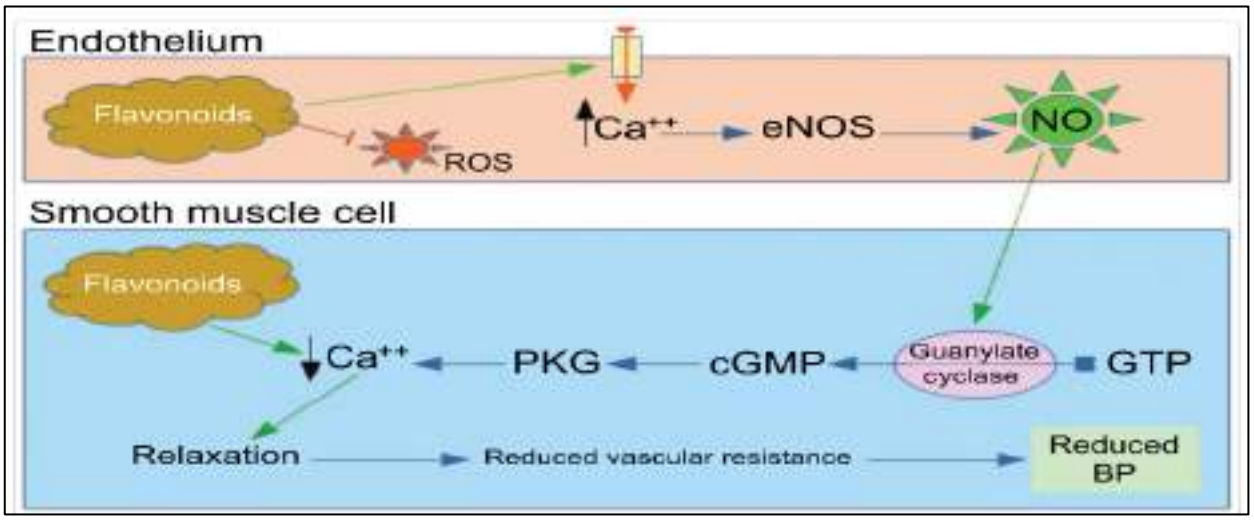

Figure 3. The effect of flavonoids on the endothelium: reducing oxidative stress and enhancing nitric oxide production. Within smooth muscle, nitric oxide plays a role in reducing blood pressure by inhibiting calcium and promoting relaxation. This relaxation is facilitated by the diffusion of nitric oxide from the endothelium

Flavones, a subgroup of luteolin-rich flavonoids, exert their antihypertensive effect by activating the cAMP/protein kinase A cascade, which increases endothelial NO concentration and promotes vasodilation. Flavonols like kaempferol and quercetin modulate the renin-angiotensin-aldosterone system, improve endothelial dysfunction, and regulate smooth muscle contraction in vessels by activating NO-synthase 3. Naringenin, a flavanone, activates potassium channels to induce vasodilation, while hesperetin, another flavanone, increases NO levels and blocks calcium channels. Epicatechin reduces superoxide production and increases NO-synthase activity to lower blood pressure. Soy isoflavones, such as daidzein and genistein, promote vasodilation through similar mechanisms and also have additional effects on prostaglandin production and calcium ion channel regulation, respectively. Genistein can act as antihypertensive by inhibiting tyrosine kinase Pyk2, the enzyme responsible for the regulation of calcium ion channels and activation of signaling pathways and also reduce smooth muscle hypertrophy in pulmonary arteries, leading to a decrease in pulmonary hypertension.[23,24]

7. The synergy between exercise and flavonoids in the treatment of hypertension

The combination of physical activity and flavonoid intake holds considerable promise in the treatment of hypertension, offering a multifaceted approach to blood pressure management. Regular physical exercise, particularly aerobic activities of moderate to high intensity, has been shown to stimulate the production of nitric oxide (NO) by endothelial cells within blood vessels. NO is a potent vasodilator, which means it relaxes blood vessels, reducing vascular resistance and consequently lowering blood pressure. In parallel, flavonoids found in various natural foods and beverages, such as fruits, vegetables, green tea, and dark chocolate, possess vasodilatory and antioxidant properties. They can enhance the vasodilation effect and shield endothelial cells from oxidative stress. The combination of regular exercise and flavonoid consumption appears to provide a synergistic benefit by reducing oxidative stress, controlling inflammation, and supporting weight management and glucose metabolism.[16,25]

Sephia et al. conducted a study to investigate the effects of a catechins-enriched diet on arterial blood pressure. The results showed that the hypotensive effects were observed only during the period of diet administration, similar to the effects of moderate physical activity. However, in the group that only underwent training, the beneficial effects on blood pressure tended to diminish over time. This decline may be attributed to the oxidative stress induced by physical exercise, which counteracts the benefits on the cardiovascular system. To counteract these side effects, the simultaneous administration of antioxidants such as catechins can be effective. This combination can prolong the hypotensive effect of physical activity, as demonstrated in the training + catechins-enriched diet group in this study. Futheremore, Moonikh et al. showed that eight weeks of high-intensity interval training alone or with quercetin by reducing oxidative stress (increasing total antioxidant capacity (TAC) and reducing malondialdehyde (MDA) reduces level of concentric pathologic hypertrophy in men with hypertension.[26,27]

8. Conclusion

The combination of diets rich in flavonoids and regular exercise presents a synergistic approach to managing hypertension. Flavonoids enhance endothelial function and reduce oxidative stress, thereby complementing the vasodilatory and blood pressure-lowering effects of exercise. Studies have shown that the simultaneous intake of flavonoids and exercise can extend the hypotensive impact of physical activity and decrease oxidative stress, thereby aiding in improved blood pressure management. In summary, the interaction between physical activity and flavonoids in the treatment of hypertension is a fascinating area of research. Their complementary mechanisms suggest that combining exercise and

diets rich in flavonoids may offer a more comprehensive and effective strategy for managing hypertension and promoting overall cardiovascular health. Further studies are needed to explore this synergy in greater detail and to establish practical recommendations for individuals with hypertension. Nonetheless, these findings emphasize the potential benefits of incorporating both regular exercise and a flavonoid-rich diet into hypertension management.

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Literature Review

The effectiveness of tempuyung extract (*Sonchus arvensis*) in the herbal treatment of gout arthritis: Literature review

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Abstract: Gout arthritis is a chronic inflammatory disease caused by disorders of nucleic acid metabolism and *monosodium urate* (MSU) deposition with hyperuricemia as one of the main causes. The incidence of gout arthritis in Indonesia is known to have increased, which in 2013 was 11.9%. Pharmacology management for gout arthritis can cause serious side effects so it is necessary to find alternative drugs that have minimal side effects. One of the types of plants known to have antihyperuricemia activity is tempuyung *(Sonchus arvensis)* that were found in some of the major islands in Indonesia. Tempuyung is useful in preventing the inflammation process and accumulation of monosodium urate (MSU) in joints. The mechanism is still uncertain, but it is allegedly due to the presence of flavonoid compounds in tempuyung which act as an inhibitor of the xanthine oxidase enzyme that causes gout arthritis. In addition, the extracts of tempuyung leaves and roots also have anti-inflammatory, antioxidant, antibacterial properties and so on.

Keywords: Gout arthritis, *Sonchus arvensis*, antihyperuricemia, flavonoid, xanthine oxidase.

1. Introduction

Gout arthritis is a chronic inflammatory disease that occurs as a result of disturbances in nucleic acid metabolism and accumulation of monosodium urate (MSU) crystals in the joints. Gout arthritis is described by severe pain and swelling of one or more synovial joints.[1] Hyperuricemia is one of the main causes of gout.[2] Increased uric acid metabolism, and decreased uric acid excretion, or a combination of both are the causes of hyperuricemia.[3]

Based on data by the Global Health Data Exchange (GHDx) by the World Health Organization (WHO), cases of gout in worldwide is estimated 7.44 million people with incidence around 0.097%, and the prevalence in Canada is estimated 41.22 million cases (0.54%).[4] The prevalence of arthritis gout in Indonesia is still unknown with certainty.[5] However, the incidence rate is known to be increasing.

In 2013, the incidence of gout arthritis was 11.9% (Kemenkes RI, 2013). Research on the Sangihe ethnic group on the island of North Minahasa by Ahimsa & Karema K. (2017) found a prevalence of gout was 29.2% while research conducted by Raka Putra et al. (2017) showed that the prevalence of hyperuricemia in Bali was 14.5%.[5]

First-line treatments of gout arthritis include non-steroid anti-inflammatory drugs (indomethacin and naproxen), corticosteroids, and colchicine.[1] Gout arthritis can be cured by using uric acid discharge which works by blocking xanthine oxidase such as allopurinol, febuxostat - or by increasing renal uric acid excretion.[6] However, these drugs can cause serious reactions or side effects, such as nephropathy, kidney toxicity, gastrointestinal toxicity, liver damage myelosuppression, allergic reactions and increased 6-mercaptopurine toxicity. Therefore, it is necessary to find new alternative agents with better efficacy to reduce uric acid that have minimal side effects.[1,7]

Exploration of natural materials in Indonesia can be carried out by using plants as herbal medicines with therapeutic potential as anti-hyperuricemia.[8] Xanthine oxidase inhibitors need to be developed as an alternative treatment because they have lower side effects than allopurinol, for example, tempuyung leaves.[9]

The purpose of writing this literature review is to determine the potential use of tempuyung (*Sonchus arvensis*) in the herbal treatment of gout arthritis and to determine the content of bioactive compounds that have anti-hyperuricemic activity.

2. Methods

The research method uses a descriptive approach by reviewing the literature from research articles. Search for articles was carried out through the google site (www.google.co.id), google scholar (www.scholar.google.co.id) and PubMed (pubmed.ncbi.nlm.nih.gov) with keywords namely tempuyung, *Sonchus arvensis* and gout arthritis.

3. Results and Discussion

Sonchus arvensis (Tempuyung)

Tempuyung, a plant from the genus Sonchus and in the Asteraceae family, is a traditional plant that grows in Indonesia and found in Sumatra, Java, Bali, Sulawesi and Papua. Therefore, tempuyung is also known as a native Indonesian medicinal plant (OAI).[10] Tempuyung is found to grow at an altitude of 50 to 2400 meters above sea level and is often found in rice fields, roadsides and cliffs.[11] There are several Sonchus species found, namely *Sonchus arvensis, Sonchus oleraceus, Sonchus asper,* and *Sonchus erzincanicus.* However, *Sonchus arvensis* is the easiest to obtain and use as research materials. The difference is not very visible, both in morphology and anatomy.[12]

Tempuyung has a spear-like with a long shape, jagged leaf edges, smooth and thin surface. The stems of tempuyung are round and erect, the roots are taproot type and the fruit is small brown with a hard and wrinkled texture (Wahyuni *et al*.,2019). In Chinese pharmacology, it is stated that tempuyung has a dominant bitter taste.[13]

Tempuyung has many beneficiences, including treating gout, asthma, diuretics, cough, stone urination, fever and others so it is often referred to as an anti-hyperuricemia and anti- inflammatory plant[1], antioxidants[14], antibacterial[15] and so on. In fact, research by Wadekar et al in 2012 found that tempuyung can be consumed for the treatment of helminthiasis, diarrhea and dysentery.[13]

Tempuyung (*Sonchus arvensis*) is a traditional medicinal plant that has benefits to reduce uric acid levels in the body due to its nature as an inhibitor of the xanthine oxidase enzyme.[9] Several studies have found that tempuyung contains secondary metabolites such as tannins, phenols and flavonoid.[16] Flavonoid compounds that contains in tempuyung are apigenin 7-O- glucoside as the highest levels compound with 0.5% in total, 5,7,4-trihydroxy flavone (apigenin), luteolin 7-O-glucoside and 5,7,3,4'-tetrahydroxy flavone (luteolin). [10,17]

Gout Arthritis

Gout arthritis is a disease that is often found around the world. Gout arthritis is a heterogeneous group as a result of uric acid supersaturation in extracellular fluid or due to deposition of MSU crystals in the tissue.[18] Gout arthritis mostly occurs in adult men to old age and in women usually occurs in the post-menopausal period.[19]

Pathogenesis

Purine metabolism arises from the conversion of adenosine and guanosine to uric acid in the human body. Initially, adenosine is converted to inosine by the role of adenosine deaminase. In higher primates, uric acid is converted to alatoin (a water-soluble product in mammals) by uratase (*uricase*). Humans do not have uratase, so the end product of purine metabolism is uric acid.[20]

The cause of gout is a metabolic disorder of purine catabolism in which the production and excretion of various purine catabolites becomes excessive. This overproduction and excretion is caused by various genetic defects in PRPP synthetase. When uric acid levels exceed the solubility limit, sodium urate / *monosodium urate* in soft tissues and joints crystallizes. This is what causes an inflammatory reaction in the form of gout arthritis.[20]

Phagocytosis by macrophages occurring in the joint cavity causes an inflammatory response in gout arthritis. Furthermore, there is the formation of the inflammasome as a protein complex that mediates the enzymatic process of pro-IL-1β which is initially inactive to biologically active IL-1β which is

then secreted from cells. MSU crystals with a co-stimulating role and lipopolysaccharides trigger the activation of IL-1β.[21]

The onset of gout attacks will be associated with changes in the increase and decrease of uric acid serum levels. Attacks rarely occur when uric acid serum levels are stable. The decrease in uric acid serum can precipitate the release of monosodium urate crystals from their deposits. In some patients with gout arthritis or with asymptomatic hyperuricemia urate crystals are found in the knee area that has never had an acute attack and in the metatarsophalangeal joint.

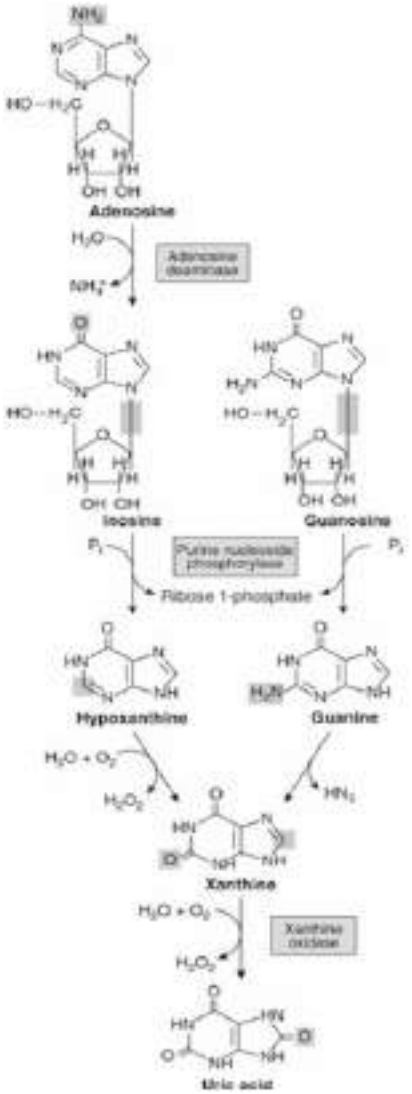

Figure 1. Uric acid metabolism[20]

Management

The management of gout arthritis must be done early to prevent the complications. The goal of treating acute gout arthritis is to eliminate complaints of joint pain and inflammation with drugs such as non-steroidal anti-inflammatory drugs (NSAIDs), colchicine, corticosteroids, or the ACTH

hormone.[22] In the intercritical and chronic stages, the goal of treatment is to lower uric acid levels to normal levels to prevent recurrence by administering a low- purine diet and taking the drug allopurinol together with other uricosuric drugs. However, long-term use of these medicines can cause serious side effects, including nephropathy, kidney toxicity, gastrointestinal toxicity, liver damage, myelosuppression, allergic reactions and increased 6-mercaptopurine toxicity. Therefore, it is necessary to find alternative new agents with better efficacy to reduce uric acid that have minimal side effects, for example, such as herbal treatment with tempuyung.[1,7]

Activity of *Sonchus arvensis* Extract as Xanthine Oxidase Inhibitor

Research on the use of tempuyung showed that compounds that have xanthine oxidase enzyme inhibitor activity are flavonoids. The flavonoid content in tempuyung leaves was 0.1044%, while in the tempuyung root it was 0.5% and the largest flavonoid was apigenin 7-0- glucoside.[3,10] There is a change in decreased uric acid levels from each different dose of tempuyung extract which shows the activity of tempuyung leaves as an anti-hyperuricemia with the most effective dose of 0.35 mg/gBB.

Apart from the tempuyung leaf extract, anti-hyperuricemic activity was also found in the tempuyung root infusion. In the research of Retnowati et al. (2014) found that tempuyung root infusion had an effect on reducing uric acid levels where the concentration of 40% of tempuyung root infusion was almost equivalent to allopurinol. The 40% concentration group was the group with the most people where the average uric acid level measurement was the greatest when compared to the other dose groups (10% and 20%).

Flavonoids

Flavonoids are one of a group of phenolic compounds that are commonly found in plant tissue.[23] Flavonoids can also be found in fruits, vegetables, whole grains and bark.[24]

Flavonoids consist of a solitary benzene ring along with a benzo-gamma- piron structure that are shaped by three acetic acid derivation units and phenylpropane units (through shikimic acid route). More than 500 compounds from a total of 2000 compounds undergo formation in the free state (aglycones) and the rest as O- or C- glycosides.[25] Classifications of flavonoids include flavones, flavonols, flavanols, flavanones, isoflavonoids, neoflavonoids, chalcone, and catechins. The difference in the substitution of the flavonoid structure causes this classification. This difference also results in diverse pharmacological activities.[26] Some of its pharmacological activities are as antioxidants, anti-inflammatory, anti- mutagenic, and even anti-cancer.[23,24]

One of the pharmacological activities in this case is as an antioxidant which works as an inhibitor of the xanthine oxidase enzyme so that there is inhibition or reduction of uric acid formation in the body.[3] Flavonoids have potential as antioxidants by acting as electron donors to prevent reactive

oxygen species (ROS). ROS is a trigger for inflammation due to accumulation of *monosodium urate* (MSU) in joints, which causes complaints and disorders of gout arthritis.[1]

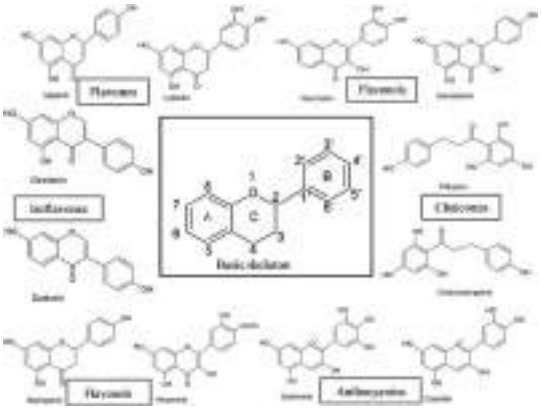

Figure 2. Basic skeleton structure of flavonoids and their classes[24]

The xanthine oxidase pathway is an important pathway that contributes to oxidative injury to tissues, particularly after the ischemia-reperfusion stage. Xanthine dehydrogenase is a type of the compound that exists under physiological conditions, however its design is changed to xanthine oxidase during ischemic conditions. Xanthine oxidase is a provenance of oxygen free radicals. Xanthine oxidase reacts with molecular oxygen and releases superoxide free radicals in the reperfusion phase.[24]

According to research conducted by Lin, et al. (2015) known that apigenin has strong activity as a xanthine oxidase inhibitor, followed by luteolin, and kaempferol.[27]

4. Conclusions

Based on the literature review, it can be concluded that tempuyung, which is a native Indonesian medicinal plant (OAI), is effective as an herbal treatment for gout arthritis. Tempuyung leaf and root extracts can be useful for reducing uric acid levels because they prevent inflammation and accumulation of *monosodium urate* (MSU) in joints. The mechanism is still uncertain, but it is allegedly due to the presence of flavonoid compounds in tempuyung which act as an inhibitor of the xanthine oxidase enzyme that causes gout arthritis. Therefore, consuming tempuyung can be an alternative therapy in addition to medical and healthy lifestyle behaviors.

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Research Article

Association between balancing nutrition with dental and skeletal development in East Java stunting children

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Abstract: Stunting can lead to a child having a shorter stature compared to what is expected for their age group may affect a child's overall health, cognitive development, and future well-being. Stunting and nutrition are closely linked, inadequate nutrition during the critical stages of early childhood development including in the dental and skeletal development. Adequate and balanced nutrition during early childhood is essential for the proper growth and formation of teeth and bones. The aim of this study is to analyze association between balancing nutrition with dental and skeletal development in east Java stunting children. Elementary school children 78 (39 stunted, 39 normal control) between 7-15 years of age. Stunting was determined by relating height and age, according to the World health organization. Survey was conducted in Surabaya and Sumenep to know the statues of balancing nutrition and tooth erupsion age and also observed Stunting children the skeletal and dental development. Correlation test is using Pearson's Test. There is a significant negative relationship between balancing nutrition and tooth eruption age in East Java Stunting Children with p= 0.009 ($p<0.05$), with correlation coefficient 0.28. There is significant relationship indicates between balancing nutrition and dental development (dental malocclusion) in East Java Stunting Children with p=0.003, with positive relationship (r=0.32); but not significant relationship was found between balancing nutrition with skeletal development in stunting children p=0.864 ($p>0.05$), with negative correlation -0.19. Nutrition in stunting children associated with tooth eruption age and dental development

Keywords: dental and skeletal development; nutrition; stunting

1. Introduction

Addressing the issue of stunting in developing nations like Indonesia necessitates dedicated and ongoing attention as it poses a significant public health concern. According to data gathered by the

World Health Organization (WHO) on stunting rates among children under five, Indonesia ranked third among countries in the South-East Asia Region (SEAR) with the highest prevalence of this problem.[1] Stunting is an indicator that a child has experienced long-term inadequate nutrition, typically during the critical periods of growth, such as in the first 1,000 days of life which includes pregnancy and the first two years after birth (from conception to the child's second birthday).[2] This critical growth phase encompasses a total of 270 days during pregnancy and the subsequent 730 days after birth.[3] When a child is stunted, it means their height-for-age falls significantly below the average height for children of the same age, reflecting a failure to achieve their full growth potential.

In line with the UNICEF framework, growth retardation is attributed to three primary factors: an imbalanced diet, low birth weight, and medical history.[4] Stunting is a visible manifestation of chronic malnutrition and is often a result of insufficient intake of essential nutrients such as protein, vitamins, and minerals over an extended period.[5,6] A mother's pre-pregnancy weight, her height, and the weight she gains during pregnancy directly correlate with maternal nutrition and the growth of the fetus. Maternal nutrition significantly impacts fetal growth, particularly in developing nations. While multiple factors interact and affect fetal development, maternal malnutrition is recognized as a primary contributor to intrauterine growth retardation (IUGR) in these regions.[7]

Most of the fetal weight gain happens during the third trimester, but the impact of these nutrients isn't limited to just the second or third trimester. Research in animals has demonstrated that nutritional deficiencies around the peri-implantation period can significantly hinder fetal growth.[8] Infections and malnutrition reinforce each other in a harmful cycle during pregnancy, leading to unfavorable obstetric outcomes, such as low birth weight, which in turn affects the likelihood of infant growth retardation.[4]

Furthermore, infants born with low birth weight often experience intrauterine growth restriction, resulting in slow growth and frequently an inability to catch up with the growth rates expected after birth.[8] Consequently, intrauterine growth restriction (IUGR) leads to stunted growth, and insufficient catch-up growth also influences the likelihood of stunting.[9] Therefore, consistent care during the perinatal period, encompassing pregnancy and lactation, is crucial for ensuring the best outcomes and the newborn's health. It is expected that infants will catch up with their growth in the first few months of life without any further complications.[7]

The consequences of stunting can be long-lasting and encompass not only physical growth but also cognitive development, immunity, and overall health [6], Incorporating the formation and maturation of teeth. While undernourished children frequently encounter delayed tooth eruption, dental cavities are prevalent during early childhood, especially when malnutrition coincides with the formation of teeth.[10] In Indonesia, children experiencing growth stunting have reported severe primary dental

cavities as a common issue. Moreover, there is an association between cavities and growth retardation in children's deciduous (baby) teeth.[11] Data reveals that in 2013, approximately 24% of infants in Indonesia were born with stunted growth, and by 2018, nearly 90% of children under the age of five were affected by dental cavities.[12]Addressing stunted children's treatment remains challenging due to the fact that the root causes and physiological mechanisms behind it are not yet fully understood.[13] The aim of this study is to analyze association between balancing nutrition with dental and skeletal development in east Java stunting children.

2. Methods

The methodology of a research study is an observational correlational analytic study with a cross-sectional approach is a type of study that observes and analyzes data to determine correlations between variables at a single point in time, without intervening or manipulating those variables. The time frame which the research was conducted during July 2023. Sampling was carried out by using a random sampling method to select elementary school children from total population sampling stunting children in Ngagelrejo Surabaya and Sumenep is 78 children (39 stunted, 39 normal control) between 7-12 years of age.

The inclusion criteria of this study were children aged 7-12 years who indicated their willingness to participate as research subjects by signing informed consent document by the parents of children, and children who had a height-for-age Z-score <-2 SDS from WHO. The study's exclusion criteria included children with congenital conditions like Down's syndrome, cleft lip, and cleft palate, children with hormonal disorders affecting tooth eruption, skeletal and dental, and children on long-term medication.

The equipment and materials employed in this research encompassed research information sheets, children's data sheets, parental consent forms, dental and skeletal development status forms, nutritional evaluation sheets for recording height and age measurements, a standing height measuring device (microtoise), disposable dental examination tools such as probes, tweezers, mouth mirrors, and excavators, disinfectants (70% alcohol), trays, towels, cotton rolls, flashlights, masks, gloves, and stationery.

Stunting was determined by relating height and age, according to the World Health Organization. Stunting: Severely stunted: Number of children whose height-for-age z-score is below minus 3 (-3.0) standard deviations (SD) below the mean on the WHO Child Growth Standards (hc70 < -300). Moderately or severely stunted: Number of children whose height-for-age z-score is below minus 2 (-2.0) standard deviations (SD) below the mean on the WHO Child Growth Standards (hc70 < -200). Mean z-score for height-for-age: Sum of the z-scores of children with a non-flagged height-for-age score

(∑ hc70/100, if hc70 < 9990). Survey was conducted in Surabaya and Sumenep to know the statues of balancing nutrition and tooth erupsion age and also observed Stunting children the skeletal and dental development. Information recorded in the document, including name, gender, birth date, height, and examination date. The child's nutritional status is classified as adequate if they consume all nutrients in their entirety. The parameter of the nutritional status was scored as follows: a score of 1 is given if the child consumes solely fish, meat, vegetables, or a single fruit. A score of 2 is assigned if the child consumes a minimum of two types of nutrients. The child's balancing nutritional status is classified as adequate if they consume all nutrients in their entirety. Dental development consists of the two parameters; first the age at which the initial tooth erupt's and the malocclusion detected in the child's teeth. Their initial teeth erupt's were assessed using anamnesis, and to dental development was scored using Dental maloclusion criteria are evaluated by counting the malocclusions detected in the child's teeth. A score of 1 is assigned if two or more malocclusions are present, a score of 2 is given if there is one malocclusion, and a score of 3 is recorded if no malocclusions are observed in the child's teeth. Skeletal development through intra-oral examinations was evaluated with a score of 1 if there is a skeletal class 2/3 and a score of 2 if there is a skeletal class 1. Class I skeletal" relationship, as assessed, typically refers to a balanced and harmonious alignment of the upper and lower jawbones, where the upper jaw (maxilla) and lower jaw (mandible) are in proper positioning in relation to each other. This is often considered the ideal or normal jaw relationship. Class II skeletal relationship, the upper jaw (maxilla) is positioned more forward in relation to the lower jaw (mandible). This results in an overjet, which is the horizontal gap between the upper and lower front teeth when the molars are in contact. Class III Skeletal Relationship (Mesiocclusion): In a Class III skeletal relationship, the lower jaw (mandible) is positioned more forward in relation to the upper jaw (maxilla). This results in an underbite, where the lower front teeth are in front of the upper front teeth when the molars are in contact.

Data analysis correlation test using Spearman's Test correlation to examine the correlation between balancing nutritional status and age of eruption of the first teeth and also dental maloclusion and skeletal development. This research has been reviewed and approved by the Research Ethics Commission (KEP) Universitas Hang Tuah.

3. Results and Discussion

The study findings indicated that, based on the descriptive test, the nutritional status parameter stunting children had a median score of 2, whereas the dental development parameter at the time of first teeth eruption had a median score of 8. For dental malocclusion, the median score was 3, and for skeletal development, the median score was 1. Meanwhile, among non-stunted children, the descriptive

test outcomes indicate that the nutritional status parameter has a median score of 3, while the dental development parameter during the permanent molar eruption phase has a median score of 7. For dental malocclusion, the median score is 2, and for skeletal development, it registers a median score of 1 as shown in table 1.

Table 1. Median of nutritional status, dental development, and skeletal development

	Stunting children Median	Non-stunted children Median
Balancing Nutritional status	2	3
Dental development: Time of first teeth erupt's	8	7
Dental development: Dental malocclusion	2	3
Skeletal development	2	2

The results of the Shapiro-Wilk test indicate that all the data follow a normal distribution, necessitating the use of a parametric test ($p \geq 0.05$) but the data value is interval necessitating the use of Sperman's corelation

Table 2. The results of the correlation between Spearman's nutritional status and first eruption teeth in East Java stunting children

	Correlation coefficient	p-value
Spearman's test correlation balancing nutritional status with first teeth eruption	-0.28	0.009

There is a significant negative relationship between balancing nutrition and tooth eruption age in East Java Stunting Children with p value= 0.009 ($p<0.05$), with correlation coefficient is negative and weak - 0.28 as shown in table 2

Table 3. The results of the correlation between Spearman's nutritional status and dental development East Java stunting children

	Correlation coefficient	p-value
Spearman's test correlation balancing nutritional status with dental development (dental malocclusion)	0.32	0.003

Table 4. The results of the correlation between Spearman's nutritional status and skeletal development East Java stunting children

	Correlation coefficient	p-value
Spearman's test correlation balancing nutritional status with skeletal development	-0.19	0.864

There is a significant relationship indicates between balancing nutrition and dental development (dental malocclusion) in East Java Stunting Children with p=0.003 showed moderate positive correlation relationship (r=0.32). Table 3 shows the result of the Spearman rank correlation using SPSS software. The correlation was not found between balancing nutrition with skeletal development in stunting children p value is not significant p=0.864 (p>0.05), with negative coefisient correlation -0.19 which is weak corelation.

In stunting children, the association between nutrition and dental and skeletal development becomes even more critical. Stunting, which is the impaired growth and development of children due to chronic malnutrition, can have significant effects on both dental and skeletal health. In this research, the nutrition status of stunting children in East Java showed a median with a score of 2 is assigned if the child consumes a minimum of two types of nutrients. Corelation test results showed there was a correlation between balancing nutritional status and eruption of first teeth in stunted children aged in East Java (Table 2) with correlation coefficient is negative and weak.

Stunting leads to impaired physical growth, resulting in shorter stature than what is expected for a child's age. Children who experience stunting may have a reduced adult height potential, and it can be challenging for them to catch up to a normal height once stunting has occurred. Stunted children may experience delays in cognitive development. Malnutrition and the lack of essential nutrients during early childhood can affect brain development, leading to cognitive deficits, learning difficulties, and decreased cognitive function.[14] Around the age of 10, a child's brain makes up about 5–10% of their total body mass, requiring twice as much glucose and 1.5 times more oxygen per gram of tissue compared to an adult's brain. Additionally, the child's brain contributes up to 50% of the entire basal metabolic rate of the body.[14,15] Stunted children may be more susceptible to emotional and behavioral problems, including increased irritability, anxiety, and attention issues. These challenges can affect their overall well-being and social interactions. Stunting can weaken the immune system, making children more vulnerable to infections and diseases. This can create a vicious cycle where frequent illnesses further hinder proper growth and development. Stunted children may face difficulties in school due to cognitive and developmental delays. They might struggle to keep up with their peers academically, leading to poorer school performance and lower educational achievements. Stunting can affect muscle development and overall physical strength, which might limit a child's ability to engage in physical activities and affect their motor skills. Long-term Health Consequences: Stunting during childhood can increase the risk of chronic health problems in adulthood, such as obesity, diabetes, cardiovascular disease, and other non-communicable diseases. Inter-generational

Impact: if stunted girls become mothers in the future, they may be more likely to give birth to undernourished babies, perpetuating the cycle of malnutrition and stunting.[16]

The regulation of weight and food intake is intricate, encompassing various pathways. It seems to revolve around the hypothalamus, especially the medial central area, serving as a central control hub, as well as peripheral cellular regulation through the Mechanistic Target of Rapamycin Complex 1 (mTORC1). When it comes to responses in the hypothalamic and mTOR systems due to food deprivation, they offer a reversible natural experiment that sheds light on comprehending the impact of different factors like nutritional status, psychosocial stress (including poverty, maternal deprivation, and abuse), the endocrine system, as well as linear and skeletal growth, and how they interact with one another.[17] A dietary pattern consisting of low-quality protein that is linked to stunted growth results in notably lower levels of essential amino acids circulating in the bloodstream compared to children who are not stunted. These insufficient essential amino acid intakes can have a negative impact on growth, primarily because they affect the primary growth regulation pathway, known as the mechanistic target of rapamycin complex 1 (mTORC1) pathway. This pathway is highly sensitive to the availability of amino acids and integrates various signals, including nutrients (particularly proteins and amino acids), growth factors, oxygen, and energy, to regulate growth in several areas like the chondral plate, skeletal muscle development, myelination of the central and peripheral nervous systems, cellular growth and differentiation in the small intestine, hematopoiesis, iron metabolism, and organ size through the Hippo pathway. These organs play a crucial role in child stunting and the associated health issues such as anemia, impaired cognitive function, environmental enteric dysfunction, and immunity against infectious diseases. [17] When there is a shortage of amino acids, mTORC1 inhibits the synthesis of proteins and lipids, as well as the growth of cells and the entire organism. When amino acid concentrations are low, mTORC1 is spread out throughout the cytoplasm and becomes inactive. Autophagy, which is a response to nutrient deficiency, is a process where damaged or surplus proteins and other cellular components are transported to the lysosome and subsequently broken down, releasing free amino acids into the cytoplasm. Proteins act as a reservoir of amino acids that can be mobilized through autophagy when amino acids are scarce. Furthermore, in the absence of amino acids, other signals like growth factors and energy cannot compensate for the amino acid deficiency to activate mTORC1.[18,19]

Maintaining a balanced nutritional is essential for the proper eruption of teeth. In children with stunting in East Java, it's established that the emergence of the first teeth at the age of 8 months is delayed compared to non-stunted children. Balancing nutrition refers to the practice of consuming a diet that provides the body with the right proportion of essential nutrients to support overall health

and well-being. It involves making food choices that ensure an adequate intake of carbohydrates, proteins, fats, vitamins, minerals, and water while maintaining an appropriate caloric balance. Persistent malnutrition in children who are stunted is linked to a decrease in the production of insulin-like growth factor 1 (IGF-1). Even a temporary 50% decrease in calorie intake or a 33% drop in protein availability can lead to a reversible decrease in IGF-1 levels. The reduced IGF-1 levels cause a secondary increase in growth hormone (GH) due to the negative feedback caused by the low IGF-1 levels on the pituitary gland's synthesis of GH. As a result, there is a shift of resources away from growth towards maintaining metabolic balance. The widely recognized metabolic effects of growth hormone, which do not rely on IGF-1, would clearly be beneficial when facing reduced nutrient intake. These effects include increased breakdown of fats and the release of free fatty acids from fat tissue reserves, as well as the inhibition of glucose uptake by muscle tissue.[20] Balanced nutrition aims to meet the body's nutritional requirements for various functions, including energy production, growth, repair, and maintenance. It also helps prevent nutritional deficiencies and related health issues. A balanced diet typically includes a variety of foods from different food groups, such as fruits, vegetables, grains, lean proteins, and dairy products, in proportions that support optimal health.[21] Quality proteins, such as those found in products like milk, when included in complementary, supplementary, and rehabilitative food items, have proven to be effective in promoting healthy growth. Specific amino acids like lysine and arginine have been identified as factors that influence the release of growth hormones in young children through the somatotropic axis. Higher intake of these amino acids is associated with lower fat mass index in pre-pubertal lean girls. Maintaining stable glucose levels is crucial for effectively coping with chronic malnutrition that results in stunted growth. It is essential to ensure sufficient blood glucose levels until the brain and other vital glucose-dependent functions can adjust to using ketones as an alternative fuel source. One contributing factor is an increase in gluconeogenesis, which is partly driven by cortisol. Additionally, reduced glucose uptake by body tissues is a significant factor. In this context, chronic malnutrition has been associated with both reduced insulin secretion and/or heightened insulin resistance. Elevated levels of cortisol and growth hormone counteract the effects of insulin and prevent low blood sugar levels during malnutrition. A study investigating the impact of malnutrition during the initial year of life on glucose tolerance and plasma insulin levels revealed that early undernourishment in the period following birth, regardless of the infant's birth weight, was linked to increased levels of insulin in the blood and decreased sensitivity to insulin. Numerous studies have documented elevated cortisol levels in undernourished children. The increased cortisol levels observed during malnutrition signify the body's effort to adapt to reduced dietary protein and/or energy intake by breaking down muscle protein to supply the liver with the essential amino acids required for

gluconeogenesis and the synthesis of albumin.[14] Moreover, consuming an adequate amount of protein during early childhood is positively linked to both height and weight at the age of 10.[22] Sumenep is renowned for its fish production; however, it's paradoxical that not all stunted children in the area consume fish.

There is a significant relationship indicates between balancing nutrition and dental malocclusion in East Java Stunting Children with p=0.003 showed moderate positive corelation relationship (r=0.32). Among stunted children, it's recognized that the dental malocclusion score reaches level 2, a score of 2 is given if there is one malocclusion, compare with normal children a malocclusion score is 3 when observed in the child's teeth which is noted when there are no observed malocclusions in the child's teeth.

There are nutritional needed for dental development such as calcium and phosphorus: these minerals are essential for the development of strong teeth. Adequate calcium and phosphorus intake help in the mineralization of tooth enamel, making it strong and resistant to decay. Vitamin D: Vitamin D is necessary for the absorption of calcium, and without it, the body cannot effectively utilize calcium for tooth development. Also, vitamin A: Vitamin A supports the development of healthy gums and oral tissues, helping to maintain the integrity of the tooth-supporting structures. Vitamin C: Vitamin C is crucial for the synthesis of collagen, which is an essential component of the periodontal ligament that anchors teeth to the jawbone.[23] Nutritional needed for skeletal development such as calcium and Phosphorus: These minerals are vital for the development of strong bones. Calcium is the primary building block of bones, and phosphorus plays a role in bone mineralization. Vitamin D: As with dental development, vitamin D is crucial for the absorption and utilization of calcium in bone formation. It helps maintain proper levels of calcium and phosphorus in the blood, which are necessary for bone health. Vitamin K: Vitamin K is involved in the regulation of bone mineralization and supports the synthesis of specific proteins essential for bone formation. Magnesium: Magnesium is required for the proper function of enzymes involved in bone metabolism and mineralization. Also zinc: zinc is necessary for bone growth and plays a role in the synthesis of collagen, which is a structural protein in bones.[24]

Skeletal score among stunted children and normal children reaches the same skeletal (score 2) that means stunted and normal children have relationship class 1 skeletal. Class I skeletal" relationship, as assessed, typically refers to a balanced and harmonious alignment of the upper and lower jawbones, where the upper jaw (maxilla) and lower jaw (mandible) are in proper positioning in relation to each other. This is often considered the ideal or normal jaw relationship. The correlation was not found between balancing nutrition with skeletal development in stunting children p-value is not significant

p=0.864 (p>0.05), with negative coefficient correlation -0.19 which is very weak. The lack of a harmonious connection between nutrition and skeletal growth in stunted children in East Java suggests that, aside from nutrition, there are additional factors influencing the skeletal development of these children. Apart from nutrition, consistent physical activity such as exercise program during growth appears to be one of the key factors that significantly impact the attainment of peak bone mass.[25] Also the role of growth hormones such as parathyroid hormone (PTH), gut hormones (gastric inhibitory peptide (GIP), glucagon-like peptide.[26]

To address the association between nutrition and dental and skeletal development in stunting children, it's crucial to implement comprehensive interventions that focus on improving their overall nutritional status. These interventions include: Promoting exclusive breastfeeding for the first six months of life, introducing appropriate complementary feeding with nutrient-rich foods after six months, ensuring access to a diverse and nutritious diet, providing nutritional supplementation, if necessary, Improving maternal nutrition and health during pregnancy, Enhancing access to clean water, sanitation, and healthcare.[27] A holistic approach that integrates nutrition-sensitive and nutrition-specific interventions is essential to reduce stunting and promote optimal growth and development in children. It requires collaboration between governments, communities, healthcare providers, and other stakeholders to address the multifaceted causes of stunting and ensure a healthier future for children. This includes promoting a diverse and nutrient-rich diet, providing necessary supplements when needed, and ensuring access to proper healthcare. Early identification and management of stunting, along with appropriate nutritional support, can help mitigate the impact on dental and skeletal health, leading to better overall growth and development in affected children. To combat stunting and improve child nutrition, it is crucial to focus on interventions that address both immediate and underlying causes of malnutrition.[28]

4. Conclusion

Balancing Nutrition in stunting children associated with tooth eruption age and dental malocclusion.

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Literature review

Future trends for physiology research to support better health and quality of life

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Abstract: Needless to say, conducting research is essential to support better health and quality of life. There are several important aspects in the research. For example, through research experience, medical professionals can acquire the skill to think logically when making a diagnosis, not just by referring textbooks. In addition, since each ethnic group may have different susceptivity to certain diseases due to different genetic background, one cannot simply apply database of disease created by other country. Thus, I would like to emphasize the importance of conducting research in each country to have own original data for the improvement of health. Among research categories, I also would like to emphasize that the Physiological Scinece research my play the center role.

Keywords: health; physiology; quality of life

1. Introduction

In the dynamic landscape of medical science, the pursuit of knowledge through research stands as a cornerstone for the evolution of healthcare systems worldwide. This article explores the future trends in physiological research, aiming to underscore its pivotal role in fostering better health and an enhanced quality of life. As we delve into the significance of integrating early exposure to research in medical education and the establishment of dedicated research facilities in each country, a compelling narrative emerges — one that advocates for the indispensable connection between research endeavors and the advancement of medical practices.

2. Importance of including early exposure to research in the curriculum of medical education

Medical professionals, including clinicians, are responsible to keep the society in healthy conditions. To take care of diseased patients, they may make an appropriate diagnosis and treatment just by referring clinical guidelines published in certain textbooks or medical journals. However, such attitude may limit their skill to deal with complicated cases. They may have to refer further the research articles to solve problems. Without research experiment, however, applying research findings to their daily clinical activity is not easy. Physician Scientists, who have an experience of conducting basic research, can apply their knowledge for diagnosis and treatment with confidence, because, through research

activity, they can acquire the skills of critical thinking, communication, and team works [1], in addition to their skills to integrate research findings into clinical practice.[2] With this regard, research experience is very important. Particularly, it would be important for health professionals or candidates such as medical students to be exposed to research when they are young. Thus, I suggest integrating research project into the medical education curriculum.

In the medical education curriculum, on the other hand, we can also emphasize the importance of medical research for the advancement of medicine and medical care, and support innovation in medicine through involvement in academic and research activities, and developing one's scientific thinking skills. We should never forget that medical science is a part of biological science. Among medical science, Physiological Science is particularly important. Many (actually, I would like to mention "most") laboratory tests are created based on physiological research findings, ranging from the electrocardiogram to a simple measurement of electrolytes.[3] Thus, conducting research activity, particularly Physiological Science research, can contribute greatly the advancement of healthcare system.

Figure 1. In Japan, students should spend for at least 4 weeks in the basic research department. Some students enroll M.D. & Ph.D. course thereafter

3. Importance of having the own research facility in each country

As mentioned above, medical professionals may just refer diagnostic criteria published in the textbooks or medical journals. In many cases, most of such criteria were generated other regions or countries. It should be noted, however, each ethnic group have different susceptibility to certain diseases. For example, etiology of the type 2 diabetes (T2D) differs greatly between Western countries and Asia-pacific region. While insulin resistance is the most common cause of T2D in Western countries, b cell disfunction to secrete enough amount of insulin is the most common in Asia-Pacific region.[4] In addition, climate, lifestyle, eating habit, and socio-economic system differ greatly among regions. Thus, appropriate therapeutic approach could be different between different ethnic groups and countries. Under such circumstances, obtaining own study result from patients of each country is important. For

such purpose, conducting research in each country by considering region-specific factors may be particularly important. Again, because of the reason mentioned above. Physiological Science-related research may play a center role.

4. Conclusion

Conducting research can help obtaining skills for critical thinking as well as for integrating basic science knowledge into clinical settings. Such skills are important for medical professionals to support healthcare in each region. Physiological Science research have greatly contributed to develop diagnostic and therapeutic approaches of various diseases. Thus, I suggest that all medical professionals should be involve in research, particularly Physiological Science, for certain amount of time during under- or post-graduate education.

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Literature Review

Role of aerobic exercise in sarcopenia treatment for older adults: a literature review

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Abstract: Regular aerobic exercise has been shown to address many aspects of sarcopenia. Aerobic exercise induces various adaptations and improvements in skeletal muscle, including increased satellite cell content, resulting in increased myonuclear number per fiber. It also leads to gene expression changes, mitochondrial volume/density alterations, increased mitochondrial enzyme activity, and enhanced lipid oxidation in the muscles. This literature review aims to evaluate the role of aerobic exercise in the treatment of sarcopenia in older adults by examining its impact on muscle strength, muscle mass, and physical performance. Fourteen systematic reviews and meta-analyses published from 2013 onwards were included in the comprehensive search. The findings suggest that aerobic exercise alone does not significantly improve muscle strength or mass in older adults with sarcopenia. Similarly, aerobic exercise alone did not demonstrate a positive effect on muscle mass. However, aerobic exercises like Taichi or Traditional Chinese Exercise showed promise in improving physical performance measures. The role of aerobic exercise as a standalone treatment for sarcopenia remains inconclusive, but when combined with other exercise modalities and nutritional supplementation, it has the potential to enhance muscle strength and physical performance in individuals with sarcopenia. Further research is needed to better understand the effectiveness of aerobic exercise in sarcopenia treatment and its optimal combination with other interventions.

Keywords: aerobic exercise; sarcopenia; muscle mass; muscle strength; physical performance

1. Introduction

Sarcopenia, the age-related loss of muscle mass and strength, is a significant public health concern in aging populations.[1] It is associated with various adverse health outcomes, including decreased physical function, increased risk of falls and fractures, and reduced quality of life.[2] Although exercise is recognized as a key strategy for managing sarcopenia, most studies have focused on resistance training as the primary intervention. Aerobic exercise has been shown to induce various adaptations in skeletal muscle, including increases in satellite cell content, changes in gene expression, improvements in mitochondrial function, and modulation of inflammatory pathways.[3] However, its specific impact on muscle mass, muscle strength, and physical performance in individuals with sarcopenia remains

unclear. Currently, there is limited research specifically examining the association between aerobic exercise alone and its effects on muscle mass, muscle strength, and physical performance in older adults with sarcopenia. The majority of studies have explored the combined effects of aerobic exercise with other modalities such as resistance training or balance training. The unique contribution of aerobic exercise as a standalone intervention in the context of sarcopenia has not been extensively investigated. Furthermore, systematic reviews and meta-analyses have demonstrated that aerobic training, resistance training, whole-body vibration training, and mixed training have positive effects on increasing muscle mass, strength, and physical performance in older people with sarcopenia.[4] Additionally, nutritional supplementation combined with exercise has been found to benefit muscle mass gain and function enhancement in elderly individuals.[5]

Incorporating aerobic exercise into comprehensive interventions for the management of sarcopenia in elderly individuals can lead to improved muscle strength, prevention of frailty, management of comorbidities, enhanced overall health and quality of life, and individualized care.[6] This highlights the importance of exercise as a key component in the management of sarcopenia and emphasizes the need for tailored exercise programs that consider the specific needs and capabilities of older individuals. Despite advancements, there are still gaps in the literature that require further research. These include determining optimal exercise protocols, investigating long-term effects, elucidating underlying mechanisms, developing individualized approaches, conducting comparative effectiveness studies, exploring nutritional interventions, and assessing functional outcomes. Understanding the potential benefits of aerobic exercise alone in sarcopenia management is crucial for developing effective and comprehensive interventions for older adults. By conducting a literature review focused on aerobic exercise as a standalone treatment for sarcopenia, this study aims to fill the existing research gap and provide valuable insights into the potential benefits and limitations of aerobic exercise alone in the management of sarcopenia. The findings of this review will contribute to the current knowledge base, guide future research efforts, and inform the development of evidence-based guidelines for the prevention and treatment of sarcopenia in older adults.

2. Methods

A systematic search was conducted across six electronic databases from 2018 to 2023: PubMed, Sciencedirect, Sagepub, Tripdatabase, Cochrane Library, Embase, and CINAHL. Only studies in the English language were included. The following keywords were utilized: "aerobic exercise" OR "aerobic training" OR "aerobic" AND "treatment" OR "prevention" AND "sarcopenia" OR "presarcopenia" OR "pre-sarcopenia" OR "muscle mass" OR "muscle strength" OR "physical performance" AND "elderly" OR "geriatric". The flowchart for systematic search was shown in Figure 1. The search strategy utilized

a combination of keywords and search terms related to the population (elderly individuals), condition (sarcopenia), intervention (aerobic exercise), and study design (systematic review and meta-analysis). The search terms included variations and synonyms to ensure comprehensive retrieval of relevant articles. The inclusion criteria for study selection were as follows:

a) Systematic reviews or meta-analyses published from 2018 onwards.
b) Focused on the efficacy of aerobic exercise as a treatment for sarcopenia in elderly individuals.
c) Included studies involving human participants aged 60 years and older with a diagnosis or criteria for sarcopenia.
d) Evaluated outcomes related to muscle mass, muscle strength, physical performance, or quality of life.
e) Written in English.

The exclusion criteria included:

a) Non-systematic reviews or meta-analyses.
b) Studies not specifically assessing the effectiveness of aerobic exercise as a treatment for sarcopenia.
c) Animal or in vitro studies.
d) Studies with participants outside the defined age range of 60 years and older.
e) Studies not reporting relevant outcomes.

The initial screening of search results was based on the titles and abstracts of the articles. Full-text articles were then assessed for eligibility based on the inclusion and exclusion criteria. Two independent reviewers conducted the study selection process, and any discrepancies or disagreements were resolved through discussion with third reviewer. References lists of the included systematic reviews and meta-analyses were manually screened to identify additional relevant studies that may have been missed in the electronic database search. The search strategy and study selection process were performed in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure transparency and rigor in the literature search and selection process. The final selection of systematic reviews and meta-analyses meeting the inclusion criteria will form the basis for the literature review. The included reviews/meta-analyses were evaluated for the assessment of risk of bias in the included studies. This involves determining if the reviews/meta-analyses employed appropriate tools, such as the FAITH Critical appraisal tool, to assess the quality of the individual studies. Assessing the risk of bias helps in identifying potential limitations and weaknesses in the primary studies and allows for a more informed interpretation of the results. The statistical methods used for data analysis and synthesis were examined. This includes assessing if appropriate statistical techniques were applied, such as random-effects models for meta-analyses, to

account for heterogeneity among the included studies. Additionally, the appropriateness and transparency of the methods used to pool and present the results were evaluated. The reviews/meta-analyses were evaluated for the assessment of heterogeneity among the included studies. This includes examining if measures of heterogeneity, such as I^2 statistics, were reported and if appropriate sensitivity analyses were conducted to explore potential sources of heterogeneity. Understanding and addressing heterogeneity ensure the validity and robustness of the conclusions drawn. By conducting these methodological assessments, the reliability and validity of the included systematic reviews and meta-analyses were evaluated. This process helps in assessing the overall quality of the evidence synthesized and enhances confidence in the conclusions and implications drawn from these reviews/meta-analyses.

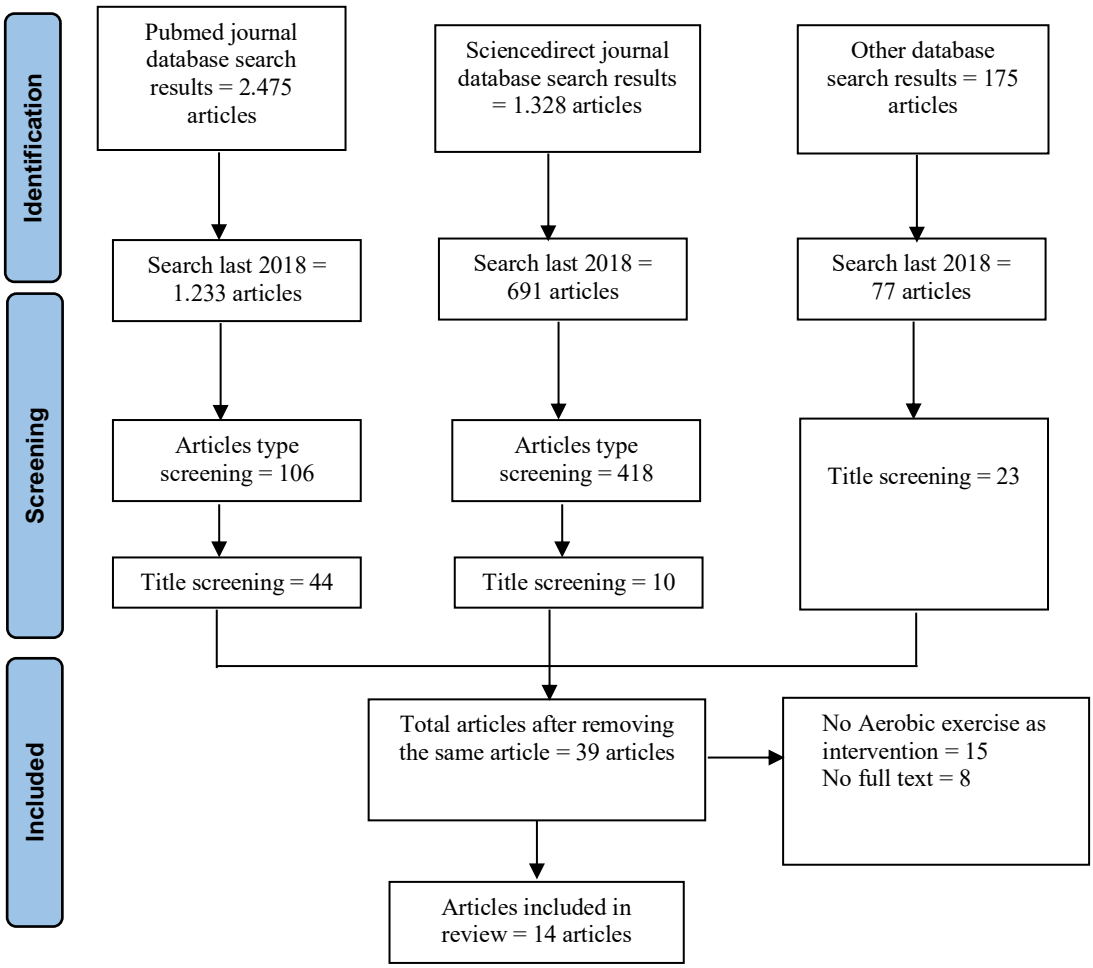

Figure 1. Search strategy flowchart

3. Results and Discussion

Table 1 showed summary of included studies. The findings from the 14 systematic reviews included in this analysis shed light on the effectiveness of aerobic exercise alone and combination exercises in managing sarcopenia in elderly individuals. Regarding aerobic exercise alone, four systematic reviews

[7–10] concluded that it does not significantly increase muscle strength in elderly individuals with sarcopenia. However, one systematic review [5] reported contrasting results, suggesting that aerobic exercise alone can indeed improve muscle strength. Similarly, concerning muscle mass, two systematic reviews [7,11] found that aerobic exercise alone does not lead to a significant increase in muscle mass among elderly individuals with sarcopenia. Interestingly, none of the systematic reviews specifically reported that aerobic exercise alone could enhance muscle mass. In terms of physical performance, two systematic reviews [7,8] revealed that aerobic exercises, such as Taichi or Traditional Chinese Exercise, can lead to improvements in the Chair Stand Test and Time Up and Go Test in elderly individuals with sarcopenia.

Additionally, this analysis considered various systematic reviews on combination exercises, which encompassed a mix of resistance training, aerobic exercise, balance training, and strength training. Five systematic reviews [12–16] indicated that combination exercises can increase muscle mass in elderly individuals with sarcopenia, while three others [5,11,17] reported no significant effect on muscle mass. Furthermore, eight systematic reviews [4,10,12–17] concluded that combination exercises can increase muscle strength in elderly individuals with sarcopenia, while only one systematic review [9] presented opposing findings. Regarding physical performance, six systematic reviews [4,5,9,10,13,14] found a significant improvement with combination exercises, whereas one systematic review [9] reported different results. Notably, combination exercises when combined with nutritional supplementation were found to increase both muscle mass and muscle strength in elderly individuals with sarcopenia based on two systematic reviews.[9,18]

Sarcopenia, characterized by the waning of skeletal muscle mass and function with age, looms as a significant health concern among the elderly. Aerobic exercise has been investigated as a potential treatment for sarcopenia, and current research suggests that it can be beneficial in improving muscle mass, strength, and physical performance in elderly individuals with sarcopenia. The available evidence suggests that aerobic exercise may not have a significant effect on muscle mass and strength in the elderly due to several potential mechanisms or physiological pathways. Firstly, the primary effects of aerobic exercise may lie in cardiovascular function and oxidative metabolism, with minimal impact on muscle mass and strength.[19] Secondly, aerobic exercise may not provide a sufficient overload to activate muscle protein synthesis and subsequent muscle hypertrophy. [20] Thirdly, aging could be associated with a reduced response to muscle protein synthesis stimulation, potentially limiting the effectiveness of exercise in stimulating muscle growth.[21] Fourthly, low-volume aerobic exercise may not be adequate to promote increases in muscle strength and power in elderly subjects.[22] These potential mechanisms collectively contribute to the intricate relationship between aerobic exercise and muscle-related outcomes in the elderly population.

Although current evidence showed that aerobic exercise alone has not been proven to be able to improve muscle mass and muscle strength in older people with sarcopenia, aerobic exercise has been shown to be able to improve physical performance in older people with sarcopenia and this improvement can be attributed to several potential mechanisms. One significant mechanism is the positive impact on protein metabolism. Research has indicated that low-intensity aerobic training, typically performed at around 40% to 60% of maximum heart rate, contributes to improved protein metabolism in older adults dealing with sarcopenia.[23] This metabolic enhancement plays a pivotal role in maintaining muscle mass and function, consequently leading to notable advancements in physical performance. Another crucial pathway through which aerobic exercise enhances physical performance is the modulation of insulin resistance. High-intensity aerobic training, usually conducted at 60% to 80% of maximum heart rate, has been demonstrated to circumvent protein insulin resistance and preserve muscle protein synthesis.[23] Given that insulin resistance is a prevalent issue in sarcopenia and can hinder muscle protein synthesis, the ability of aerobic exercise to regulate insulin resistance becomes a critical factor in promoting muscle protein synthesis and, in turn, elevating physical performance among older individuals with sarcopenia. Furthermore, the augmentation of mitochondrial function stands out as another mechanism by which aerobic exercise contributes to enhanced physical performance. Mitochondrial dysfunction is intricately linked to age-related sarcopenia and is a key contributor to muscle weakness and fatigue. Studies have unveiled that aerobic exercise triggers the enhancement of mitocondrial biogenesis and subsequently improves mitochondrial function in the elderly population.[24] This heightened mitochondrial efficiency translates to increased energy production and decreased muscle fatigue, ultimately culminating in improved physical performance. Moreover, the systemic effects of aerobic exercise on cardiovascular health and oxygen delivery also play a pivotal role in elevating physical performance. As cardiovascular fitness and oxygen delivery improve through regular aerobic exercise, overall muscle function and endurance are positively influenced, allowing older individuals to execute physical tasks more efficiently.[24] This systemic improvement not only contributes to better physical performance but also enhances the overall functional capacity of elderly individuals struggling with sarcopenia.

Table 1. Summary of the included studies

No	Study detail	Aim	Intervention detail	Results	Limitation
1	Contreras, FH et al (2018)[16] No of studies: 9 (7 RCT) Age: 66.8±5.4 - 81.4 ± 4.3 years	Assessing the influence of Exercise OR exercise plus dietary supplements on body composition and physical performance in elderly individuals with sarcopenic obesity	Exercise Interventions: AT, RT, MT, WBEMS Combined Interventions: WBEMS + Exercise and Supplements, RT + AT + Supplements. Supplements: (i) 40g/day of protein with 21g of high-leucine whey protein (2.8g per serving) and essential amino acids (27g), (ii) 1.7-1.8g/kg of protein, (iii) amino acid supplementation (3g leucine and 20g vit D) and the catechin, once daily, for 3 months.	Exercise and exercise + supplementation reduce body fat percentage (MD - 0.85%; 95% CI -1.61 to -0.08%; P=0.005; I2=62%), increase ASM (MD 0.40 kg; 95% CI 0.18-0.63%; P=0.0005; I2=0%), improve grip strength (MD 1.30 kg; 95% CI 0.58-2.01; P < 0.0004; I2=20%), and gait speed (MD 0.05 m/s; 95% CI 0.03 to 0.07; P < 0.00001; I2=0%). Exercise alone and exercise + supplementation reduce waist circumference (MD - 1.40 cm; 95% CI -1.99 to -0.81; P=0.00001; I2=12%), decrease total fat mass (MD - 1.77 kg; 95% CI -2.49 to -1.04; P < 0.0001; I2=21%), and trunk fat mass (MD - 0.82 kg; 95% CI -1.22 to -0.42; P=0.0001; I2=16%).	The heterogeneity of exercise interventions, the limited number of studies, and the lack of a clear definition of sarcopenic obesity used.
2	Yin, YH (2020)[18] No. of studies: 16 (12 RCT) The age ranged from 41 to 90 years with a mean of 72.01 ± 7.76 years. Out of 16 studies, 2 included only male participants, 8 included only females, and 2 included both genders.	Describing the criteria for identifying sarcopenic obesity (SO) and the non-pharmacological intervention components used for its management, as well as assessing the effectiveness of these interventions.	The interventions employed in the studies included: ● Exercise: aerobic, resistance, machine-based training. ● Nutrition: supplements, controlled diet. ● Combination of exercise and nutrition. ● Electric acupuncture. From the 16 studies, 3 of them conducted aerobic exercise interventions.	Both exercise with or without nutritional therapy showed an impact on grip strength and reduction in body fat percentage. Exercise + nutrition had a significant effect in increasing ASM.	There are challenges posed by the heterogeneity of exercise interventions, the limited number of studies, and the lack of a clear and consistent definition of sarcopenic obesity used across the research.
3	Li, LM (2023)[17] No. of studies: 11 RCT No. of subjects: 1.136	Summarizing the effectiveness of home-	Home-based exercise interventions: Resistance Training (RT), Aerobic Training (AT),	● Home-based exercise resulted in a non-significant increase in Skeletal Muscle	The sample size is small, and there is a moderate to high risk of bias.

	Age: 60-89 years	based interventions on sarcopenia	balance exercises, and gait training. Intervention duration: 3 to 6 months, with each session lasting 30 to 90 minutes, conducted 1 to 2 times per week.	Index (SMI) by 0.12 kg/m2 compared to the control group. ● Home-based exercise led to an improvement in grip strength (MD = 1.25 kg, 95% CI: -2.10, 4.60, p = 0.46, I2 = 78%) and Chair Stand Test (CST) performance in the intervention group (MD = 0.56 kg, 95% CI: 0.09, 1.03, p = 0.020). ● Home-based exercise showed a non-significant increase in gait speed (GS) by 0.03 m/s and a non-significant decrease in CST time (1.15 seconds).	
4	Lu, Linqian (2020)[4] No. of studies: 25 RCT No. of subject: 1.191 older people with sarcopenia A total of 1,191 older adults with sarcopenia participated, with 613 in the exercise group and 578 in the control group. The average age ranged from 60.6±2.3 to 89.5±4.4 years.	Assessing the effects of various exercises (Resistance Training - RT, Whole Body Vibration Training - WBVT, Mixed Training - MT) on knee extension strength (KES) and physical performance (Timed Up and Go - TUG) in older adults with sarcopenia.	Out of 14 studies that performed Mixed Training interventions: RT+AT+BT: 2 studies. RT+AT: 5 studies. Duration ranged from 60 to 90 minutes, 1 to 5 times per week, lasting 8 weeks to 6 months. RT: 20 studies. Duration ranged from 20 to 60 minutes, 1 to 5 times per week, lasting 6 weeks to 6 months. WBVT: 6 studies. Duration ranged from 15 to 40 minutes, 3 to 5 times per week, lasting 3 to 8 months.	The exercise group exhibited significantly stronger knee extension strength compared to the control group (SMD = 0.86, 95% CI: 0.55 to 1.16, p < 0.00001, I2=75%). RT and MT increased KES compared to the control group (RT, SMD = 1.36, 95% CI: 0.71 to 2.02, p < 0.0001, I2 = 72%; MT, SMD = 0.62, 95% CI: 0.29 to 0.95, p = 0.0002, I2= 56%). However, WBVT did not show a significant difference in KES scores between the exercise and control groups (SMD = 0.65, 95% CI: −0.02 to 1.31, p = 0.06, I2 = 80%). The exercise group demonstrated better Timed Up and Go (TUG) performance compared to the control group (SMD = -0.66, 95% CI: −0.94 to −0.38, p < 0.00001, I2 = 60%). (RT, SMD = -0.92, 95% CI: −1.30 to −0.55, p < 0.00001, I2= 22%; WBVT, SMD = -0.30, 95% CI: −0.60 to 0.00, p = 0.05, I2= 0%; and	The effect of various exercises on muscle mass was not evaluated due to differences in criteria, indicators, and assessment methods. High heterogeneity may be attributed to the variation in assessment and types of exercises used.

				MT, SMD = -0.69, 95% CI: −1.22 to −0.15, p = 0.01, I2 = 70%). The exercise group also exhibited better gait speed (GS) compared to the control group (SMD = 0.82, 95% CI: 0.43 to 1.21, p < 0.0001, I2 = 87%). RT and MT increased gait speed compared to the control group (RT, SMD = 2.01, 95% CI: 1.04 to 2.97, p < 0.0001, I2 = 84%; MT, SMD = 0.69, 95% CI: 0.29 to 1.09, p = 0.0008, I2 = 81%). However, WBVT did not have a significant effect on gait speed (SMD = 0.12, 95% CI: −0.15 to 0.39, p = 0.38, I2= 0%). There was no significant difference in Sit-to-Stand Test (STS) performance between the exercise and control groups (SMD = 0.11, 95% CI: −0.36 to 0.57, p = 0.65, I2 = 87%). (RT, SMD = 0.80, 95% CI: −0.79 to 2.39, p = 0.32, I2= 95%; WBVT, SMD = -0.25, 95% CI: −0.52 to 0.02, p = 0.07, I2= 0%; MT, SMD = -0.04, 95% CI: −0.63 to 0.55, p = 0.89, I2 = 79%).	
5	Zhang, Yanjie (2021)[14] No. of studies: 17 No. of subjects: 985 older people with sarcopenia Average age range: 67.6 - 86 years. Approximately 74% of the participants were female (7 out of 17 studies included an all-female population).	Assessing the effects of exercise on muscle strength, body composition, and physical performance in older adults with sarcopenia.	The interventions included Aerobic Training (AT), Resistance Training (RT), and Mixed Training (MT) with durations ranging from 30 to 60 minutes, 1 to 7 times per week, lasting 8 to 36 weeks. Out of 17 studies, 4 used AT interventions.	The exercise group showed significant improvements in grip strength (SMD = 0.30, 95% CI [0.15, 0.45], I2 = 6%, p < 0.01), Chair Stand Test (CST) performance (SMD = 0.56, 95% CI [0.30, 0.81], I2 = 36%, p < 0.01), and knee extension strength (SMD = 0.32, 95% CI [0.15, 0.50], I2 = 0%, p < 0.01) compared to the control group. The exercise group also demonstrated better performance in the Timed Up and	There is no consistent criterion for determining sarcopenia, and the reviewed studies used different measurement instruments.

				Go (TUG) test (SMD = 0.74, 95% CI [0.48, 1.00], I2 = 0%, p < 0.01) and gait speed (GS) (SMD = 0.59, 95% CI [0.35, 0.82], I2 = 62%, p < 0.01) compared to the control group. Furthermore, the exercise group exhibited improved Skeletal Muscle Index (SMI) (SMD = 0.37, 95% CI [0.15, 0.58], I2 = 16%, p < 0.01) and Appendicular Skeletal Muscle Mass (ASM) (SMD = 0.31, 95% CI [0.13, 0.49], I2 = 20%, p < 0.01) compared to the control group. Moderate-intensity exercise (SMD = 0.81, 95% CI [0.57, 1.05], p < 0.01) significantly improved TUG compared to high-intensity exercise (SMD = 0.23, 95% CI [-0.26, 0.71], p = 0.37). High-intensity (SMD = 1.39, 95% CI [0.73, 2.06], p < 0.01) and moderate-intensity exercise (SMD = 0.41, 95% CI [0.10, 0.72], p < 0.01) significantly increased SMI compared to light to moderate-intensity exercise (SMD = 0.29, 95% CI [-0.20, 0.79], p = 0.25).	
6	Ni, Hsiang-Jung (2021)[13] No of studies: 34 RCT No of subjects: 2.168 older people with sarcopenia Average age range: 64.3 - 88.6 years.	Synthesizing evidence on the effects of exercise on muscle mass, muscle strength, and physical performance in older adults with muscle wasting.	Intervention duration ranged from 1.5 to 12 months, with a median of 3 months, conducted 1 to 7 times per week, lasting 15 to 75 minutes. Out of the studies reviewed: 13 Randomized Controlled Trials (RCTs) used Resistance Training (RT).	Combination training increased Appendicular Skeletal Muscle Index (ASMI) but not significantly (SMD 0.04, 95% CI -0.13 to 0.20, P = 0.66, I2 = 57%). Combination training increased Appendicular Skeletal Muscle Mass (ASM) significantly (SMD 0.45, 95% CI 0.03-0.87, P = 0.04, I2 = 0%). Combination training improved grip strength significantly (SMD 1.42, 95% CI 0.23 to 2.60, P = 0.02, I2 = 45%).	There was considerable heterogeneity due to variations in exercise regimens, different standards, and measurement instruments used. Only short-term effects of exercise were observed, and long-term effects were not assessed.

			12 RCTs used a combination of stretching, strength training, RT, Aerobic Training (AT), gait training, balance training, endurance training, etc. 5 studies used Whole Body Vibration Training (WBVT). 1 study used AT. Out of the 5 studies using AT (1 pure AT and 4 AT as part of a combination training)	Combination training did not significantly increase Knee Extension Strength (KES) (SMD 4.56, 95% CI -2.04 to 9.35, P = 0.06, I2 = 51%). Combination training did not significantly improve gait speed (GS) (SMD 0.12, 95% CI -0.06 to 0.31, P = 0.20, I2 = 92%). Combination training improved Timed Up and Go (TUG) performance significantly (SMD -1.42, 95% CI -2.71 to -0.12, P = 0.004, I2 = 82%). RT showed significant improvements in ASMI, grip strength, and gait speed.	
7	Galindo, David (2021)[11] No of studies: 12 RCT Participants were older adults (>65 years) diagnosed with sarcopenia.	Analyzing the effects of physical exercise, while excluding the role of other treatments, including nutrition, that may play a role in sarcopenia.	Strength training alone and combination training (Resistance Training - RT, Aerobic Training - AT, neuromuscular exercises) were studied. Two studies used AT as part of the combination training.	In studies where AT (walking) dominated the training program, muscle mass did not show improvement. In fact, the number of individuals meeting the criteria for sarcopenia increased by the end of the study. Muscle mass increased with strength training. Aerobic training (walking) did not achieve significant benefits. However, combination training, such as aerobic and balance exercises, had significant positive effects on anthropometric and muscle function parameters. The exercises should be tailored to the individual subjects' characteristics.	Limitations in drawing conclusions include difficulties in analyzing and comparing results due to the lack of established consensus for measurement and standardization, especially in functional tests and anthropometric measurements. Each study assessed different variables. Another significant limitation is the large variability in the age range of the participants across studies, from 65 to over 90 years. No study evaluated potential efficacy differences based on age ranges.
8	Wang, Haolin (2022)[10] No of studies: 91 (23 RCT) No of subjects: 1.252 The participants' ages ranged from 60 to 101, with an average of 63.2±1.4 years and 89.5±4.4 years.	Analyzing the efficacy of exercise on muscle strength, muscle mass, and physical performance in older adults with sarcopenia.	Various exercise methods were used, such as Resistance Training (RT), Aerobic Training (AT), and combination training, with durations ranging from 8 to 24 weeks, mostly lasting 12 weeks.	RT (MD = 4.31, 95% CI = 3.22–5.39, p < 0.001) and combination training (MD = 1.59, 95% CI = 0.62–2.56, p = 0.001) increased grip strength. AT did not significantly increase grip strength (MD = 0.83, 95% CI = -0.58–2.24, p	The quality of the included Randomized Controlled Trials (RCTs) was not good due to high bias and inadequate blinding methods. Most studies were

	Out of the 15 studies: 12 studies included both male and female participants. 1 study included only male participants. 2 studies did not provide gender information.		The most common frequency was 2 times per week. Out of the 8 studies using AT (all as part of combination training)	= 0.25) and Knee Extension Strength (KES) (SMD = 0.23, 95% CI = -0.06–0.51, p = 0.12). Overall, exercise increased grip strength (MD = 2.38, 95% CI = 1.33–3.43, p < 0.001), KES (SMD = 0.50, 95% CI = 0.36–0.64, p < 0.001), muscle mass (MD = 0.28, 95% CI = 0.01–0.56, p = 0.04), and gait speed (SMD = 0.88, 95% CI = 0.49–1.27, p < 0.001).	conducted on female participants.
9	Niu, Kun (2022)[8] No of studies: 13 RCT No of subjects: 718 The average age ranged from 64+3 to 82.8+8.5 years.	Assessing the effectiveness of TCE on sarcopenia.	TCE includes Aerobic Training (AT) and has a duration of 8 weeks to 18 months.	TCE did not significantly increase grip strength [MD = 1.43, 95% CI (-0.54, 3.41), P = 0.15, I2 = 2%]. TCE improved Chair Stand Test (CS) performance [MD = 2.45, 95% CI (1.88, 3.01), P < 0.00001, I2 = 38%]. TCE improved gait speed (GS) [MD = 0.31, 95% CI (0.30, 0.32), P < 0.00001, I2 = 13%]. TCE improved Timed Up and Go (TUG) test performance [MD = -1.91, 95% CI (-3.64, -0.19), P = 0.03, I2 = 81%].	
10	Bao, Wangxiao (2020)[5] No of studies: 22 No of subjects: 1.041 The average age ranged from 60.51 to 85.9 years, with approximately 80.75% being female.	Evaluating the effects of exercise on muscle mass, muscle strength, and physical performance in older adults with sarcopenia.	The types of exercise interventions included Resistance Training (RT), Aerobic Training (AT), strength training, Whole Body Vibration Training (WBVT), and home-based exercise. The durations of the interventions ranged from 6 to 36 weeks, lasting for 30 to 80 minutes. Out of the 3 studies using AT (all as part of combination training)	There was no significant effect of exercise on Appendicular Skeletal Muscle Mass (ASM) (SMD 0.15, 95% CI -0.05 to 0.36, P = 0.15, I2 = 34%) and Appendicular Skeletal Muscle Index (ASMI) (SMD 0.21, 95% CI -0.05 to 0.48, P = 0.12, I2 = 66%). AT significantly increased grip strength (SMD 0.48, 95% CI 0.13 to 0.83, P = 0.007, I2 = 83%). Exercise significantly improved gait speed (GS) (SMD 0.44, 95% CI 0.26 to 0.61, P < 0.00001, I2 = 67%) and Timed Up and Go (TUG) test performance (SMD -0.97, 95% CI -1.22 to -0.72, P < 0.00001, I2 = 91%).	There was high heterogeneity among the studies.
11	Lee RN, Szu-Ying (2018)[12] No of studies: 10 studies (7 RCT)	Integrating and analyzing research on physical activity's	The types of exercise interventions included Aerobic Training (AT), Resistance Training (RT), Strength	It was observed that increasing physical activity is an effective protective strategy against sarcopenia. This is because	

	The age range of the participants is between 60 and 89 years.	impact on sarcopenia in the geriatric population	Training, Balance Training, and Flexibility Training. The exercise frequency ranged from 2 to 6 times per week, with durations lasting 6 to 12 months. Out of the 5 studies using AT (all as part of combination training)	enhanced physical activity can lead to an increase in muscle mass and muscle strength	
12	Huang, Chia-Yu (2022)[7] No of studies: 11 RCT No of subjects: 1.676 The average age ranged from 70 to 89.5 years.	Assessing the effects of Tai Chi on muscle mass, muscle strength, physical performance, and other geriatric syndromes in older adults with sarcopenia and frailty.	9 studies used Yang-style Tai Chi, and 1 study did not specify the style used. The duration of the Tai Chi training ranged from 30 to 90 minutes, conducted 2 to 7 times per week, and lasted for 8 to 48 weeks.	There was no significant increase in muscle mass (WMD: 0.53 kg, 95% confidence interval (CI) -0.18 to 1.24; P = 0.14; I2 = 0%). There was no significant increase in grip strength (WMD: -0.06 kg, 95% CI -1.98 to 1.86; P = 0.95; I2 = 0%). There was no significant increase in gait speed (WMD: 0.05 m/s, 95% CI -0.11 to 0.20; P = 0.55; I2 = 99%). There was a significant increase in Chair Stand Test (CST) performance (WMD: 2.36 (times), 95% CI 1.50–3.21; P < 0.00001; I2 = 87%). There was no significant increase in Sit-to-Stand Test (SST) performance (WMD: -1.21 (seconds), 95% CI -3.26 to 0.85; P = 0.25; I2 = 95%). There was a significant improvement in Timed Up and Go (TUG) test performance (WMD: -0.72s, 95% CI -1.10 to -0.34; P = 0.0002; I2 = 0%).	The studies showed high heterogeneity
13	Shen, Yanjiao (2023)[9] No of studies: 42 RCT No of subjects: 3.728 The average age was 72.9 years, with the majority being women (73.3%).	Comparing the effectiveness of various exercises in older adults with sarcopenia.	The types of exercises included Resistance Training (RT), Aerobic Training (AT), Balance Training, Combination Training, and Nutritional interventions. The median follow-up duration was 12 weeks (ranging from 12 to	AT did not have a significant impact on muscle strength (MD 0.46 kg, CI95 -1.13 to 2.04). RT+AT did not have a significant impact on muscle strength (MD 1.94 kg, CI95 0.79 to 3.08) but showed some effectiveness in improving the Chair Stand Test (CST)	There was high heterogeneity among the studies. The Cochrane risk of bias tool was used to assess the risk of bias, but this tool has not been validated yet.

			16 weeks), and the intervention duration ranged from 8 to 144 weeks.	performance (MD 1.72s, CI95 -3.17 to -0.27). RT + AT + Nutrition was effective in increasing muscle strength (MD 3.04 kg, CI95 1.64 to 4.4) and moderately effective in improving CST performance (MD -2.28, CI95 -3.73 to -0.83). RT + AT + Balance Training was not effective in increasing muscle strength (MD 0.2kg, CI95 -3.5 to 3.9), gait speed (MD 0.04s, CI95 -0.14 to 0.22), and Timed Up and Go (TUG) test performance (MD -1.7s, CI95 -3.99 to 0.59).	
14	Carcelen-Fraile, Maria DC (2023)[15] No. of studies: 13 No. of subject: 571 Age: 63 ± 3.1 years – 80.8 ± 4.7 years	Determine the effects of different combinations of resistance training-based interventions on the musculoskeletal health of older male adults with sarcopenia	The types of exercises included RT combined with protein and vitamin, Aerobic RT combined with protein only, and RT combined with AT.	It was found that resistance training combined with low-intensity aerobic training improved handgrip strength, quality of life, muscle mass, musculoskeletal mass total fat percentage, weight, BMI, lower and upper limb power, VO2Max and generated favorable modifications in the Satellite Cells related markers. Additionally, it was observed that low-intensity aerobic training is more effective than high-intensity training when combined with resistance training, irrespective of AT and RT order.	The articles were published in Eurasia and, specifically, more than 60% of the articles were published in Germany, the results cannot be extrapolated to other populations such as Latin American or African populations. Also, because of the variety in the methodological quality of the articles and the lack of a specific PEDro score inclusion criteria, the quality of this systematic review may be affected.

AT: Aerobic training; BMI: Body Mass Index; CST: Chair stand Test; GS: Gait speed; RT: Resistance training; SST: Sit to stand test; SO: Sarcopenia obesity; TCE: Traditional Chinese exercise; TUG: Time up and go; WBVT: Whole body vibration training; WBEMS: Whole body electromyostimulation

The augmentation of aerobic exercise effects through combination with resistance exercise as a treatment for sarcopenia in older individuals is substantiated by various compelling mechanisms, as underpinned by recent research.[25] A pivotal mechanism lies in the synergistic interplay between resistance exercise and aerobic exercise. While resistance exercise targets muscle strength and hypertrophy through weightlifting or resistance band training, its integration with aerobic exercise can further amplify muscle mass and strength.[25] This combination offers a holistic strategy to address the multifaceted nature of sarcopenia, leading to enhanced physical performance and functional capacity.[25] An intricate mechanism involves the stimulation of muscle protein synthesis. Resistance exercise activates the mTOR signaling pathway, a key driver of muscle protein synthesis.[25] This activation propels the generation of fresh muscle proteins, fostering muscle growth and improved functionality.[25] Such an anabolic environment complements the effects of aerobic exercise, providing a robust musculoskeletal foundation for aerobic activities. Moreover, the impact of exercise, including resistance exercise, transcends localized effects, exerting systemic influences on physiological processes. Exercise enhances insulin sensitivity, bolsters mitochondrial function, and mitigates chronic inflammation.[25] These systemic enhancements bolster muscle health and function, magnifying the benefits of aerobic exercise within the realm of sarcopenia treatment.[25]

Nutritional supplementation holds the potential to significantly amplify the efficacy of exercise as a treatment for sarcopenia in older individuals, as showed by our review. One pivotal mechanism is the supplementation's ability to furnish essential nutrients indispensable for muscle growth and repair. Notably, essential amino acid (EAA) supplements containing constituents like leucine have exhibited promising effects in enhancing muscle mass and functional parameters.[26] Protein supplementation, though yielding inconsistent results, also contributes vital nutrients for muscle health.[26] By providing these critical building blocks, nutritional supplementation effectively bolsters anabolic processes integral to muscle protein synthesis, thereby augmenting the impact of exercise on both muscle mass and function.[26] A parallel mechanism revolves around optimizing energy availability. Through offerings like high calorie shakes or meal replacement drinks, nutritional supplements furnish supplementary energy crucial for sustaining exercise performance and counteracting muscle wastage.[27] This strategic energy intake is pivotal for supporting the intensified energy requirements during exercise, ultimately fostering muscle adaptation. Furthermore, nutritional supplementation addresses the prevalent nutrient deficiencies frequently observed among older individuals grappling with sarcopenia. Micronutrient scarcities, including but not limited to vitamin D, calcium, and vitamin B12, can exert detrimental effects on muscle health and functionality.[27] The act of supplementing with these essential nutrients effectively optimizes muscle function, thereby reinforcing the advantages

garnered from exercise. Importantly, nutritional supplementation can exert a positive cascading influence on overall health and well-being, indirectly heightening the impact of exercise on sarcopenia. For instance, an improved nutritional status, often facilitated by protein supplementation, correlates with an elevated health-related quality of life among older individuals residing in nursing homes.[28] By elevating the overall health and well-being, nutritional supplementation cultivates an environment conducive to exercise-induced adaptations. It's vital to acknowledge that the effectiveness of nutritional supplementation remains contingent on individual requirements and the specific supplements employed. Moreover, meticulously designed and standardized studies evaluating the combined effects of exercise and nutritional interventions remain paramount in formulating precise treatment guidelines for sarcopenia.[26] Consequently, personalized strategies and collaborative consultations with healthcare professionals or registered dietitians stand as prudent measures to define the most fitting and efficacious nutritional supplementation regimen for older individuals contending with sarcopenia. This strategic amalgamation of exercise and nutritional supplementation signifies a promising path toward optimizing the treatment outcomes and overall quality of life for the elderly population grappling with sarcopenia.

Despite substantial progress, there are still significant gaps in our understanding of aerobic exercise's impact on sarcopenia. Long-term effects and sustainability remain underexplored, urging the need for comprehensive longitudinal studies to assess the enduring benefits of exercise interventions. Moreover, the variability in study methodologies, exercise protocols, and participant characteristics contribute to controversies and conflicting findings. To address these issues, future research should focus on developing standardized protocols and conducting comparative effectiveness studies to identify the most efficient exercise modes for managing sarcopenia.

This literature review boasts a robust level of evidence owing to its methodological strengths. By employing systematic reviews and meta-analyses as the included articles, alongside a comprehensive search strategy to ascertain the chosen studies, this review ensures a rigorous evaluation of the subject matter. Such an approach lends significant credibility and reliability to the findings. Notably, the scarcity of comprehensive guidelines delving into the role of aerobic exercise as a treatment for sarcopenia underscores the importance of this review. By synthesizing existing evidence, this review strives to bridge this knowledge gap, providing valuable insights into the potential efficacy of aerobic exercise in combating sarcopenia among older individuals.

Limitations of this literature review include the high heterogeneity among the included systematic reviews, which may be due to variations in exercise regimens, measurement tools, and diagnostic criteria across studies. The limited number of studies specifically addressing the impact of aerobic

exercise alone as a treatment and prevention for sarcopenia also contributes to the lack of conclusive evidence. Additionally, the potential mechanisms or physiological pathways involved in the response of muscle mass and strength to aerobic exercise are still not fully understood. The primary effects of aerobic exercise may lie in cardiovascular function and oxidative metabolism, with minimal effects on muscle mass and strength. The age-related diminished response to protein synthesis stimulation in muscle and the potential lack of sufficient overload from low-volume aerobic exercise could further limit its effectiveness in stimulating muscle growth. Lastly, the low volume of aerobic exercise may not be adequate to induce significant improvements in muscle strength and power in older individuals.

4. Conclusion

In conclusion, the systematic reviews and meta-analyses conducted from 2018 onwards suggest that while aerobic exercise may not have a significant impact on muscle mass and strength in elderly individuals with sarcopenia, it holds promise in improving physical performance outcomes. The findings emphasize the importance of incorporating aerobic exercise as part of comprehensive interventions targeting sarcopenia in older individuals, as it can contribute to enhanced physical function and quality of life. However, the heterogeneity among the included studies and the need for further research to determine optimal exercise parameters highlight the necessity for tailored exercise prescriptions and individualized approaches. These findings have implications for clinical practice, emphasizing the potential benefits of aerobic exercise in the management of sarcopenia and the importance of ongoing research to refine our understanding of its effects and establish clearer exercise guidelines for this population.

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Literature Review

Maternal comprehensive care on stunting prevention in preconception, prenatal and postnatal phase

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Abstract: Stunting or short stature (shortness) is a growth failure due to malnutrition in the first thousand days of a child's life, affecting growth and development during adulthood. This paper will briefly discuss the challenges to maternal comprehensive care for reducing stunting in child, the various strategies that can be used to improve nutrient intake in these target groups, the evidence for an impact on linear growth or stunting from preconception, prenatal and postnatal nutrition interventions and the need for integrated approaches that address the multifactorial aetiology of stunting.

Keywords: care; comprehensive; malnutrition; maternal; stunting

1. Introduction

Stunting is measured by a height-for-age z-score of more than 2 standard deviations below the World Health Organization (WHO) Child Growth Standards median, showing a restriction of a child's potential growth. Child stunting can happen in the first 1000 days after conception and is related to many factors, including socioeconomic status, dietary intake, infections, maternal nutritional status, infectious diseases, micronutrient deficiencies and the environment. The World Health Organization (WHO) in 2019 shows that the stunting prevalence in the Southeast Asia exhibited the second highest globally, about 31.9%. Interestingly, the prevalence in Indonesia is relatively high compared to other middle-income countries. Based on Basic Health Research 2018, the prevalence of stunting in children under three years old was 10.2% and in children under five years old was 30.8%. The high prevalence must be reduced because stunting has a long-lasting negative impact on the country, such as hampering economic growth, increasing poverty, and widening inequality.

The World Health Assembly and the UN's Sustainable Development Goals call for a 40% reduction in childhood stunting by 2025 with the ultimate goal of eradicating all childhood malnutrition. A several part series on Stunting in childhood: an overview of global burden, trends, determinants, and drivers of decline is a systematic review of 89 studies from which basic, underlying, and immediate determinants of stunting were identified. Basic determinants are: an asset index of household income

and parental education, particularly maternal education. Underlying determinants are numerous: sanitary disposal of stool, clean water, bed nets, vaccination coverage, attendance of antenatal clinic visits, optimal breastfeeding practices, and household food security. Immediate stunting determinants are: reduction in fertility, birth spacing, maternal height, infant birthweight, dietary diversity, and diarrhea.

This body of work and future directions are especially important as the COVID-19 pandemic is predicted to worsen malnutrition globally. It is estimated that the prevalence of wasting could increase 10–50% causing an excess of ≤2 million child deaths.

One of the causes of stunting is the maternal condition of the mother prior to and during pregnancy. Maternal factors affecting stunting included mothers with an arm circumference of less than 23.5 cm, a lack of iron consumption during pregnancy, birth of the first child while under 21 years of age, low maternal education, and family economic status.

According to Indonesian Basic Health Research, the prevalence of stunting among children aged 5-12 years was 22.6% in 2018, a decrease from 30.7% in 2013. Similarly, the prevalence of anemia among children aged 5-14 decreased from 29.4% in 2013 to 26.8% in 2018. Stunting is considered a high and moderate public health problem, respectively.[1–6]

2. Literature review

2.1. Pathophysiologic of stunting altering intrauterine fetal growth

Intrauterine growth disorders can cause proportional (if the disturbance starts in the second trimester) or disproportionate (if the disturbance starts in the third) fetal growth. Fetal growth is regulated by a complex interaction between maternal nutritional status, endocrine-metabolic signals, and placental development. Physiologically, there is a transfer of energy toward homeostasis of metabolism in failure to thrive, which ends in stunting. The energy used for linear growth is limited, along with the relative insulin resistance that occurs in periods of starvation. Insulin resistance contributes to the addition of energy through catabolic processes, especially during starvation and sepsis. Several hormonal changes occur in catabolic conditions, namely, an increase in serum GH with a decrease in IGF-1 levels and their expression. In children, malnutrition and illness are associated with decreased growth rates, whereas recovery and re-feeding result in accelerated linear growth, often called "catch-up" growth. Optimal linear growth generally only occurs in healthy and well-nourished individuals. The growth deficit accumulated during this period will only partially recover if the disease is cured. When conditions that inhibit growth are overcome, linear growth generally returns to normal and exceeds normal levels for their age. This phenomenon is called "catch-up growth." Stunting is caused by accumulated stress episodes that have been going on for a long time, which are not balanced

by catch-up growth. This results in decreased growth compared to children who grow up in a supportive environment.

In utero, insulin, IGF-1, and IGF-II are responsible for fetal growth. Insulin primarily affects fetal adiposity. IGF-II is the main hormone responsible for early fetal growth, and after organogenesis, IGF-1 is more important for fetal growth. The production of IGF-1 in the fetus is influenced more by nutrition than endocrine factors. Low levels of IGF-1 and IGFBP-3 were found in low birth weight and small for gestational babies. Growth hormone (GH) and thyroid hormone do not affect fetal growth because the differentiation of these two hormones does not result in short newborn length.

The hormones that play a role in growth are growth hormone (growth hormone, somatotropin), IGF, sex hormones, thyroid hormones, and glucocorticoids. Linear growth occurs due to the elongation of long bones due to ossification chondrogenesis in the growth plates. Growth plates are thin cartilage layers present at the ends of long bones. The growth plate consists of 3 zones, namely the resting zone, the proliferation zone, and the hypertrophy zone. The rate of chondrogenesis, which is identical to the rate of linear growth, is regulated by a complex interaction between nutrition, hormones, inflammatory cytokines, local growth factors, extracellular matrix, and intracellular proteins. Thus, gene mutations that play a role in growth plate chondrogenesis can result in growth disorders.

In humans, brain and body growth, especially during the rapid fetal growth phase, requires a relatively high supply of energy and metabolism. Cellular energy metabolism depends on oxygen. Iron (Fe) deficiency decreases oxygendependent cellular energy metabolism due to decreased heme and Hb synthesis, decreased red blood cell (RBC) synthesis, and decreased RBC survival due to increased oxidative stress in RBC, Hb autoxidation, generation of toxic oxygen radicals scramble and increased uptake by macrophages. As a result, iron deficiency anemia causes impaired cognitive abilities and linear growth.

In caloric deficiency, most effects on growth plates are mediated by the endocrine system, such as decreased levels of IGF-1, sex steroids, and thyroid and increased glucocorticoids. Malnutrition can also reduce growth plate response because increased fibroblast growth factor (FGF) in low-calorie intake conditions inhibits the action of growth hormone on chondrocytes. Insuline-like growth factor (IGF) acts as a growth-promoting factor in the growth process and mediates growth hormones. Inflammatory cytokines such as Tumor Necrosis Factor-a (TNF-a), Interleukin-1B (IL-1), and Interleukin-6 (IL-6) inhibit chondrogenesis and suppress IGF-1 secretion. The GH-insulin-like growth factor-I (GH/IGF-1) axis is central to linear growth and is susceptible to various defects. GH action is mediated by the IGF system either directly (on the growth plate) or indirectly. Prenatal growth depends primarily on maternal nutrition, while postnatal growth is controlled by GH receptor (GHR)

/ GH interactions that stimulate IGF-1 production. Disruption of the GH/IGF-1 axis results in an IGF-1 deficiency syndrome characterized by growth retardation due to failure of GH production or GH resistance. GH resistance arises from GHR mutations, defects in post-receptor signaling, and defects in IGF-1 synthesis or may be secondary to chronic disease, malnutrition, or circulating GH or GHR antibodies. Serum protein levels of the IGF system have been reported to be altered in malnutrition. The primary clinical use of IGF-1 measurement is in assessing pituitary GH status, but serum levels have also been known to reflect nutritional status, decreasing during fasting and starvation. After one week of starvation, levels were comparable to those observed in hypopituitarism. The decrease in levels appeared to be greater in those with protein-energy malnutrition than in those with only energy malnutrition.[1–3]

2.2. Maternal factors and stunting

Maternal health factors such as process of pregnancy, mother's height, and mother's nutritional status, can affect the child nutritional status. Poor maternal factors can lead to poor quality of children's health at birth and insufficient nutritional components of breast milk. Poor nutritional status before and during giving birth can cause babies born with low birth weight, which we known as a risk factor for stunting. Besides that, supplementation and food intake during pregnancy will also affect the macronutrient components of breast milk which are the main source of nutrition for children in the first 6 months of birth. Data of chronic energy deficiency based on Indonesian Basic Health Research 2018 was still quite high about 14.5% in women of childbearing age and 17.3% in pregnant women. This high prevalence may be associated with various risk factors affecting stunting, including maternal and child medical history, maternal and child nutritional status, the gender of the child, environment, economic condition of household, the mother’s educational background, and the number of toddlers in a family. Inadequate nutrition, which may contribute to stunting, is associated with ineffective feeding practice, in which an increase in nutritional demand is not fulfilled with proper quality and quantity of feeding practice.

Despite dramatic economic advances in the last two decades, child undernutrition and stunting remain serious public health problems in Indonesia. Of the 24.5 million children under 5 years of age in Indonesia, approximately 9.2 million (37%) are stunted. Regions with large rural populations exceed the national average, including West Kalimantan (39.7%), Central Kalimantan (39.6%) and South Sumatra (38.9%). The high stunting rate in Indonesia is associated with a combination of complex factors, including nutrition, hygiene and childcare practices characterized by poor dietary diversity and sub-optimal feeding practices, low maternal and paternal education inadequate maternal nutrition, shorter maternal height, lower per-head household expenditure, low birthweight, insufficient birth

spacing, low levels of exclusive breastfeeding, open defecation and insufficient hygiene practices, and household food insecurity. Improving mothers' behavioral beliefs, behaviors, and education are key to addressing stunting. Improved maternal education, especially, has consistently been associated with reductions in child stunting. This complex relationship between education and stunting is likely mediated by other, more proximal factors.

Malnutrition in pregnant women has significantly contributed to the high stunting prevalence in Indonesia. Poor nutrition during pregnancy implicates 85% of the stunted infants. It caused low birth weight, stunted fetal growth, and death due to a low immune system. Malnutrition in pregnant women is a poorer quality of life and health status because inadequate nutrition during pregnancy causes decreased immune system, weakness, fatigue, and lethargy, like anemia symptoms and signs. Inadequate iron levels frequently accompany malnutrition, whereas iron presents essential minerals for increasing pregnant women's health status, enhancing the immune system, and supporting fetus growth and development. During pregnancy, good intake and iron supplements are essential to reduce stunting risk.

Two maternal gender inequities that appear to be associated with stunting in different settings are girl child marriage and intimate partner violence (IPV). Maternal child marriage has been found in multiple contexts to be associated with increased child malnutrition and infant mortality potentially through the mechanisms of early childbearing and reductions in maternal education, which can impact maternal behaviors affecting child health, such as choosing to breastfeed, immunize, and educate children.[1–8]

2.3. Maternal roles in preventing stunting

The mother's important role in preventing stunting in the child lies in three phases: the preconception phase, prenatal phase, and post-natal phase (baby phase – toddlerhood). The mother's role in these three phases becomes a key factor in preventing stunting events in the child. Although the conception period is not yet a fetus, maternal nutrition's early strengthening must be done. The mother's body is ready in the prenatal phase for fetal development, continuing in the post-natal phase until adolescence.

- **Preconception Phase**

The mother's role during preconceptions varies from optimal nutritional fulfillment to the mother's role. Most mothers with a high level of nutritional knowledge can practice how to provide their food to meet their nutritional needs. Periconception conditions, including the mother's nutritional status before pregnancy and the mother's energy and nutritional status intake, affect the initial process of growth and development. 24 In addition to nutrition, other maternal factors play a role in determining

the growth of offspring, for which all of these factors are the mother's responsibility. These maternal factors include infection, pregnancy in adolescence, short birth distance and hypertension during pregnancy and genetics, maternal infections associated with malaria, worms, HIV/AIDS, and other conditions that can cause IUGR and then inhibit growth in infants. Teenage pregnancy interferes with the availability of nutrients for fetuses due to maternal growth's constant competition demands. Hypertension during pregnancy can also lead to adverse nutritional outcomes for spring. The mother's height is an indicator of preconception nutritional status that is most closely related to the child's linear growth. The mother's height is an important indicator that may reflect the combination of the mother's genetics and the nutritional and environmental factors she experienced during her childhood. Therefore, genetic factors and the mother's nutritional status during the first 1000 days are essential for her child's growth. Although genetic factors cannot be changed, strengthening nutrition in children during the growth period can increase the child's growth. Maternal marital status was associated with stunting. Children with married parents had a lower risk of stunting, and parents who were never married or divorced/widowed had a higher chance of stunting children.

- **Prenatal Phase**

The mother's role in the prenatal phase is vital in preventing stunting events in the child. Maternal nutrition plays a key role in fetal growth, infant health and survival, and long-term child health and development. During the first half of a critical 1000 days period (conception up to 6 months), the mother is the only source of nutrition for a developing child; first in the womb and then during the first six months of life when exclusive breastfeeding is recommended.

Balanced energy and protein supplementation, which provides roughly 25% of the total energy supplement as protein, is an important intervention for the prevention of adverse perinatal outcomes in undernourished women. It increases birth weight by 41 grams and reduces the risk of stillbirths by 40% and small-for-gestational-age births by 21%. The WHO recommends nutrition education and increasing daily energy and protein intake for pregnant women in undernourished populations, to reduce the risk of low-birth-weight neonates. In highly food-insecure areas or in populations with little access to a variety of foods, additional complementary interventions are recommended to reduce the risk of stillbirths and small-for-gestational age neonates, such as balanced energy and protein dietary supplementation for pregnant women. Monitoring of programmes for energy and protein supplementation, to assess their effects, feasibility, acceptability and equity implications, is encouraged.

The mother is responsible for the fulfillment of fetal nutrition during the prenatal period; critical nutrients that are very important for mothers to pay attention to during the prenatal period are omega-3 fatty acids, iron, iodine, calcium, zinc, magnesium, and vitamins. Therefore, the mother's

responsibility during the prenatal period lies not only in the fulfillment of optimal nutrition for the fetus but also provides a conducive environment that can increase maternal factors so that the mother is ready for fetal development until the delivery period so that the optimal growing fetus can be spared stunting.

- **Postnatal Phase**

Also called infant and toddlerhood phase: 6-24 months. The role of mothers in toddlerhood ranges from early breastfeeding initiation, exclusive breastfeeding, and providing proper breast milk companion food. This role is essential because inappropriate feeding of infants and young children is one of the leading causes of stunting. Key recommendations for infant and young child feeding practices include the minimum duration of continued breastfeeding, minimum diversity of food, and minimum acceptable food. Babies who do not initiate early breastfeeding are associated with future stunting events. Proper feeding of infants and young children is very much related to the mother's knowledge. Research proves that mothers who have a better knowledge of infant and young child feeding practices can lower stunting incidence ($P < 0,001$).

Strengthening the economic aspect must also be done, which is the mother and the family's responsibility. Research in India shows that children from low-income groups have high levels of difficulty buying food. This finding suggests that poverty is not the only contributing factor to malnutrition, but lack of food intake, poor hygienic habits, and low environmental and educational levels can also contribute to child malnutrition in low-income groups. However, optimizing the local food which is more accessible and cheaper as local-food based complementary could be affordable for family. Meanwhile, employed mothers were one of the risk factors for stunting in children under two years, and employed mothers had a higher risk of stunting children. Moreover, boys were more likely to be stunted than girls, and several studies found similar findings in Indonesia. Sex and follicle-stimulating hormones might play a role in further growth.

Some strategies applied, both prenatal and post-natal, can positively impact a child's growth, but the results are mixed, and growth responses are not always observed. The role of health workers to degrade and prevent stunting is also needed, growing evidence that improving the accessibility and quality of nutritional education through health services can improve maternal knowledge and child food intake, both of which can lower the prevalence of stunting.[9–13]

3. Conclusion

Maternal comprehensive care for reducing stunting in child impact on linear growth or stunting from preconception, prenatal and postnatal nutrition interventions and the need for integrated approaches that address the multifactorial aetiology of stunting. Interventions to improve maternal,

newborn and child health can be delivered through community based service-delivery platforms and prevent child stunting. Some examples include programmes for iron and folic acid supplementation, multiple macronutrients and micronutrient supplementation, initiate early breastfeeding, exclusive breastfeeding, family planning as well as antenatal, perinatal and postnatal care. Combining nutrition-specific interventions with steps for women's empowerment is very important. Increased food intake and women's health services, prevention of early marriage and conception, completion of secondary education, increased purchasing power, reduction of tedious work, and elimination of domestic violence deserve special attention.

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Literature Review

Changes in reproductive endocrinology with ageing

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Abstract: According to the guidelines of the World Health Organization (WHO), menopause is defined as the permanent cessation of menstruation due to loss of activity from the ovarian follicles. In addition, menopause was considered to occur after 12 consecutive months of amenorrhea, for which no other pathological or physiological cause could be determined. The 2017 Indonesian Health Demographic Survey (IDHS) shows that the number of women 30-34 experiencing menopause is 9.7%, 35-39 years is 11.0%, 40-41 years is 12.7%, 42-43 years is 14.2%, 44-45 years is 17.1%, 46-47 years is 26.7% and 48-49 year as much as 43.1%. In postmenopausal women, the decline in the role of the ovaries gradually reduces the ability of the pituitary gland to produce steroid hormones. With age, the number of follicles decreases. When the number of follicles reaches a critical level, there is a disruption in the functioning of the hormonal regulatory mechanisms, resulting in luteal insufficiency, irregular menstrual cycles,and the end of the menstrual cycle. Signs and symptoms of menopause can not only be seen physically, but also psychologically. In diagnosing a woman experiencing menopause, several examinations will be carried out in the form of anamnesis, physical examination, and if necessary, laboratory tests can be carried out.

Keywords: hormones; menopause; menstruations

1. Introduction

1.1. Definition of menopause

The World Health Organization (WHO) defines menopause as the cessation of menstrual periods permanently as a result of the ovarian follicle's activity ceasing. In addition, menopause often occurs after 12 months of continuous amenorrhea, with no other identifiable causes due to a physiology or illness. Menopause occurs during the final menstrual period, which can only be diagnosed retrospectively and occurs one year or more after the onset of the condition. Menopause frequently happens in women who experience reduced fertility. Conversely, induced menopause is characterized as the halt of menstruation subsequent to the surgical extraction of both ovaries (known as oophorectomy) or the deliberate suppression of ovarian function due to medical intervention (such as chemotherapy or radiation).[1]

The Stages of Reproductive Aging Workshop (STRAW) introduced a system dividing female reproductive aging into stages preceding and following the final menstrual period:

a. Preceding: Early (-5), peak (-4), and late (-3) reproductive stages describing years before perimenopause. Stage -3 involves regular cycles with elevated follicle-stimulating hormone (FSH).
b. Early menopause transition: Characterized by varying cycle lengths.
c. Late transition: Involves two or more skipped cycles and 60 or more days of amenorrhea.
d. Following: Stage +1 signifies the first 5 years after the final menstrual period, and stage +2 represents the late post menopause.

1.2. Physiology of menopause

In postmenopausal women, the ovaries' declining function gradually diminishes the pituitary gland's capacity to generate steroid hormones. As women age, the follicle count decreases. Once the follicle count reaches a crucial point, it disrupts the operation of the hormonal regulatory mechanisms, leading to insufficient luteal activity, irregular menstrual patterns, and the cessation of menstruation. The aging process and decreased ovarian functionality result in the ovaries becoming unresponsive to the pituitary gland's attempts to stimulate steroid hormone production. In an attempt to produce estradiol, the pituitary gland endeavors to prompt estrogen synthesis in the ovaries, consequently boosting the secretion of FSH and LH. Elevated levels of FSH and LH during this life phase serve as indicators of ovarian dysfunction.[2]

In women with regular cycles, FSH levels progressively rise with age, particularly in those over 40–45 years (STRAW stage -3). During this period, circulating estradiol and inhibin A levels slightly increase. Inhibin B levels remain relatively constant until around age 40, after which they decrease, inversely correlated with rising FSH levels. Recent research underscores inhibin B's role in regulating FSH levels in women with normal cycles after age 40. Consequently, reproductive aging after 40 is characterized by a rapid decline in primordial follicles and a subsequent drop in inhibin B secretion. FSH increases, maintaining or elevating estradiol and inhibin A secretion until follicular depletion.[3]

Changes in FSH, estradiol, and inhibins during the menopausal transition involve an early decline in early follicular-phase inhibin B (STRAW stage -2). FSH levels further increase, and women with intervals of over 3 months between menses (late perimenopause, approximately STRAW stage -1) experience higher FSH levels and significantly reduced estradiol and inhibins. By the time of the final menses, FSH levels reach around 50% of their postmenopausal concentrations, which are 10–15 times higher than those in reproductive-age women. Estradiol levels decrease by about 90% post-menopause. The postmenopausal state (STRAW stages +1 and +2) is characterized by elevated FSH and LH, low estradiol and progesterone, and relatively preserved testosterone levels. DHEAS decreases gradually.[4]

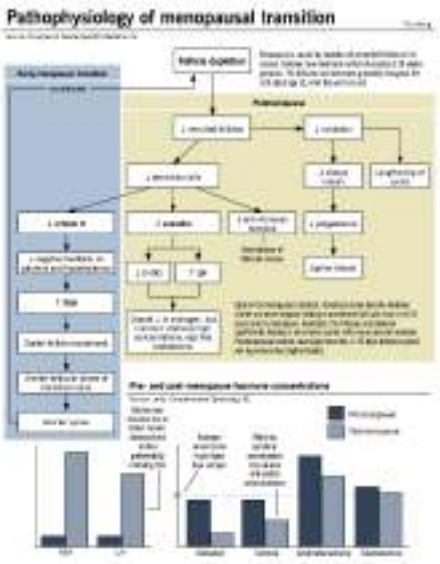

Figure 1. Pathophysiology of menopausal transition

1.3. Epidemiology of menopause

According to the Central Bureau of Statistics, in 2015, there were 17.21 million women between the ages of 40 and 50 who had begun to experience premenopausal, and there were 21.22 million women over the age of 50 who had begun to experience menopause. Out of the 152.69 million women in Indonesia, it is predicted that up to 20.36 million women may experience premenopausal by 2035.[5]

According to the 2017 Indonesian Health Demographic Survey (IDHS), 9.7% of women aged 30-34 experience menopause, followed by 11.0% of women aged 35 to 39, 12.7 % of women aged 40 to 41, 14.2 % of women aged 42 to 43, 17.1% of women aged 44 to 45, 26.7% of women aged 46 to 47, and 43.1% of women aged 48 to 49. Agency for Statistics Center 2021.

Every year, 1.3 million women in the US go through menopause, which occurs between the ages of 51 and 52, but not just at those times; 5% of women also go through menopause between the ages of 40 and 45, and 1% of women go through early menopause, which occurs before the age of 40.

1.4. Sign and symptoms of menopause

Manifestations of menopause can manifest both in the physical and psychological realms. Physically, observable indications encompass occurrences such as bleeding irregularities, occurrences of hot flashes and night sweats, diminished vaginal elasticity leading to dryness, alterations in body composition involving increased fat deposition, sleep disturbances such as insomnia, and the persistence of headaches. Conversely, psychological manifestations may encompass reduced memory capabilities, alterations in emotional processing and cognitive retention, as well as heightened levels of stress.

Menopause, a natural biological process, marks the end of a woman's reproductive years and is associated with various physiological changes that result from hormonal fluctuations. These changes often lead to a range of symptoms and health effects that can impact a woman's physical and emotional well-being. Some of the key signs and symptoms of menopause include vasomotor symptoms, sleep disruption, cognitive changes, mood changes, urogenital symptoms, sexual function changes, metabolic and cardiovascular changes, muscle loss, bone remodeling, skin aging, and hair changes.

a. Vasomotor symptoms and sleep disruption

During menopause, vasomotor symptoms like hot flashes and night sweats are common. These symptoms are triggered by changes in thermoregulation due to alterations in hormonal levels. Menopause leads to a reduction in the thermoneutral zone, making minor increases in core body temperature trigger excessive thermoregulatory reactions. Fluctuations in sex hormones like estrogen and serotonin can lead to vasomotor phenomena. Sleep difficulties are also common during the menopausal transition, with lower inhibin B levels linked to poor sleep quality. Melatonin release changes, potentially causing early morning awakenings. Obstructive sleep apnea might worsen sleep issues in menopausal women.

b. Cognitive changes

Oestradiol, a type of estrogen, plays a significant role in cognitive performance. The decline in estrogen levels during perimenopause is associated with transient cognitive deficits. Fluctuations in LH levels and ovarian failure might drive cognitive dysfunction and spine density loss. Persistently high levels of FSH and LH are linked to Alzheimer's disease, possibly contributing to increased amyloid-β production. Stressful life events during menopausal transition, in the presence of estrogen variability, can also impact cognitive function.

c. Mood changes

Fluctuations in steroid hormones, particularly estrogen and progesterone, are believed to trigger perimenopausal depression. Individuals with increased sensitivity to these hormone changes are at a higher risk of perimenopausal depression. Estrogen's role in regulating serotonin and noradrenaline pathways can impact mood. Fluctuations in progesterone-derived neurosteroids might underlie menopause-associated depressive symptoms. Dysregulation of the hypothalamus–pituitary–adrenal axis, coupled with stress, can contribute to mood changes.

d. Urogenital symptoms and sexual function

Hypoestrogenism during menopause affects lower genital and urinary tracts. Vaginal dryness, reduced lubrication, and thinning of vaginal tissues can lead to dyspareunia and urinary symptoms like urge incontinence. Sexual dysfunction is also common, with testosterone and DHEAS levels

influencing sexual desire and arousal. Vaginal changes due to waning estrogen levels can cause pain during intercourse.

d. Metabolic and cardiovascular changes

The menopausal transition involves hormonal shifts, including a shift from predominantly estrogenic to androgenic hormonal states. This shift can lead to fat accumulation, particularly visceral fat, which is associated with insulin resistance and metabolic syndrome. Oestrogen deficiency can also lead to negative changes in endothelial cell function, increasing the risk of cardiovascular disease (CVD) and atherogenesis.

e. Muscle loss and bone remodeling

Oestrogen deficiency during menopause is linked to muscle loss and bone remodelling. The decline in oestrogen levels affects muscle tissue, leading to reduced muscle mass and strength. Growth hormone (GH) secretion, essential for maintaining muscle mass, decreases with age and is further affected by oestrogen deficiency. Bone mineral density decreases due to increased bone resorption outweighing bone formation, leading to an increased risk of osteoporosis and fractures.

f. Skin aging and hair changes

Skin changes during menopause include reduced collagen content, resulting in thinner, less elastic skin. Melanocyte levels decline, leading to uneven skin tone. Hair loss can occur due to altered estrogen-androgen ratios, leading to shorter hair growth cycles and transformation of terminal hairs to vellus hairs. Androgen activity can also lead to facial hair growth in some women.

In conclusion, menopause brings about a complex array of physical and emotional changes due to hormonal fluctuations. These changes can impact various aspects of a woman's health and well-being, including vasomotor symptoms, sleep disruption, cognitive changes, mood changes, urogenital symptoms, sexual function changes, metabolic and cardiovascular changes, muscle loss, bone remodeling, skin aging, and hair changes. It's important for women to understand these potential effects and seek appropriate medical guidance and support to manage their symptoms and overall health during this transitional phase of life.

1.5. Menopause diagnosis

Menopause women undergo transitions away from menstruation, accompanied by other menopausal indications spanning urogenital, vasomotor, and psychogenic aspects. To diagnose menopause in a woman, a series of evaluations are conducted, including medical history, physical examination, and, if deemed necessary, laboratory assessments. The medical history relies on the patient's age and experienced symptoms, with menopause acknowledged when menstrual cessation extends for 12 months, typically emerging around ages 40 to 45 and beyond. Physical evaluations

indicative of menopause encompasses amplified blood pressure, escalated body mass, diminished breast size, decreased stature linked with osteoporosis, vaginal atrophy, and more.

For enhanced diagnostic certainty or if supplementary evaluations are warranted regarding menopause, ancillary tests encompassing the evaluation of Follicle Stimulating Hormone (FSH) and estrogen levels, the scrutiny of Thyroid Stimulating Hormone (TSH) and thyroid hormones, the examination of Anti-Mullerian Hormone (AMH), and the assessment of vaginal pH can be conducted. In instances of menopausal women, FSH levels exhibit elevation while estrogen levels dwindle, with FSH exceeding 40 IU/L. Subdued estrogens is attributable to the absence of maturing egg cells, whereas heightened FSH stems from the underutilization of follicles for development. TSH and thyroid hormone evaluations ensure the exclusion of alternative causes mirroring menopausal symptoms, such as hypothyroidism. AMH, produced by granulosa cells within developing follicles, serves as a gauge for ovarian follicle counts; consequently, during menopause, AMH levels decline owing to reduced follicular reserves. A normal vaginal pH measures <4.5; however, during menopausal states, vaginal pH elevates in the absence of vaginal infections, ascribed to lowered estrogen levels within the body.

Laboratory examinations and physical assessments are scarcely employed as sole techniques for menopausal diagnosis; often, menopause can be affirmed through clinical evaluation, identifying a cessation of menstruation spanning 12 months. Nevertheless, other assessments can be conducted to eliminate the possibility of alternative clinical conditions imitating menopausal symptoms.[6]

2. Methods

This literature review delves into the multifaceted aspects of menopause, encompassing its definitions, epidemiology, manifestations, and diagnostic procedures. The review employs a comprehensive and systematic approach to explore existing knowledge on menopause. The design involves synthesizing information from various reputable sources, including research articles, surveys, and official health organizations such as the World Health Organization (WHO).

The subject of the literature review revolves around menopause, particularly focusing on its definition, stages, epidemiology, and clinical manifestations. The review also delves into the diagnostic methods used to identify menopause in women.

The primary location of this literature review is not limited to a specific geographic area, as it draws information from a wide range of sources worldwide. The studies and surveys referenced in the review encompass diverse populations and regions, including Indonesia and the United States. The sources cited provide insights into menopause prevalence, symptoms, and diagnostic practices across different demographics.

By meticulously analyzing and synthesizing information from various sources, this literature review offers a comprehensive understanding of menopause, its various stages, associated symptoms, and the diagnostic processes involved.

3. Results and Discussion

a. Hormonal measures taken at a single point in time have minimal diagnostic relevance since the menopausal transition is a period of considerable hormone volatility.
b. Recent research has revealed that ovarian inhibin B plays a crucial part in the endocrine alterations associated with menopause.
c. FSH and estradiol have gotten to about half of their postmenopausal final levels at the time of the final menstruation.
d. The menopausal transition results in an increase in free androgen levels.

4. Conclusion

The research delves into the multifaceted domain of menopause, a phase characterized by the permanent cessation of menstrual periods due to the decline in ovarian follicle activity, as defined by the World Health Organization (WHO). This study not only sheds light on the definition but also examines the intricate process of diagnosing menopause. It underscores the importance of the absence of menstruation for a continuous 12-month period without discernible physiological or pathological triggers. The research employs a comprehensive approach that encompasses medical history, physical examinations, and potential laboratory tests to effectively diagnose menopause. Focusing primarily on women aged 40 to 50 undergoing the stages of premenopause and menopause, the study is geographically situated in Indonesia. Utilizing data derived from the Central Bureau of Statistics and the Indonesian Health Demographic Survey, the research provides valuable insights into the prevalence of menopause within this region. Furthermore, a separate analysis conducted in the United States provides an in-depth exploration of menopause occurrences across distinct age groups. The study not only explores the physical manifestations of menopause, such as irregular bleeding, hot flashes, and vaginal changes but also delves into the psychological shifts, including memory alterations and heightened stress levels. The diagnostic process encompasses the evaluation of crucial indicators including FSH, estrogen, thyroid hormones, AMH, and vaginal pH. While clinical assessment is often adequate, supplementary tests are employed to ensure accurate diagnosis by eliminating potential alternative conditions. Ultimately, this research significantly advances our understanding of menopause, offering key insights into its epidemiology, manifestations, and the intricacies of its diagnostic procedures.

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Literature Review

The impact of the CODE STEMI program on enhancing service quality and clinical outcomes in ST-Elevation myocardial infarction patients: A systematic review

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Abstract: STEMI is one of the classifications of acute coronary syndrome that requires immediate intervention to prevent tissue ischemia. Management of STEMI, based onthe 2017 ESC guidelines, should be performed within 90-120 minutes after the patient arrives at the hospital. The CODE STEMI program is essential to achieve timely treatment goals, reduce mortality rates, Major Adverse Cardiac Events (MACE), length of hospital stay, and cost efficiency. This literature review aims to determine the impact of the CODE STEMI program on improving service quality and clinical outcomes in ST-elevation myocardial infarction patients. Method: This research study utilized a Systematic Review using the PRISMA (Preferred ReportingItems for Systematic Reviews and Meta-analyses) design method. The quality assessment of articles was conducted using the CEBM (Critical Evidence-Based Medicine) Cohort Study Checklist and CEBM Critical Appraisal Tool Case Control. Results: Based on the analysis of the articles, it was found that the CODE STEMI program had a significant influence on improving service quality by reducing treatment time with a mean value of 52.53 minutes, shortening hospital stay by 1 day, and achieving inpatient cost efficiency up to USD 1,052.02. Other results also indicated that the CODE STEMI program had an impact on clinical outcomes, with adecrease in mortality rate by 13.3% and a reduction in MACE by 7.36%. Conclusion: The CODE STEMI program has a positive effect on improving service quality and clinical outcomes in ST-elevation myocardial infarction patients and can be applied in healthcare services to reduce mortality rates, complications, and healthcare costs.

Keywords: CODE STEMI; PPCI; Quality of Life

1. Introduction

The main cause of death in the world is cardiovascular disease.[1] Coronary heart disease accounts for half of the deaths caused by cardiovascular disease. In 2017, the WHO reported around 17.8 million deaths caused by cardiovascular disease each year.[2] STEMI, which is one of the classifications of acute coronary heart disease, accounts for 4-12% of mortality in the national registries of the ESC countries.[3]

Because morbidity and mortality are directly dependent on myocardial ischemia, rapid reperfusion of coronary artery obstruction is essential for optimal acute ST-elevation (STEMI) management. [4]

PPCI is an intervention recommended as reperfusion therapy for patients with myocardial infarction with acute ST elevation.[5] Revascularization with PCI is widely recognized as the most successful treatment for vessel restoration, salvaging compromised myocardium, and preserving cardiac function. PPCI that is carried out with an appropriate onset time will reduce mortality and the incidence of MACE (Major Adverse Cardiac events).[6] However, in practice, there are five potential problems in the management of STEMI patients: patient delays, delays in diagnosis and treatment decisions, delays in transportation, and a lack of collaboration between hospital management and doctors. The establishment of STEMI CODE as a STEMI notification system activated by the IGD is expected to solve this problem.[7]

This research was conducted in connection with the Hermina Depok program plan, which will be made a cardiac center by the Hermina Hospital Group, which has 44 branches throughout Indonesia. In addition, Hermina Depok is also used as a hospital with standard services and quite complete cardiac examination facilities. Hermina Depok has examination facilities ranging from the simplest, such as an EKG, to CT angiograms and cathlabs where to intervene with a diagnosis of AMI. Hermina Depok also has an Emergency Botton application, which is used when people experience a medical emergency.[8]

A preliminary study was carried out from July to September 2022 at Hermina Depok, where for patients who experienced attacks of total occlusion of the coronary arteries (STEMI), as many as 37 people (26.61%) out of a total of 139 patients with a diagnosis of AMI (Acute myocarditis and infarction) participated. The total average monthly visits in 2022 will reach 968. This figure is not a small number and needs serious attention considering the effect of occlusion is ischemic, which then becomes infarction. The speed of treatment will determine how extensive the myocardial lesion is. Ischemic onset of 0–24 hours is described as early cardiac muscle necrosis; dark spots appear.[8]

In this millennial era, where nurses are supposed to be partners who accompany doctors in treating patients, this is what makes nurses also have the skills to identify patients with STEMI symptoms, perform a 12-lead ECG, and also read an ECG description as a basis for diagnosing STEMI.[9]

The purpose of this research is to produce a standard operating procedure for handling STEMI using the CODE STEMI method. The operational standards will be slightly elaborated in the form of modules that can later be used by Hermina Depok Hospital.

2. Methods

This research study used a Systematic Review using the PRISMA (Preferred Reporting Items for

Systematic Reviews and Meta-analysis) design method. In this study, PICOT (Population, Intervention, control or comparison, Outcome, and time) was used. Where the population is STEMI patients, Intervention is PPCI (Primary Percutaneous Coronary Intervention), control or comparison is a comparison of patients using CODE STEMI and not using CODE STEMI, Outcome is reducing the risk of complications and death in STEMI patients, and Time journal selection is carried out between the distance of 10 years back or from 2012–2022.

This study has inclusion criteria and exclusion criteria to avoid confusion.[10] The inclusion criteria in this study were (1) articles with STEMI patient study subjects using the CODE STEMI comparison protocol and not using the CODE STEMI protocol, and (3) English articles. While the exclusion criteria were (1) articles with a literature review research design; (2) acute myocardial infarction patients with diagnoses other than STEMI, namely NSTEMI and UAP; and (3) Management of STEMI patients who not using CODE STEMI, (4) articles that do not explain p-values.

The prism method used in this study resulted in 93 articles from PubMed and 91 articles from ScienceDirect, for a total of 184 articles. After that, the researcher conducted a duplication test for the total number of articles and found that there were 27 articles that experienced duplicates and were deleted from the study. So that as many as 140 articles were selected based on inclusion and exclusion criteria. According to the inclusion criteria, 31 articles were obtained. Then 31 articles were reviewed in depth and selected based on due diligence; 7 were found to be suitable, and 24 were eliminated. So, a total of seven articles will be discussed in this study.

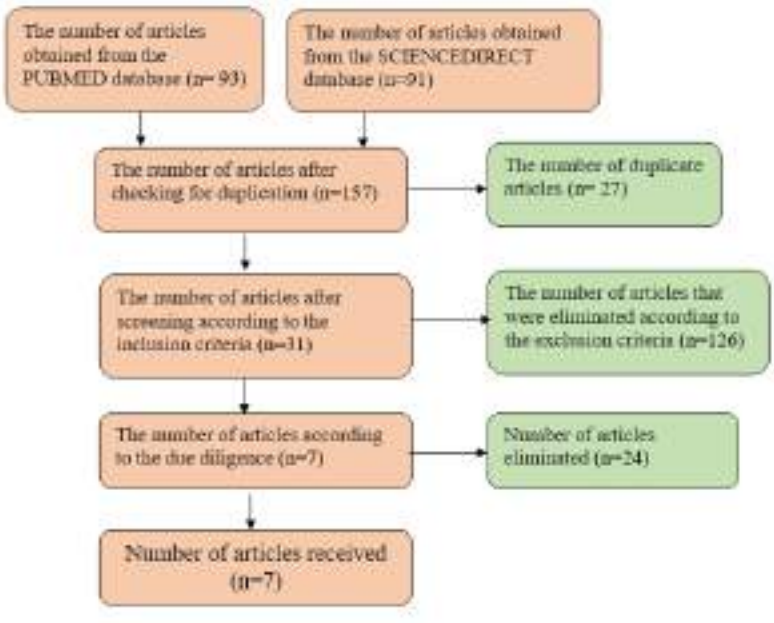

Figure 1. Literature selection process

3. Results and Discussion

In table 1, the result of the study conducted by Ji Quan KOH et al. (2017), the percentage of men when CODE STEMI had been implemented was 73.8%, and before using CODE STEMI, it was 87.8%.[11] Another study was conducted by Eka Ginanjar et al. (2021) with male respondents before and after using CODE STEMI (88%), and after using CODE STEMI (86%).[12] Research conducted by Sharad Bajaj et al. (2012) shows that the percentage of men is also higher than that of women, namely 70.37% before the period of using CODE STEMI and 75% during the period of using CODE STEMI.[13] This shows that men have a greater chance of experiencing coronary syndrome with total occlusion than women.

The risk factors that were experienced by many respondents were hypertension, diabetes mellitus, dyslipidemia, and smoking (table 1). In a study conducted by Alberto Cordero et al. (2015), hypertension was experienced by 71.1% before using CODE STEMI, and after using CODE STEMI, it had a percentage of 63.1%; diabetes mellitus before CODE STEMI 38.9% and the CODE STEMI protocol period 29.1%; dyslipidemia 52.5% and after CODE STEMI 48.3%; smoking 28.4% and after the STEMI protocol 35.5%.[14]

Based on the average age in the research conducted by Christopher J. et al. (2015), the respondents before the CODE STEMI intervention were 57 years old; after the CODE STEMI intervention, they were 55 years old.[15] Another study conducted by Abdulah malik et al. (2017), who often experienced STEMI before implementing the CODE STEMI protocol, namely 53 years, found that after implementing CODE STEMI, the average age of respondents was 54 years.[16] The aging and elderly population is very vulnerable to cardiovascular disease. Age is an independent risk factor for cardiovascular disease (CVD) in adults, but this risk is compounded by additional factors, including frailty, obesity, and diabetes.[17]

CODE STEMI is a protocol designed to improve the STEMI patient management system with a predetermined standard time to produce a good outcome and reduce mortality according to guidelines. Reducing reperfusion time for ST-segment elevation myocardial infarction is critical to improving outcomes.[11] The time from hospital arrival to the PPCI procedure is called door-to-balloon time (DTBT). DTBT is very important to determine the clinical outcome of STEMI patients. The international recommendation in the guideline is 90 minutes.[3] There are significant differences in DTBT between each article analyzed before and after using CODE STEMI (Figure 2).

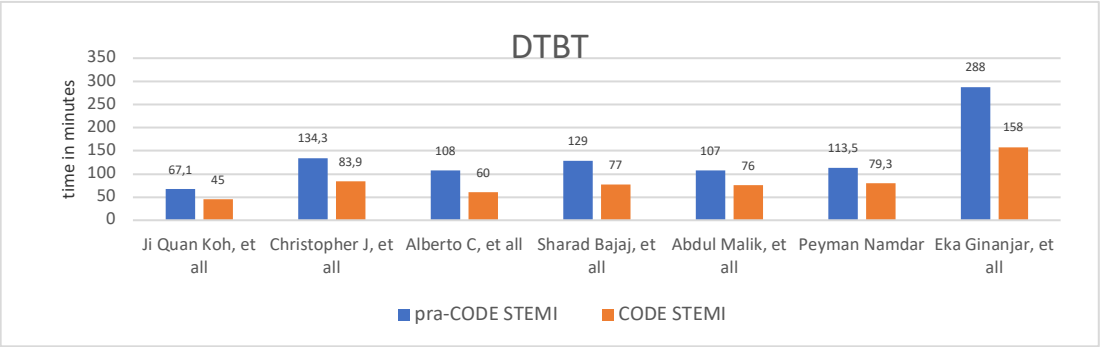

Figure 2. Comparison of DTBT before and after using CODE STEMI

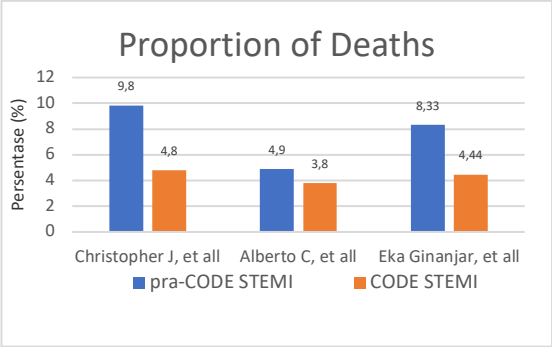

Figure 3. Comparison of the percentage of deaths before and after using CODE STEMI

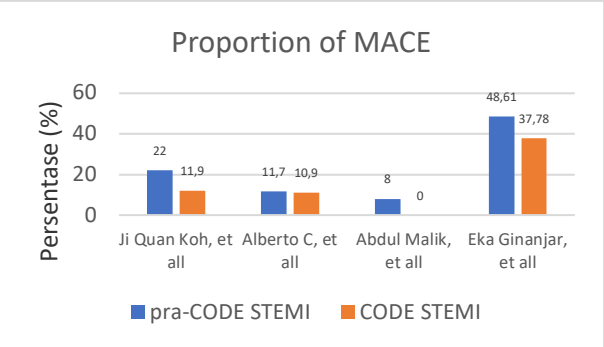

Figure 4. Comparison of the percentage of MACE before and after using CODE STEMI

Reperfusion therapy plays an important role in the management of Acute Coronary Syndrome to treat the etiology and improve the patient's clinical condition.[16] Therefore, delays in performing PCI for STEMI patients are a high cause of death and increase the incidence of MACE (Major Adverse Cardiac events).[18] The main complications that occur in the hospital include heart failure, bleeding, recurrent myocardial infarction, and death. In this study, there was a reduction in mortality (Figure 3) and MACE values after using CODE STEMI (Figure 4).

The speed and accuracy of handling STEMI patients greatly affect the average hospital stay. Originally, in Alberto et al.'s (2016) study, before using CODE STEMI, the length of stay of patients with STEMI was 7 days; after using CODE STEMI, it was 5.9 days. Then, in the study of Eka Ginajar et al. (2021), the length of treatment was originally 7 to 6 days (Figure 5).

The length of stay in the hospital also has an impact on the total costs spent on these patients. Of the seven journals in this review, only one discussed the total costs during the hospital stay. Before using the STEMI CODE, Eka Ginajar et al. (2021) found a total cost of USD 4870.97, and after using the STEMI CODE protocol, USD 3818.95.[12] Of the existing articles, there are two that examine the total length of stay and costs before and after using CODE STEMI (Figure 6).

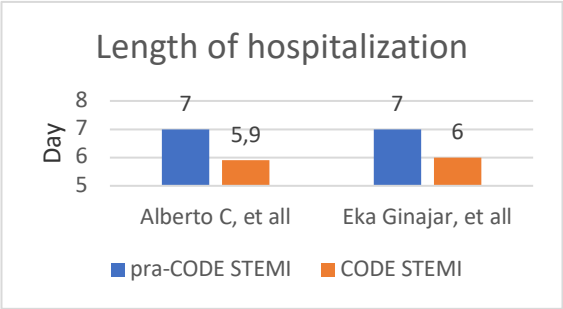

Figure 5. Comparison of length of stay before using CODE STEMI and after using CODE STEMI

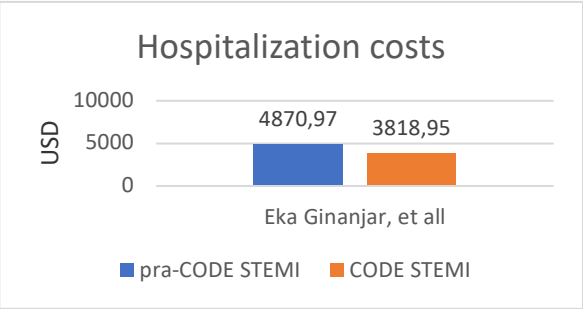

Figure 6. Comparison of total hospitalization costs before and after using CODE STEMI

In this study, almost all of the journals reviewed had male respondents who were 70% larger than women, especially during their productive age. In accordance with the theory put forward by Thomas F. Luscher (2021), men have a greater risk factor for experiencing cardiovascular disease due to their smoking habits.[19] Another phenomenon that causes men to suffer from cardiovascular disease more often is that fertile women who have not experienced menopause have the hormone estrogen. which makes high levels of HDL (high-density lipoprotein). High levels of HDL can protect against atherosclerosis.[20] Atherosclerosis is one of the beginnings of blood flow that is not smooth enough to provide food and oxygen supply to the tissues and heart muscle. So that there is an imbalance between supply and demand in the heart muscle, the heart muscle experiences ischemia, and the patient experiences typical chest pain.

Risk factors that are widely mentioned in the literature are hypertension (56.22%), smoking (49.76%), dyslipidemia (34.67%), and diabetes mellitus (34.30%), which make blood vessels experience atherosclerosis. These risk factors that create pressure resulting in chronic stress on the coronary artery endothelium due to these risk factors, especially hypertension, can cause endothelial dysfunction followed by an increase in inflammatory cells through the disturbed endothelium (monocytes and lymphocytes), adherence or sticking of platelets to the damaged endothelium, and inflammation of the blood vessel walls that causes the proliferation of smooth muscle cells (SMC) that attack the tunica intima. Macrophages and SMC take up oxidized LDL cholesterol and turn into so-called foam cells that accumulate to form a layer of fat that will become plaque on the walls of blood vessels.

Table 1. Systematic literature review of the implementation of CODE STEMI for improving better outcomes for STEMI patients

Author/ year	Country	Research design	Respondent characteristics	Outcome measurement instrument	Indicator	Results	Conclusion
Ji Quan KOH, et, al/2017	Australia	Cohort retrospective	The number of respondents was 83, who were divided into 2 groups: group A (pre-STEMI CODE) (41) and group B (STEMI CODE) (42). A: mean age 59.6, male 36 (87.8%), hypertension 25 (62.5%), dyslipidemia 17 (42.5%), smoking 26 (72.2%), diabetes 8 (20%). B: mean age 61.6, male 31 (73.8%), hypertension 17 (45.9%), dyslipidemia 15 (40.5%), smoking 21 (54.6%), diabetes 4 (10.8%).	Door-to-balloon time (DTBT) according to STEMI guidelines	Median door-to-baloon time (DTBT), MACE, and hospital readmission rate at 30 days to 12 months	There were 41 and 42 patients in the pre-CODE STEMI and CODE STEMI groups, respectively. The median DTBT decreased significantly by 22.1 minutes from 67.1 to 45.0 (p = 0.001). Patients who had a DTBT of 90 minutes (CODE STEMI: 95.2% vs. Pre-CODE STEMI: 73.2%, p = 0.007) Lower in-hospital mortality rate, pre-CODE STEMI 4 (9.6%) CODE STEMI 2 (4.8%); mortality rate from 30 days to CODE STEMI 4 (9.8%); CODE STEMI 2 (4.8%). MACE at 30 days and 12 months (4.8% vs. 9.8%, p = 0.43; 11.9% vs. 22.0%, p = 0.25)	The hospital's CODE STEMI notification system significantly reduced DTBT time in patients undergoing PPCI. In addition, CODE STEMI makes mortality and MACE rates lower at 30 days and 12 months
Abdulmalik Abdullah Alyahya, *et al* / 2017	Saudi Arabia	Quantitative cohort, observational, retrospective	There were 100 respondents who were divided into 2 groups: group A (50), pre-STEMI CODE (50), and group B, STEMI CODE.	Door-to-balloon time (DTBT) according to STEMI guidelines	Symptom onset time to door, ECG time to door, ECG activation time, activation time	Code STEMI had a significantly lower D2BT with a median of 76.5 minutes and an interquartile range (IQR) of 63–90 minutes,	Implementation of the Code STEMI protocol was associated with significant reductions in DTBT

			Group A: Average age: 53.70 men 43 (86%) women 7 (14.0%) Medical history: DM 26 (52%) hypertension 29 (59%) dyslipidemia 6 (12%) CHF 2 (4%) angina 3 (6%) family history of CAD 7 (14%) smoker 23 (46%) Group B: Average age 54.18 male 43 (86%) female 7 (14.0%) Medical history: DM 24 (48%) hypertension 31 (62%) dyslipidemia 12 (24%) CHF 0 (0%) angina 3 (6%) history of the CAD family 1 (2%) smoker, 29 (58%)		to device, time from door to MACE devices in hospitals and the percentage of false activations	compared with a median of 107 minutes and an IQR of 74–149 minutes in preCODESTEMI patients. MACE decrease (8% vs. 0%, p =0.043)	and decreased in-hospital MACE rates.
Eka Ginanjar, *et al*/ 2021	Indonesia	Quantitative and qualitative analyzes used observational methods and retrospective cohort designs	The number of respondents was 207 patients who were grouped into groups A (non-Code STEMI) (72 patients) and B (Code STEMI) (135 patients). Group A: Male: 63 (88%) Female: 9 (12%) Median age: 59.7; history of diabetes mellitus 34 (47%) hypertension 41 (57%) dyslipidemia 24 (33%) obesity 2 (3%) Group B: Male: 116 (86%) Female: 19 (14%). Median age 56.1; history of diabetes mellitus 55 (41%)	Door-to-balloon time (DTBT) according to STEMI guidelines	Response time (DTBT), clinical outcome (major adverse cardiac events ((MACE)) mortality), total cost during hospitalization and length of hospital stay	Decreased DTBT CODE STEMI (288 [120 to 1,376] minutes vs 158 [66 to 640]) minutes, $p < 0.001$). Time reduction up to 130 minutes (45%). Apart from that, a decrease was found. MACE percentage was 10.83%, from 35 (48.61%) in non-CODE STEMI to 51 (37.78%) in CODE. Reduction in DTBT time, total costs, and length of stay for patients treated with the code STEMI	The CODE STEMI program has successfully demonstrated full improvement in seven components of quality of care and fulfilled marketing mix principles, thereby improving the quality of care and restoring clinical management in patients with STEMI.

			hypertension 74 (55%) dyslipidemia 51 (38%) obesity 9 (7%)			program (p <0.001, p < 0.001, and p <0.009)	
Peyman Namdar, *et al*/ 2020	Iran	Controlled Clinical Trial	The number of respondents was 58. Pre-code STEMI (29); intervention group using code STEMI (29). Pre-code STEMI: The median age is 59 years. Male 25 (86.1%) female 4 (13.9%) hypertension 10 (34.4%) diabetes mellitus 11 (37.9%) dyslipidemia 0 (0%) smoking 11 (37.9%) History of angiography: 9 (31%) History of PCI 6 (20.8%) died 0 (0%) Code STEMI: Median age: 57.4 years Male: 22 (75.8%); female: 7 (24.2%) hypertension 15 (51.7%) have diabetes mellitus. 6. (20.8%) dyslipidemia 2 (6.2%) smoking 15 (51.7%) History of Angiography 11 (37.9%) History of PCI 11 (37.9%) died 1 (3.5%)	Door-to-balloon time (DTBT) according to STEMI guidelines	Time interval DTBT, ECG diagnosis time, STEMI diagnosis time to cath lab and cathlab to balloon time	The average DTBT time in the pre-code STEMI and code STEMI groups was 113.5–43.6 minutes vs. 79.3-27.4 (p = 0.001). From the time the patient was in the ED to the time the patient was in the ECG, the pre-code STEMI vs. code STEMI group had an average time of 9.4–13 minutes vs. 8.1-6.9 minutes (p=0.5). EKG for diagnosing AMI pre-code STEMI group and code STEMI 21.5-27 minutes, average time 13.3-8.3 (p = 0.56). Diagnosis of AMI to cathlab time control intervention: pre-code STEMI and code STEMI group: 44.1-25.7 minutes vs. 39.5-25.9 minutes (p = 0.3) Cathlab to pre-code STEMI and code STEMI group balloon time: 26.2–18.2 vs. 15.5-7.8 minutes (p = 0.008)	- Implementing the STEMI CODE can really prevent parallel work and wasted time in treating AMI patients, especially STEMI. - Alerting personnel to start the treatment process for this group of patients is carried out with prior notification, which leads to more targeted work. - increased family satisfaction levels. - Minimize the death rate, the number of readmissions to the hospital per year, and the level of disability. - Implementation of CODE STEMI has no additional costs and does not impose new costs on the system.

Christopher J. Coyne, MD, *et al*/ 2014	California, Los Angeles	Controlled Clinical Trial	The number of respondents was 232. A (pre-CODE STEMI) (91); group B (CODE STEMI) (141). Pre-CODE STEMI group Mean age: 57.1 males, 64 (70.3%) female 27 (29.7) Ethnicity: white (9.9%) African American 5 (5.5%) Asian 17 (18.7%) Latino 57 (62.6) Chest pain at initial complaint: 66 (73.3%) STEMI CODE group Average age: 55 men 102 (72.3%) women 39 (27.7) Ethnicity: white 12 (8.5%) African American 18 (12.8%) Asian 17 (12.1%) Latina 91 (64.5%) Chest pain at initial complaint: 118 (83.7%)	Door-to-balloon time (DTBT) according to STEMI guidelines	Door-to-ECG time, activation time (ECG to STEMI CODE activation), cath lab arrival time (response to patient arrival at cathlab), and balloon time (present at cathlab until balloon installation time)	PreCODE STEMI and CODE STEMI door-to-ECG times (43±93 to 30±72 min, median 23 to 14 min, p<0.01), ECG activation time (87±134 to 52±82 minutes, median 43 to 31 minutes p<0.01), time from door to balloon (134±146 to 84±40 minutes, median 85 - 75 p=0.03) after the CODE STEMI period.	With a separate cardiac triage protocol, patients are systematically processed through the ED, which is associated with decreased DBTT time as well as reduced time-sensitive interval variability from door to ECG and ECG to balloon. Finally, by retaining a designated EKG technician in the ED triage area, unnecessary delays in the diagnosis and treatment of STEMI patients are eliminated.
Sharad Bajaj, *et al*/ 2012	Amerika Serikat	Retrospective Cohort	Number of respondents: 87 STEMI patients One group consisted of 27 STEMI patients the pre-CODE STEMI period (January to December 2006), and the second group consisted of 60 STEMI patients who were treated outside of working hours (January 2007 to December 2008).	Door-to-balloon time (DTBT) according to STEMI guidelines	Comparing the average time of DTBT outside working hours for patients who came during the pre-CODE STEMI period with patients who came during the CODE STEMI period	mean DTB time pre-CODE STEMI and CODE STEMI during Outside working hours, it was 129 minutes (interquartile range [IQR] 89–155) vs. 77 minutes (IQR 67–95) (p = 0.0001) from door to ECG. decreased by 9 minutes (p = 0.0001), ECG time to the cardiac catheterization laboratory	The CODE STEMI one-call protocol makes it possible to substantially improve out-of-hours care of STEMI patients while maintaining the delivery of care by trained catheterization staff. Code STEMI is a real-life quality

			Group 1: Average age: 60.96 Female: 8 (29.62%) Men 19 (70.37%) History of CAD 8 (29.62%) Diabetes mellitus 8 (29.62%) Hypertension 17 (62.96%) Cholesterol 20 (74.07%) Family history of CAD 4 (14.81%) PCI 7 (25.92%) Smoking 14 (40.74%), cardiac arrest/shock 2 (7.40%) Group 2: Average age: 60.03 Female: 15 (25%) Male 45 (75%) History of CAD 13 (21.66%) PCI-11 (18.33%) diabetes mellitus 16 (26.66%) hypertension 30 (50%) cholesterol 27 (45%) Family history of CAD 14 (25.92%) Smoking 27 (45)% cardiac arrest/shock (13.3%)		Secondary parameters: door-to-ECG, ECG-to-Cathlab, and Cathlab-to-Balloon Other parameters analyzed were peak serum troponin levels, peak total CK levels, all-cause mortality in hospitals, all-cause mortality at 6 months, and all-cause death at 12 months.	decreased by 16 minutes ($p = 0.0009$), and arrival of the catheterization laboratory DTB had an absolute reduction of 5 minutes ($p = 0.15$). The median peak troponin-I level achieved was 62 ng/mL (IQR). 23–142) in the SAP (pre-Code STEMI) group during rest hours. A reduction to 25 ng/mL (IQR 7–43; $p < 0.002$) was observed in STEMI Code group. In-hospital mortality data, mortality No statistically significant differences were felt.	improvement initiative with successful results.
Alberto Cordero, *et al*/2016	Spanish	Observational retrospective cohort	The number of respondents in this study was 1,210, who were divided into two groups. The group before the infarction code was applied was 866 (71.6), and the group after the infarction code was applied was 344 (28.4). Pre-Code STEMI Group Average age 68.94–12.6, length of stay 6.4–5.7	Door-to-balloon time (DTBT) according to STEMI guidelines	Comparing the average time of DTBT, hospitalization time, in-hospital mortality rate, recurrent infarction, and major cardiovascular complications	After implementation, the time for catheterization is reduced. (from 1.8–2.5 hours to 1.0–1.7 hours; $P < 0.01$), causing increase in patients receiving revascularization within 48 hours, from 65.4% to 78.6% ($P < 0.01$). The average hospital stay is	Improved usage Primary angioplasty requires coordination between differences. departments involved in STEMI emergency care, and our data suggest that the implementation of the infarction

	days, male 75.1%, diabetes mellitus 38.9%, hypertension 71.1%, smoking 28.4%, dyslipidemia 52.5% STEMI CODE group Average age 67.8-13.3 length of stay 5.6 5.1 days, men 75.1%, diabetes mellitus 38.9%, hypertension 63.1%, smoking 35.5%, dyslipidemia 48.3%	significantly shorter after implementation of the STEMI infarction protocol (7.0-5.1 days vs. 5.9-4.5 days; P<0.03). There was no difference in total between in-hospital deaths. 2 periods (4.9% vs. 3.8%; P = 0.42). There aren't any difference in cardiovascular mortality (5.0% vs. 6.0%),	protocol resulting in better ACS treatment in general, but particularly for high-risk patients and/or those with STEMI. The data from this study supports the need to implement such systems in hospitals that are not integrated into local or regional plans.

The average age in this study of STEMI patients was 59.36 years. As you get older, the risk of atherosclerosis will increase. Genetics or lifestyle cause plaque to slowly build up on the artery walls. In middle age or older, the plaque that forms will cause symptoms. These symptoms are at risk of appearing in men over 45 years old, while in women the risk increases after the age of 55 years.[20] The lifetime risk of death from cardiovascular disease in white men and black men is at the age of 55 years.[19]

Effect of STEMI CODE on door-to-balloon time

In the treatment of patients in hospitals, there is often a lack of awareness by health workers of the importance of immediate treatment for STEMI patients. The Emergency Room (ER), which is the entrance for all patients, is the beginning of this problem. Crowded rooms are a very concerning issue in public hospitals, especially because the number of health workers and facilities is not proportional to the number of patients who come with a variety of different complaints. Moreover, many patients do not want to cooperate easily, adding to the workload of health workers and resulting in chaotic patient management, including STEMI patients.[21] Given the importance of prompt treatment of STEMI patients, the CODE STEMI program is urgently needed to be implemented as a solution for problems in the field.

The success of CODE STEMI in reducing DTBT time in this research study is clearly illustrated in Figure 2. In the period before using CODE STEMI, the average DTBT was 135.27 minutes, while after using CODE STEMI, the DTBT was 82.74 minutes. Code STEMI was very effective in reducing DTBT, reaching an average of 52.53 minutes. This certainly greatly affects the condition of ischemia in the heart muscles. The extent of infarcted and necrotic tissue in the myocardium depends on the vessels involved, compensation by collateral vessels, and the degree and duration of occlusion.[16] Lower DBTB is also associated with recovery of LV function, with higher LV recovery when reperfusion is performed within 2 hours compared to when reperfusion is performed after 2 hours (6.9% at <2 hours vs. 3.1% at 2 hours).[18]

Effect of STEMI CODE on length of hospitalization and treatment costs

In this study, it was found that with fast and precise management, it would certainly affect the final clinical outcome of STEMI patients. Clinical conditions with complications will certainly make the length of stay longer and the cost of treatment higher. In accordance with this study, it shows that the length of stay using CODE STEMI is shorter with an average of 1 day compared to before using the CODE STEMI protocol, and in 1 day of treatment, it can be assumed that patients can save costs of up to USD 1,052.02.

Good-quality health services include every action that will be taken to improve health services intended for patients. Quality service must provide opportunities for improvement in every aspect. Code STEMI is proven as a program to improve the quality of health services by reducing treatment time, which reduces the risk of complications, thereby shortening the length of stay and the observation period. Shortening the length of stay will certainly streamline costs during treatment. So that patients experience satisfaction in handling and also feel efficient in controlling costs during good hypertension care, this is one of the efforts to slow down the occurrence of heart failure in someone.

Effect of STEMI CODE on the death rate

Coronary heart disease accounts for half of all deaths from cardiovascular disease. Based on this study, the average death rate for stem cell patients before using CODE STEMI was greater (17.47%) than after using CODE STEMI (4.34%). The CODE STEMI program can reduce the risk of death by up to four times compared to before using CODE STEMI. The risk of death and complications in this study was higher in the pre-CODE STEMI period. This can happen because of five potential problems in the practice of handling STEMI patients in the field: patient delays, delays in diagnosis and treatment decisions, delays in transportation, and a lack of collaboration between hospital management and doctors. With the CODE STEMI procedure, time delays caused by these five problems can be minimized. So that patients get treatment faster and the death rate decreases.

Effect of STEMI CODE on major adverse cardiac events (MACE)

The cause of death in Acute Coronary Syndrome is a complication known as MACE. One of the causes of death in Acute Coronary Syndrome is a complication known as Major Adverse Cardiac Events (MACE). MACE is often defined as a combination of non-fatal stroke, non-fatal myocardial infarction, or cardiovascular death. Sometimes extended to include heart failure, coronary revascularization, and ischemic cardiovascular events. There are many predictors of MACE in patients with acute coronary syndrome.[5] The most common MACE is heart failure.[22] In line with the study of Ji Quan Koh et al., all patients using CODE STEMI had less systolic dysfunction as measured by a left ventricular ejection fraction of 40% (10% vs. 27.8%).[18]

MACE can be measured at 30 days, 3 months, and 12 months, which includes death, ACS (atypical chest pain), and stroke, as well as readmission to the hospital or hospitalization. Left ventricular (LV) function can also be measured by a transthoracic echocardiogram. From this study, MACE before and after using CODE STEMI experienced a decrease, where the average of MACE before STEMI was 22.5% and after CODE STEMI was 15.14%. This difference is not very significant, but clinically, CODE STEMI can reduce the incidence of MACE.

Table 2. Comparison of mass efficiency of non-CODE STEMI and CODE STEMI

	Non-CODE STEMI	CODE STEMI	Efficiency
DTBT	135,27 minutes	DTBT 82,74 minute	52,53 minutes
Length of Hospitalization	7 days	6 days	1 day
Treatment Costs	USD 4870,97	USD 3818,95.	USD 1.052,02
The Death Rate	17,47%	4,34%	13,13%
MACE	22,5%	15,14%	7,36%

4. Conclusion

The CODE STEMI program has a positive effect on improving service quality and clinical outcomes in ST-elevation myocardial infarction patients and can be applied in healthcare services to reduce mortality rates, complications, and healthcare costs

5. Acknowledgments

We would like to thank Hermina Depok for being the inspiration for the emergence of this research idea. We also thank the Indonesian Maju University, which facilitates our continued learning and devel.

We hope that after obtaining the facts about code STEMI through this research, further research can be carried out on the effectiveness of implementing code STEMI at Hermina Depok.

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Case Report

FEES findings on dysphagia cases of laryngomalacia patients with tracheostomy cannula

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Abstract: Laryngomalacia (LM) is a condition characterized by the abnormal flaccidity and incoordination in various structures within the larynx, including the supraglottic cartilage, arytenoid mucosa, aryepiglottic folds, and epiglottis. Lack of rigidity in these structures leads to their collapse and subsequent airway obstruction during inspiration. LM can manifest shortly after birth or within the initial weeks of neonatal life. The symptoms typically peak around 6 to 8 months of age and gradually resolve by 12 to 24 months. While mild cases are managed through observation and conservative medical therapy, severe cases, along with mild to moderate cases accompanied by other comorbidities, may require surgical intervention. Notably, tracheostomy might be necessary for severe instances, which could lead to life-threatening obstruction of the upper airway. The aim of this study is to report a case series of laryngomalacia patients with tracheostomy cannula and its characteristics of Functional Endoscopic Evaluation of Swallowing (FEES). What are the FEES characteristics of dysphagia in laryngomalacia patient with tracheostomy cannula? We reported 4 cases of laryngomalacia patients with tracheostomy cannula, with different comorbidities, and to evaluate dysphagia using FEES. FEES indicated that a significant proportion of patients, specifically 3 out of 4, exhibited penetration and aspiration issues while consuming liquids. Moreover, patients who presented with neuromuscular comorbidities had a notably more severe level of dysphagia compared to those without these additional conditions. Liquids are more at risk of causing penetration-aspiration when compared to others in laryngomalacia patients with tracheostomy cannula.

Keywords: dysphagia; FEES; laryngomalacia

1. Introduction

Laryngomalacia, the most common congenital anomaly of the larynx, is characterized by the flaccidity and incoordination of various structures such as the supraglottic cartilage, arytenoid mucosa, aryepiglottic folds, and epiglottis. This condition leads to their collapse and subsequent airway obstruction during inspiration, resulting in a distinctive high-pitched sound called inspiratory stridor. The symptoms can manifest shortly after birth or within the initial weeks of neonatal life. They typically peak around 6 to 8 months of age and resolve by 12 to 24 months.[1–3] The exact incidence in the general population is uncertain but is estimated to be around 1 in 2100-2600 children worldwide.

Laryngomalacia is the most common cause of stridor in newborns, affecting 45–75% of all infants, with male predominance (58% - 76% of cases) and no known race predilection has been reported.[4,5]

The exact causes and mechanisms of laryngomalacia is not fully understood. Multiple causal theories of laryngomalacia have been proposed including, cartilage immaturity, anatomy abnormalities and neurological/neuromuscular immaturity theories. The main symptom of laryngomalacia is high-pitched stridor and vibration during inspiration. Stridor could be worse at 8-9 months of age and disappear with age. Laryngomalacia could affect various aspects of a child's growth and development. Around 80% of babies with laryngomalacia are at risk of being underweight due to their high-calorie needs combined with feeding difficulties caused by the condition.[6,7]

Laryngomalacia with intermittent inspiratory stridor, mild to moderate stridor and without feeding difficulties can be managed with observation after making a definitive diagnosis. It is important to monitor for appropriate weight gain and the development of any severe symptoms. In patients with mild to moderate respiratory problems, positional feeding may help those infants with feeding difficulties. [2] Surgery is indicated for all patients with severe laryngomalacia, mild or moderate laryngomalacia patients who have comorbid diseases such as tracheomalacia, subglottic stenosis or patients who have failed conservative therapy and who present with complication like failed to thrive and history of recurrent aspiration. Surgical decision is based on the type of laryngomalacia: supraglotoplasty by excising redundant arytenoid mucosa in type I; incision of shortened aryepiglottic folds in type II; and epiglotoplasty in type III. Tracheostomy is performed in severe laryngomalacia that causes life-threatening upper airway obstruction. Other indications for a tracheostomy are severe laryngomalacia with complications such as failure to thrive, prolonged intubation, stenosis of the larynx and laryngomalacia that does not improve after supraglotoplasty.[4,5]

Laryngomalacia is not only leads to airway obstruction but also causes difficulty in eating, manifesting as coughing, choking, regurgitation, emesis, and slow eating. These problems arise from disruptions in the suck-swallow-breath pattern and airway protection. The clinical symptoms of laryngomalacia can be worsened by various comorbid conditions, including gastroesophageal reflux disease (GERD), pharyngolaryngeal reflux (LPR), genetic syndromes, lung and heart anomalies, failure to thrive, prematurity, and neuromuscular disorders. These comorbidities further complicate the condition and increase the likelihood of swallowing disorders in affected patients.[8,9]

The presence of tracheostomy cannula on swallowing function in the pediatric population is not fully understood. In adult patients, tracheostomy cannula anchor the larynx, preventing laryngeal elevation, and can reduce laryngeal sensitivity, leading to impaired cough mechanisms. The absence of positive subglottic pressure during swallowing increases the risk of aspiration.[10] Pediatric patients

with laryngomalacia and tracheostomy cannulas can experience swallowing disorders affecting the oral and pharyngeal phases. Laryngomalacia itself complicates swallowing, further exacerbated by the presence of tracheostomy cannulas. Objective evaluation of swallowing function is crucial in infants with laryngomalacia due to potential impacts on morbidity and mortality.[10,11]

This case series will discuss pediatric patients with dysphagia (< 2 years of age) who was diagnosed with laryngomalacia with tracheostomy cannula. Dysphagia will be assessed by FEES.

2. Case report

Case 1

A 10-month-old boy was diagnosed with laryngeal papillomas, type I-II laryngomalacia, and tracheostomy status. An elective tracheostomy was performed when the patient was 2 months old due to prolonged intubation, sepsis, and pneumonia. In the medical history, the patient can orally consume milk, and has no history of choking. During the initial physical examination, the patients's general condition was good, fully conscious and alert, with heart rate of 120 beats per minute, respiratory rate of 34 breaths per minute, and SpO2 at 99%. The patient has a size 3.0 tracheostomy cannula and the airway passage is adequate. During the examination, the patient was not using a nasogastric tube.

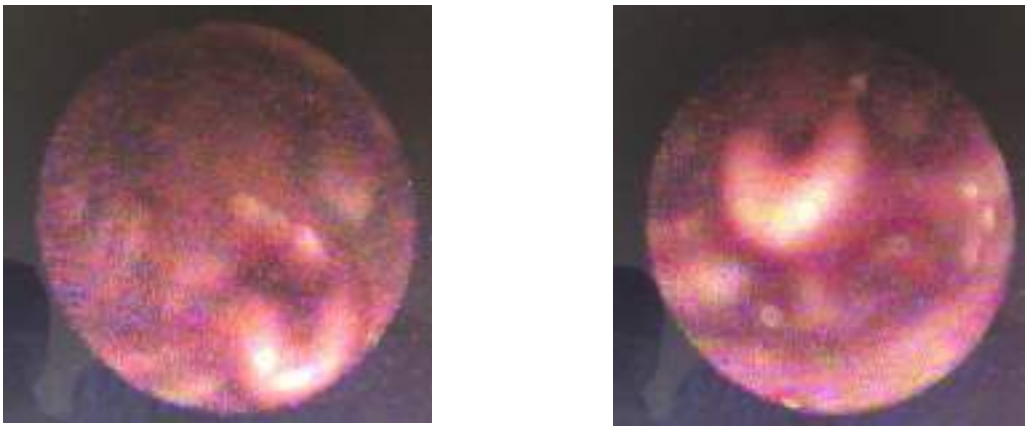
FEES was performed on February 17, 2022. During oropharyngeal examination, there was a strong lip seal and no drooling was observed. In the pre-swallowing assessment, an omega-shaped epiglottis was noted, arytenoid edema was present, and papilloma was seen on the right arytenoid. Vocal fold movement appeared symmetric, and no pooling secretions, penetration, or aspiration were found. FEES was conducted with the administration of liquid milk, and the results showed no residue, penetration, leakage or aspiration (Figure 1). FEES was not assess other food textures due to the patient's lack of cooperation. Based on FEES finding, it was concluded that the patient had type I-II laryngomalacia, laryngeal papilloma and no signs of dysphagia.

Case 2

A 6-month-old boy with various medical conditions, including chronic respiratory failure, hygroma colli, type 2 laryngomalacia, and malnutrition. The patient underwent elective tracheostomy at the age of 2 months due to prolonged intubation, pneumonia, and narrowed airway caused by higroma colli. There was no aspiration during oral feeding trial. An NGT was still inserted on the patient.

During initial examination, the patient was in good general condition, fully conscious, with vital signs such as heart rate, respiration rate, and oxygen saturation within normal ranges. FEES was conducted on April 4, 2023. The examination revealed satisfactory oropharyngeal suction power, good

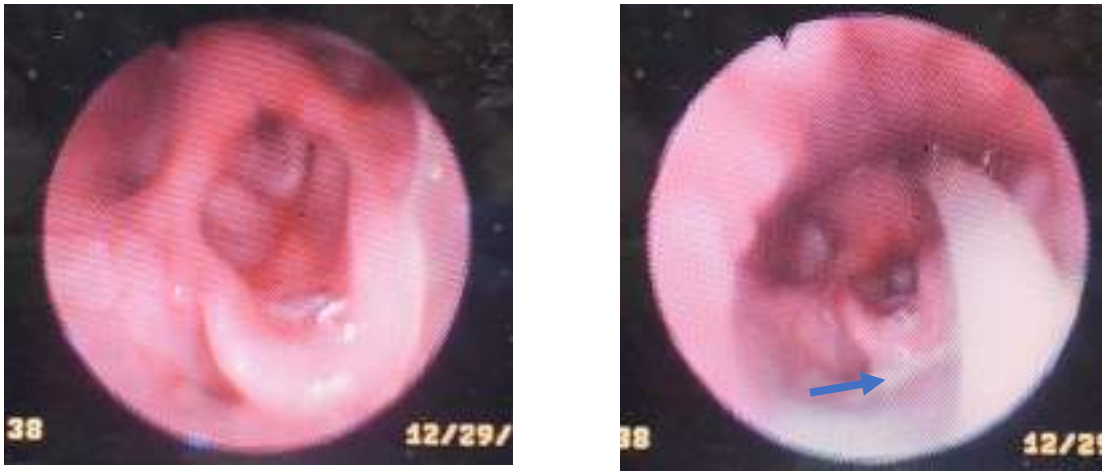
lip seal, and no drooling. Pre-swallowing assessment showed grade II lingual tonsils, omega-shaped epiglottis, edema arytenoids, and silent aspiration.



(A) **(B)**

Figure 1. FEES findings on case 1. (A) Arytenoid edema. (B) An omega-shaped epiglottis

FEES was performed with liquid milk, from the FEES findings, there was residue in the valecula, penetration above the vocal fold, and no aspiration was shown (Figure 2). Based on the FEES results, the patient was diagnosed with moderate type II laryngomalacia and oropharyngeal dysphagia.



(A) **(B)**

Figure 2. FEES findings on case 2. (A) An Omega shaped epiglotis. (B) Residu in valecula (blue arrow)

Case 3

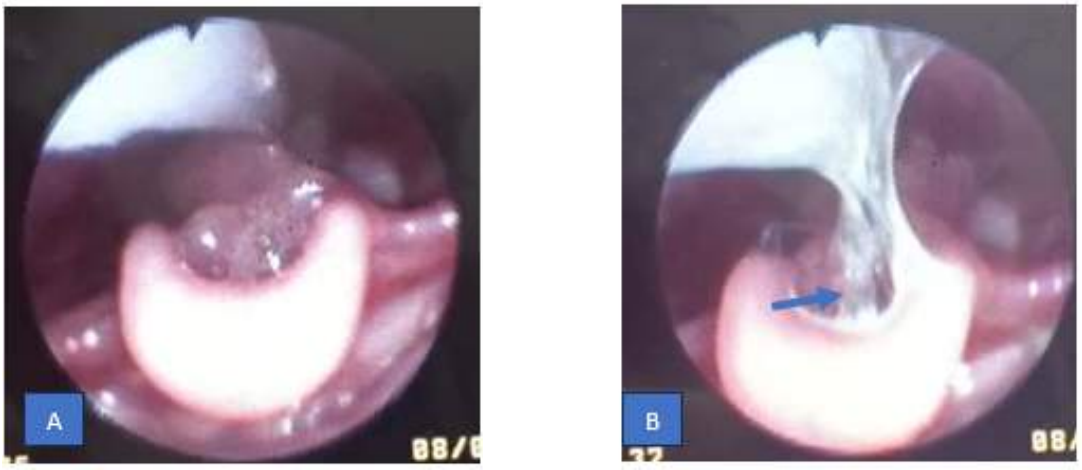
A 7-month-old girl with severe type II laryngomalacia. A tracheostomy was performed at 2 months old due to respiratory failure caused by upper airway obstruction. The patient is currently drinking

milk orally and was not choking during observation. However, the patient still had an NGT since she was 2 months old. The patient had two FEES examinations. The first FEES was perfomed on November 8, 2022, when the patient was 4 months old. The patient drank milk from the NGT.

The patient had been tried drink orally but the patient was choked. FEES examinations showed grade I lingual tonsils, stiff epiglottis, arytenoid edema, short aryepiglottic folds, symmetrical vocal and ventricular plica movements, standing secretions, minimal silent aspiration and decreased sensitivity of the hypopharynx. A penetration and aspiration was shown when patient drink milk (Figure 3). The patient's cough reflex was good. Based on the FEES results, the patients was diagnosed with type 1-2 mild laryngomalacia and pharyngeal phase dysphagia.

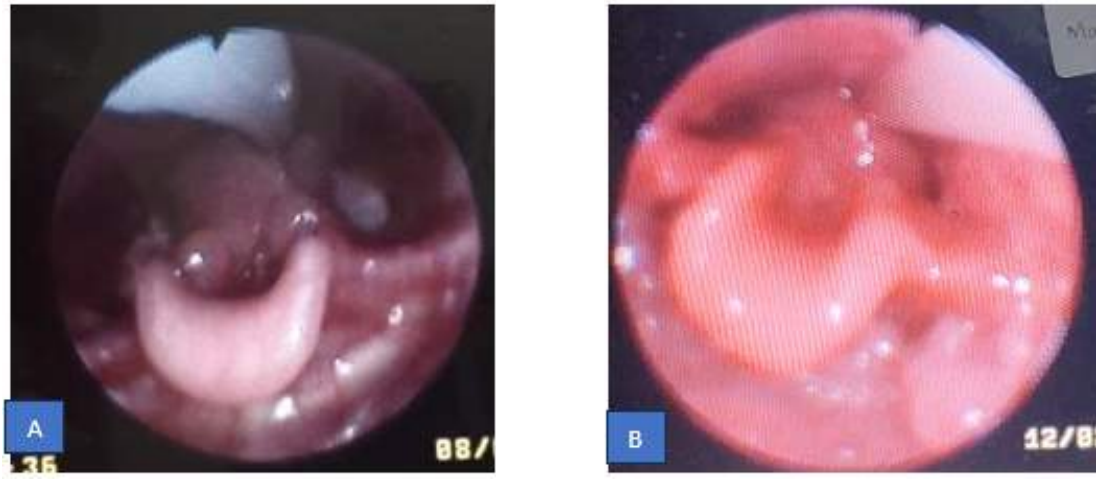
The second FEES examination was evaluated on March 8, 2023, when patient was 7 months old. There was a NGT on patient. The patient had been previously given milk orally without any complaints of choking. During initial examination, The patient's general condition was good.

Postural control was good and the patient could maintain her head position during feeding. Oropharyngeal examination showed no issues. The preswallowing assessment revealed an omega-shaped epiglottis and left aritenoid edema. FEES was performed with liquid consistency (milk), and the results shows minimal leakage, no residu, no penetration, and no silent aspiration were observed (Figure 4). The FEES examinations indicated Mild grade type I laryngomalacia. Compared to the previous FEES results, the dysphagia condition had improved. The patient was allowed to remove NGT and can be fed orally.



(A) **(B)**

Figure 3. FEES Findings in Case 3 (First FEES). (A) An omega-shaped epiglottis. Standing secretion was seen above the vocal cord (B) On FEES examination with milk, penetration and aspiration were seen (blue arrow)



(A) **(B)**

Figure 4. FEES Findings in Case 3 (Second FEES). (A) An omega-shaped epiglottis, arytenoid edema. (B) On FEES examination with milk, there is no penetration and aspiration

Case 4

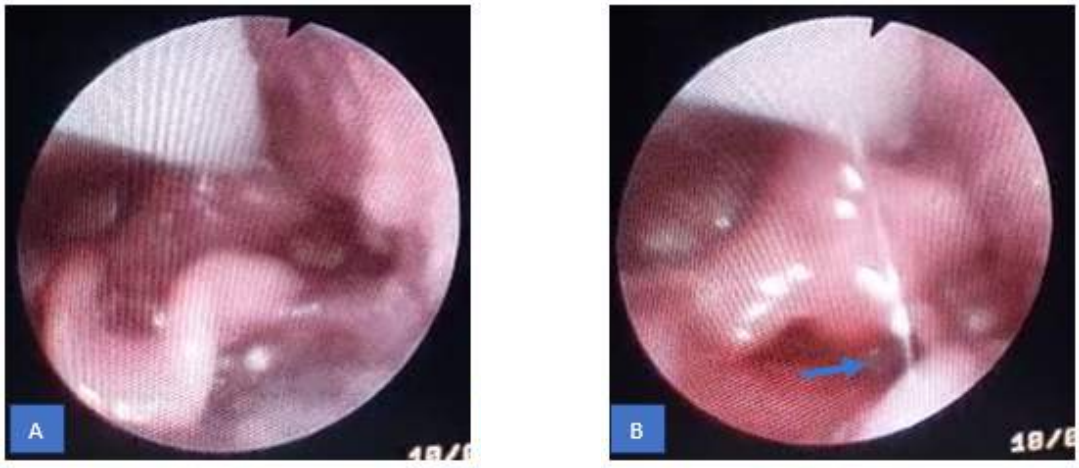
A one-year-old girl with a diagnosis of tracheostomy status due to severe laryngomalacia. The patient's medical history were cerebral palsy, epilepsy, and congenital heart disease. The patient had a tracheostomy emergency since 1 month old due to upper airway obstruction caused by severe laryngomalacia. The patient had a history of recurrent aspiration pneumonia. The patient was consuming thin-textured porridge through a nasogastric tube (NGT). An oral feeding was attempted and no choking was shown during observation.

FEES was performed on January 9, 2023. During the initial examination, the patient's general condition was good, fully conscious with compos mentis awareness, heart rate 120 beats per minute, respiratory rate 36 breaths per minute, and SpO2 levels between 95-99%.

The patient was able to maintain head position and postural control during feeding. Orofacial examination revealed a symmetrical tongue, adequate movement, adequate lip seal, and no drooling. The preswallowing assessment showed an omega-shaped epiglottis, bilateral arytenoid edema, limited vocal fold and ventricular fold movement, restricted abduction and adduction movement, no penetration, no aspiration, presence of silent aspiration, standing secretion, and sufficient hypopharyngeal sensitivity. The FEES examination began with thin-textured porridge, which showed no leakage, mild residue in the vallecula, penetration above the vocal cords with aspiration, and adequate cough reflex (Figure 5). Different food textures were not tested.

The FEES results still indicated mild grade type 1 laryngomalacia and oropharyngeal dysphagia. The management plan includes postponing the enhancement of food texture, continuing oral breast

milk feeding in an upright head position gradually, maintaining the use of feeding tube and reevaluating FEES one month after therapy.



(A) **(B)**

Figure 5. FEES Findings on case 4. (A) An omega shaped epiglottis. arytenoid edema. (B) On FEES examination with puree, there appears to be aspiration (blue arrow)

3. Discussion

In this case series, all subjects were <15 months old. A study conducted by Thompson et al.[5] showed that most laryngomalacia patients were diagnosed at 3.5 months of age, although more severe laryngomalacia was diagnosed at 6 months of age. The study conducted by Simons et al.[12] also stated that most laryngomalacia patients were diagnosed at 3 months of age. Stridor is the most common symptoms of laryngomalacia and generally appears after the first two weeks of life, and could be worse at the age of 4 to 8 months and gradually resolve by 12 to 24 months in most cases.[2]

All subjects in this study were diagnosed with type 1 laryngomalacia. This was also found in a study conducted by Cooper et al.[13] in Canada, the most common type was type 1 (40.2%), followed by type 2 (30.8%). The study conducted by Olney, quoted by Richter, stated that the most common type is type 1, which is 57%. The study conducted by Thompson showed that the type of laryngomalacia was not significantly related to the degree or prognosis of the disease.[5]

Pre-swallowing Assessment of FEES

In this case series report, on general condition, it was found that one patient with dysphagia had cerebal palsy and epilepsy that disturbed in postural control. This conditions could lead to weakness in motor tone and cervical muscle and could affect the swallowing function. Impaired postural control

would increase the risk of dysphagia 4.4 times compared to good postural control. The hyoid bone and its associated muscles, suprahyoid and infrahyoid muscles, are essential for coordinating the suck-swallow-breath cycle. These muscles stabilize the hyoid bone, contributing to synchronized movements during sucking, swallowing, and breathing. Disorders affecting the hyoid complex can disrupt this coordination, leading to feeding and swallowing issues in infants. Additionally, impaired postural control can exacerbate feeding problems and increase energy expenditure.[14]

A weak lip seal was also found on pre-swallowing assessment. Adequate sucking is facilitated by a combination of factors that create a negative intraoral pressure. The soft palate's closure against the velum, the lip's closure around the nipple (bottle or breast), and the downward movement of the lower jaw collectively contributely create the negative pressure within the oral cavity, allowing infants to effectively extract milk during breastfeeding. The presence of a weak lip seal will make it difficult to produce sufficient negative pressure. This will lead to a smaller amount of milk extracted by infants during breastfeeding. Inadequate pressure or low milk intake leads to poor bolus formation, inhibiting the reflexive swallowing response as liquid enters the pharynx. This circumstance can lead to fluid accumulation around the valleculae and pyriform sinuses, increasing the risk of penetration and aspiration.[15]

Another finding on the pre-swallowing assessment is *standing secretion*. Standing secretions can be caused by hyposensitivity of the hypopharynx so that the control of pharyngeal peristalsis is not optimal. Increasing sensory threshold in laryngomalacia patients indicates impaired function of peripheral afferent nerves from the larynx. The presence of peripheral sensation disorders will reduce the patient's level of vigilance to swallow his saliva. Standing secretion is not only caused by a lack of sensory ability but also due to a lack of motor ability to clear its saliva. Standing secretions accompanied by difficulty in swallowing can damage the Laryngeal Adductor Reflex (LAR) pathway, both afferent, central, and efferent.[7]

Swallowing Assessment of FEES

Residue is the presence of food in the pharynx after swallowing. In this case series, there were 2 patients with residue in vallecula. A study conducted by Ahmed [7] showed that viscosity of the food bolus affecting the presence of residue. Residues are generally found in two sites, epiglottis vallecula and pyriform sinuses. Residue that found in that location are signs of dysphagia. Food residue in the vallecula is caused by weakness base of the tongue to moving through the posterior pharyngeal wall. The hyposensitivity of the hypopharynx and larynx contributes to the large amount of residue in the vallecula. Residues in the pyriform sinus is due to lack of pharyngeal and upper esophageal sphincter

contraction that decrease anteroposterior diameter opening. Residue in the pyriform sinuses is relatively close to the laryngeal vestibule, increasing penetration and aspiration. Post-cricoid residues may result from weakness base of the tongue, pharyngeal contractions, and the anteroposterior diameter of the upper esophageal sphincter.[7]

In this case series, penetration and aspiration were found in liquid consistency. Penetration is a bolus that passes the laryngeal vestibule, but does not pass the vocal cords. In this case series, 3 out of 4 occur penetration and aspiration. Gasparin[16] explained that food with liquid consistency is more difficult to control and more easily causes penetration and aspiration because it can flow directly into the pharynx before the gag reflex occurs. Thickened boluses or soft solids are safer for possible passage into the larynx. In addition, thickened bolus increase tongue movement and help accelerate the initiation of the pharyngeal phase. Coughing is a protective mechanism that helps clear the airway by forcefully expelling such material. However, in some cases, laryngeal sensation can be impaired, leading to silent aspiration, where material enters the airway without triggering a cough reflex. In infants with laryngomalacia, a condition where the soft tissues of the larynx collapse during breathing, prolonged exposure to reflux can occur. This, combined with sensorimotor dysfunction of the larynx's protective mechanisms, can result in residue, penetration, and aspiration of material into the airway without the adequate cough reflex. This poses a risk of respiratory and feeding problems in affected individuals.[16,17]

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Literature Review

Effects of intermittent fasting on the liver

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Abstract: Intermittent fasting (IF) has become increasingly popular as a method of calorie restriction, primarily for weight loss purposes. However, its impact on liver health has also become a topic of interest since the liver plays a critical role in maintaining energy balance during fasting. To gain insights into IF's effects on the liver, this review is based on articles publishedin reputable databases such as Elsevier, PubMed, Google Scholar,and SAGE. IF involves a reduction in calorie intake, leading to changes in energy utilization within the body. Studies have shownthat IF has been associated with decreased liver fat accumulation,leading to improved liver function and reduced inflammation. IF can improve insulin sensitivity and reduce insulin resistance,potentially benefiting individuals at risk of developing non- alcoholic fatty liver disease (NAFLD) and type 2 diabetes. Moreover, IF appears to influence various cellular processes, suchas autophagy, which may play a role in the liver's adaptiveresponse to stress and maintenance of cellular integrity. On the other hand, there are concerns about the potential adverse effects of IF on certain populations. Proper supervision and individualized approaches are essential when considering intermittent fasting as a dietary intervention. In summary, intermittent fasting has already demonstrated its beneficial impacton liver health through crucial metabolic pathways over the years. IF holds promise as a lifestyle approach to improve overall health. Nonetheless, further research is required to fully comprehend the precise mechanisms and long-term effects of IF on liver health.

Keywords: effect; intermittent fasting; liver; metabolic

1. Introduction

Intermittent fasting (IF) has gained significant popularity as a calorie restriction method in recent years, particularly for weight loss and metabolic health benefits.[1–3] The liver, a vital organ responsible for essential metabolic processes, has been a central focus of interest in exploring the effects of intermittent fasting.[4]

As a metabolic hub, the liver plays a pivotal role in maintaining energy balance and adapting to fasting periods.[5] During intermittent fasting, the liver releases stored glucose through glycogenolysis, providing necessary energy for bodily functions during periods of reduced calorie intake. Additionally, intermittent fasting influences the metabolism of fatty acids in the liver, leading to the production of ketones as an alternative energy source.[6]

Research investigating the effects of intermittent fasting on the liver has shown promising outcomes.[7] Studies have observed reductions in liver fat content and improvements in markers

associated with non-alcoholic fatty liver disease (NAFLD), a condition characterized by excess fat accumulation in the liver unrelated to alcohol consumption.[7,8] The potential protective effect of intermittent fasting against NAFLD holds promise for managing liver-related disorders.[8]

However, despite these encouraging findings, a comprehensive understanding of the precise mechanisms underlying intermittent fasting's impact on liver health remains limited. As intermittent fasting continues to gain traction and evidence accumulates, conducting a thorough review of the existing literature on its effects on the liver is essential. By synthesizing available data from reputable resources such as such as Elsevier, PubMed, Google Scholar, SAGE, this review aims to contribute to a better comprehension of the effects of intermittent fasting on the liver. Ultimately, such insights may have implications for potential therapeutic interventions and dietary recommendations to optimize liver health and overall well-being.

2. The liver's role in energy balance and interest in liver health

The liver plays a crucial role in the human body, responsible for various functions that support metabolism, immunity, digestion, detoxification, vitamin storage, and other essential processes. It constitutes approximately 2% of an adult's body weight.[5,9]

The liver's functional unit is called a lobule, characterized by a hexagonal shape. Each lobule has a portal triad (composed of the portal vein, hepatic artery, and bile duct) located at its corners. Hepatocytes, which have physiologically distinct apical and basolateral membranes, form the base of the lobule. Hepatocytes are divided into three zones based on their function and blood supply.[5,9]

Zone I is considered the periportal hepatocyte region, receiving the best perfusion and playing a significant role in oxidative metabolism, including beta-oxidation, gluconeogenesis, bile formation, cholesterol synthesis, and amino acid catabolism. Zone II is defined as the pericentral hepatocyte area and lies between Zone I and Zone III. Zone III experiences the lowest perfusion due to its distance from the portal triad. It has a crucial role in detoxification, drug biotransformation, ketogenesis, glycolysis, lipogenesis, glycogen synthesis, and glutamine formation.[5,9]

Bile flow is facilitated by bile canaliculi, forming a network similar to chicken wire. The direction of bile flow is opposite to the blood flow. This is logical as the liver produces bile, which exits through the ducts, while the dual blood supply enters the liver for further processing. Blood flows to the central lobule through sinusoidal lumens via the hepatic vein branches.[5,9]The space between the sinusoidal lumen and the basolateral membrane of hepatocytes is known as the Space of Disse. Microvilli extend from the basolateral membrane and communicate with capillaries, allowing hepatocytes to access their blood supply. The Space of Disse contains an extracellular matrix comprising various collagens,

proteoglycans, and other proteins, providing a framework for hepatocytes and lobules. This framework is significant, as hepatocytes lack a true basement membrane. Additionally, the Space of Disse contains Kupffer cells (macrophages) and Ito cells (stellate cells). Kupffer cells filter unwanted or pathological substances from the bloodstream, while Ito cells store fat, including vitamin A, and aid in liver regeneration.[5,9]

The liver interacts with various organ systems in the body, including the endocrine and gastrointestinal systems, to support digestion and metabolism.[9] In carbohydrate metabolism, the liver serves several essential functions. It stores glycogen as a rapidly available energy source. Moreover, the liver converts galactose and fructose into glucose, the primary energy source for the body. Additionally, the liver performs gluconeogenesis, producing glucose from non-carbohydrate sources such as amino acids and glycerol. Furthermore, the liver synthesizes various chemical compounds through intermediary carbohydrate metabolism.[5,9–11]

In lipid metabolism, the liver plays a vital role, rapidly oxidizing beta-lactic acid to provide energy for other bodily functions. The liver is also responsible for synthesizing most lipoproteins essential for transporting fats and cholesterol in the bloodstream. Additionally, the liver produces significant amounts of cholesterol and phospholipids, crucial for cellular function and hormone synthesis. Moreover, the liver can convert substantial quantities of carbohydrates and proteins into fats.[5,9–11] In protein metabolism, the liver performs various important functions. It carries out amino acid deamination, removing amino groups from amino acids for protein synthesis or energy production. The liver also contributes to urea formation, which aids in the elimination of excess ammonia from body fluids. Additionally, the liver is involved in plasma protein synthesis and facilitates the interconversion of different amino acids.[5,6,9,10]

Furthermore, the liver has other metabolic functions. It synthesizes many blood components essential for the coagulation process, such as fibrinogen, prothrombin, accelerator globulin, and other critical coagulation factors. These metabolic processes in the liver rely on vitamin K for the formation of prothrombin and coagulation factors. A deficiency in vitamin K can significantly reduce the concentration of these substances, leading to impaired blood coagulation. The liver also stores iron as ferritin and releases it when needed by the body.[5,9]

The liver possesses the ability to detoxify and eliminate various drugs, including sulphonamides, penicillin, ampicillins, and erythromycins, through bile. Additionally, the liver is involved in hormonal regulation, chemically altering or inhibiting certain hormones secreted by endocrine glands. For example, the liver can influence hormone metabolism, particularly thyroid hormone (thyroxine), and

various steroid hormones like oestrogen, cortisol, and aldosterone. Liver damage can result in an excessive accumulation of hormones in body fluids, leading to hormonal imbalances.[5,9,10]

3. Effects of intermittent fasting on energy utilization

The effects of fasting on liver glycogen and glucose release play a pivotal role in maintaining energy balance during periods of food deprivation. As the body transitions from a fed to a fasting state, glycogen reserves stored in the liver are gradually depleted to sustain blood glucose levels. This depletion triggers a shift in the liver's metabolic activity, leading to the conversion of stored triglycerides into glucose through a process called gluconeogenesis.[12] This mechanism serves as a crucial source of glucose for energy-demanding tissues, such as the brain, red blood cells, and muscles, ensuring that essential physiological functions continue even when food intake is limited. The liver's dynamic response to fasting underscores its adaptability to varying energy demands and highlights its role as a central hub in maintaining glucose homeostasis.[5,9,13,14]

This adaptability aligns closely with intermittent fasting's influence on insulin sensitivity and glucose regulation which is a cornerstone of its potential benefits for liver health. Fasting periods create an environment in which insulin levels decrease, prompting tissues to enhance insulin signalling pathway as a means of efficiently utilizing available glucose. This response is particularly relevant in counteracting insulin resistance, a hallmark of NAFLD and type 2 diabetes. By improving insulin sensitivity, intermittent fasting promotes glucose uptake, reducing the strain on the liver to produce excessive glucose. Moreover, intermittent fasting's effects on insulin sensitivity extend beyond glucose metabolism to impact lipid metabolism, thereby addressing the multifaceted metabolic dysregulation that contributes to liver disorders.[14,15]

4. Impact of fasting on fatty acid metabolism and ketone production

Fasting triggers a cascade of metabolic adaptations within the liver that profoundly influence fatty acid metabolism and ketone production. In the absence of exogenous glucose supply, the liver enhances the breakdown of stored triglycerides into free fatty acids, which are subsequently utilized as an alternative fuel source for energy production. Moreover, as fatty acids are oxidized, the liver generates ketone bodies, such as beta-hydroxybutyrate and acetoacetate, through a process known as ketogenesis. Ketones serve as an essential energy substrate for extrahepatic tissues, including muscles and the brain, during prolonged fasting periods. The production of ketones not only conserves glucose but also exerts protective effects against oxidative stress and neuronal damage. This metabolic

flexibility underscores the liver's role in adapting to nutritional challenges and maintaining metabolic equilibrium.[1,13,16]

5. Impact on adipose tissue and implications for non-alcoholic fatty liver disease (NAFLD)

Non-alcoholic fatty liver disease (NAFLD) is characterized by excessive accumulation of fat in hepatocytes and is closely associated with obesity, insulin resistance, and metabolic syndrome. Intermittent fasting has emerged as a potential strategy to address NAFLD due to its capacity to reduce liver fat content.[17,18] During fasting periods, the liver depletes its glycogen stores, leading to increased fatty acid oxidation and decreased de novo lipogenesis—processes that contribute to the reduction of hepatic triglyceride accumulation. This reduction in liver fat not only improves liver function but also ameliorates the inflammatory state associated with NAFLD. Furthermore, the reduction of liver fat through intermittent fasting positively influences insulin sensitivity and metabolic health, which are central to addressing the underlying causes of NAFLD.[4,7,8,19]

Adipose tissue can be categorized into white adipose tissue (WAT) and brown adipose tissue (BAT), which are further divided into visceral and subcutaneous fat depots. WAT's primary function is to store energy as triglycerides, while BAT is involved in thermogenesis, which can counteract the effects of increased energy intake and promote weight loss. A study conducted in mice showed that intermittent fasting (IF) led to the browning of WAT and improved obesity, insulin resistance, and hepatic steatosis by altering the gut microbiome. Another preclinical study found that isocaloric IF improved metabolic balance through adipose thermogenesis, achieved by WAT browning, M2 anti-inflammatory macrophage activation, and increased vascular endothelial growth factor expression.[7,8,20]

Intermittent fasting (IF) also affects adipokines, which are signalling proteins released by adipose tissue. In obesity, there is an imbalance in adipokine production, with an increase in pro-inflammatory adipokines and a decrease in anti-inflammatory adipokines. IF has been shown to reduce weight and visceral adipose tissue (VAT), potentially further improving inflammatory outcomes and reducing the risk of non-alcoholic fatty liver disease (NAFLD). However, the specific effects of IF on adipokines are still not fully understood, and studies have shown mixed results.[21–23]

6. Previous researches on intermittent fasting and its effect on liver functions

Research conducted by Ma et al. (2021) revealed a significant decrease in blood glucose levels in IF (Intermittent Fasting) mice compared to AL (ad libitum) mice. The ALP (Alkaline Phosphatase) activity level increased significantly in IF mice. No significant changes were observed for other tested parameters. These findings suggest that intermittent fasting does not affect liver function in mice and

mainly influences metabolism regulation.[6] Sarkar et al. (2023) also supported this statement, stating that liver tissue tends to remain dormant unless chemically or mechanically injured.[24] However, according to Anton et al. (2018), the IF diet does not always lead to improved health indicators. For instance, compared to young male rats given a high-fat diet ad libitum, those on the same diet for only three hours daily over five weeks showed increased insulin resistance despite reducing body fat content.[25]

Research conducted by Munhoz et al. (2020) and Ma et al. (2021) indicated no effects on liver function parameters but showed a reduction in the liver weight of mice after IF, correlating with decreased glycogen levels.[6,26] In a study by Shahhat et al. (2022), intermittent fasting treatment in rats with ulcerative colitis significantly reduced serum AST (Aspartate Aminotransferase) and ALT (Alanine Aminotransferase) levels compared to the control group.[27] Another study by Dai et al. (2022) demonstrated that most biochemical liver function biomarkers remained unchanged during a 10-day complete fasting followed by diet restoration, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and total bile acids.[28]

Intermittent fasting (IF) has also shown a potential way to regulate autophagy and counteract its dysregulation. Studies in mice have shown that fasting can lead to increased autophagosome formation in neurons, and IF in a mouse model of Charcot-Marie-Tooth syndrome resulted in higher expression of autophagy-related proteins and decreased levels of p62 protein, indicating increased autophagy activity.[7,8,29,30]

7. Conclusion

In conclusion, intermittent fasting (IF) has garnered substantial attention as a method for promoting weight loss and metabolic improvements. The liver's significance in energy balance and adaptation during fasting has drawn particular interest. IF influences glucose release, fatty acid metabolism, and ketone production within the liver. While research highlights reductions in liver fat content and enhancements in markers linked to emerging diseases such as non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes, a comprehensive comprehension of the precise mechanisms underlying IF's impact on liver health remains limited. This review endeavours to consolidate information from reputable sources, contributing to a more profound understanding of IF's effects on the liver. While these insights hold potential for therapeutic interventions and dietary guidance, further research is imperative to fully unravel the underlying mechanisms and long-term implications. The liver's

dynamic response to fasting underscores its pivotal role in maintaining metabolic equilibrium and overall well-being.

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Systematic Review and Meta-Analysis

The effect of ramadan fasting on leptin and ghrelin levels(systematic review and meta-analysis)

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Abstract: Ramadan fasting may affect whole body metabolism by appetite-related hormones. This systematic review and meta-analysis aimed to clarify the effects of Ramadan fasting on the main hormones regulating appetite, including leptin and ghrelin. All English language papers in the PubMed, Science Direct, and Embase databases weresearched using the keywords "Ramadan fasting", "appetite", "ghrelin", and "leptin", from 2013 to 2023. Data extraction was conducted based on the main data of the studies; the primary outcomes of the analysis were mean changes in ghrelin and leptinlevels during the holy month of Ramadan in fast subjects, and meta-analysis was calculated using Medcalc. Data of 7 eligible studies, conducted between 2013 and 2023, were included in the systematic review. Of these, 4 studies with complete dataon leptin and ghrelin and 7 studies with leptin data were included in the meta-analysis. A significant decrease in leptin levels was observed after Ramadan fasting (WMD = −0.453 pg/ml, 95% CI = −2.069 to 1.163). Ramadan fasting had no significant effecton ghrelin levels (WMD = 2.19 pg/ml, 95% CI = −0.896 to 1.032). the conclusion is Ramadan fasting causes a decrease in leptin levels, without changes in ghrelin levelsin normal, overweight, or obese subjects.

Keywords: appetite; ghrelin; leptin; ramadan fasting

1. Introduction

Fasting in the month of Ramadan, which is the ninth month in the Muslim calendar, is an example of the intermittent fasting model. In some Islamic countries, Ramadan is associated with lifestyle changes that include changes in meal frequency and composition, sleep/wake patterns, and sleep duration. [1,2] During Ramadan, Muslims must refrain from eating and drinking from dawn to sunset with the length of fasting varying according to the geographic location of the area.

Ramadan follows the Islamic year (Hijri) which follows the lunar system, which is 11 days shorter than the Gregorian year. Therefore, Ramadan occurs in different seasons every nine years, which significantly affects the length of the days and nights. Furthermore, changes occur in day/night activity patterns during the month of Ramadan, such as evening prayers and waking up early for *sahur*. Moreover, in some Islamic countries, shops and shopping centers open late and stay open late at night, but eating places will close in the morning and afternoon. Additionally, eating habits may change in some cultures during the month of Ramadan; in particular, people prefer fried foods and consume

excessive amounts of sweet foods. [1] The above pattern suggests that the physiological changes that occur during Ramadan fasting may be different from those that occur during experimental fasting. [3]

Leptin, a protein product of the obesity gene, is thought to play a role in the regulation of calorie intake, fat storage, and long-term energy balance. [4,5] Leptin acts as a signal transmitter to the brain and reflects energy stores in the body. [6] A circadian rhythm of serum leptin has previously been demonstrated in normal subjects, with an average peak between 22:00 and 03:00. Morning leptin levels are relatively high; However, a significant decrease in plasma leptin occurred after breakfast, and the lowest levels occurred between 08:00 and 17:00. [7] In addition, it has been shown that the plasma leptin profile is higher in the obese group than in lean subjects and higher in women than men. [8]

Shifting meal times has been shown to increase the diurnal leptin rhythm, suggesting that eating may be associated with increased leptin at night. [8] Bogdan et al showed a shift in the circadian rhythm of leptin but no significant change in the mean plasma leptin concentration on the 23rd day of Ramadan fasting during the day in 10 male subjects. [9] One study found a significant increase in leptin during fasting, but another study found a 30-66% decrease in leptin after fasting. [10] Differences in plasma leptin levels at night during fasting may be due to changes in meal times during fasting.

Ghrelin is an appetite-stimulating peptide that appears to act as a peripheral orexigen that counteracts the action of leptin. [11] Several studies indicate a competitive interaction between ghrelin and leptin in the regulation of food intake. [12] Animal studies show that fasting and food intake affect both hormones. [13] In humans, plasma ghrelin levels increase before meals and decrease after meals. [14] Previous research has shown that Ramadan fasting can affect the circadian rhythm of several biological variables, including hormones. [3] Prolonged fasting has been shown to increase plasma ghrelin levels in rats. [15] Another study in humans found no significant change in ghrelin after a month of fasting during Ramadan

Many lifestyle changes that occur during Ramadan can affect plasma leptin and ghrelin levels, such as meal times, sleep/wake schedules and sleep duration, light exposure, and exercise. [16–18] The differences in research results that occur make it important to carry out an in-depth study of how big the changes that occur after fasting during Ramadan. Therefore, this systematic review was designed to assess how much influence intermittent fasting during Ramadan has on changes in appetite hormones by measuring plasma leptin and ghrelin levels.

2. Methods

This meta-analysis was constructed according to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement. We performed a thorough literature search of observational studies investigating the effect of Ramadan fasting on leptin and ghrelin.

Search Strategy

Literature searching was performed independently by two authors (RAT and II) in August 2023 at PUBMED, Science Direct, and ProQuest database. The keywords used in the searching strategy were Ramadan fasting, obese, overweight, leptin, and ghrelin. Search terms in databases were ("Ramadan fasting" OR “Ramadan Diurnal Fasting” OR “Ramadan Intermittent Fasting”) AND (“ appetite” OR “leptin” OR “ghrelin”) AND (“obese” OR “overweight”). The reference list in the included studies was also manually screened for other potential studies.

Inclusion and Exclusion Criteria

The inclusion criteria are observational studies of Ramadan fasting among normal weight, obese, and overweight populations which measure the interest outcome parameters at the minimum two-time points (before Ramadan, during and or after Ramadan). Studies that were written in English and recruited adult populations were included in this review. The studies such as reviews, conference reports, letters, and unpublished papers were also excluded.

Data Extraction

Data extraction was performed by one author (RAT) with confirmation from the other authors (II). We analyzed the baseline characteristics of each included study such as author, sample size, country where the study was conducted, age, sampling time, and number of fasting days. The outcome measured was the comparison of leptin, and ghrelin events before and during or after fasting during Ramadan.

Quality Assessment

Two reviewers (RAT and II) were assigned separately to evaluate the quality and eligibility of the studies. Any discrepancies would be discussed based on consensus. The quality assessment of the included observational study was conducted using a validated tool that is the Newcastle-Ottawa Scale (NOS). A NOS score of more than 7 was considered a high-quality study, a score of 6-7 was considered moderate quality, and a score of less than 6 was considered as low quality.

Statistical Analysis

Meta-analysis was performed using Medcalc. To obtain the value of the outcome measured during the time point (before Ramadan and during or after Ramadan) in mean and standard deviation of the standardized unit; mg/dl for leptin and ghrelin. If the study results were reported in different values, the results were converted accordingly to the standardized units before they were analyzed for the

funnel plot. The mean differences between the measured parameters before and after Ramadan fasting based on the sample size of each study were calculated using the Weighted Mean Difference (WMD) test, along with the 95% confidence interval. The heterogeneity of the studies was analyzed using the I^2 (square) test. I^2 of 0-30% was defined as low, 31%-60% as moderate, and 61-100% as considerable heterogeneity. Funnel plots were generated to assess the effect size of the included studies. Egger's test was also performed to test the plot asymmetry

In summary, the method used by the author is summarized in the PRISMA algorithm as follows.

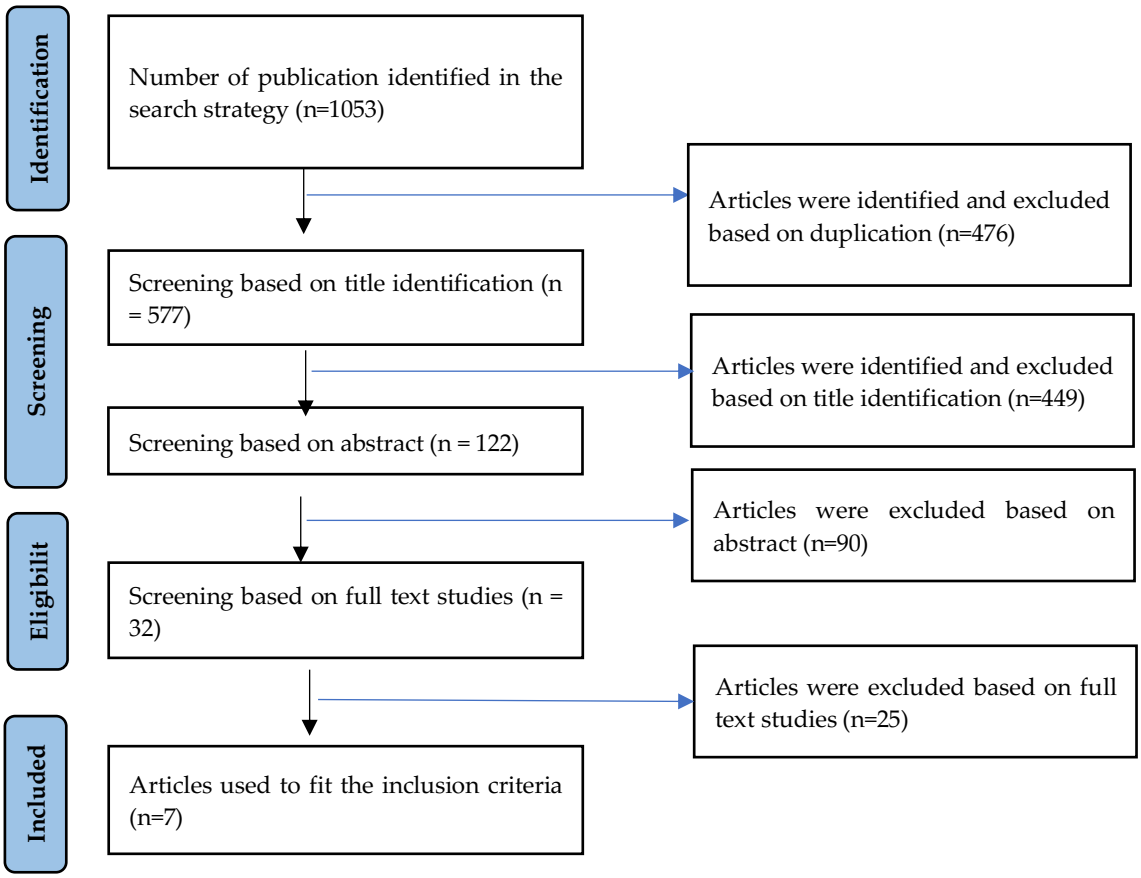


Figure 1. Systematic review of algorithm based on PRISMA. Page et al.[19]

Ramadan fasting is a type of IF that is carried out by all Muslims for one month by not eating and drinking during the day from dawn to sunset. Several health benefits have been ascribed to RIF, [9,13] including reduced blood pressure and body weight in individuals with obesity. [15] RIF has the potential to improve the secretion of peptide hormones in the gastrointestinal tract so that it can improve health.

3. Results and Discussion

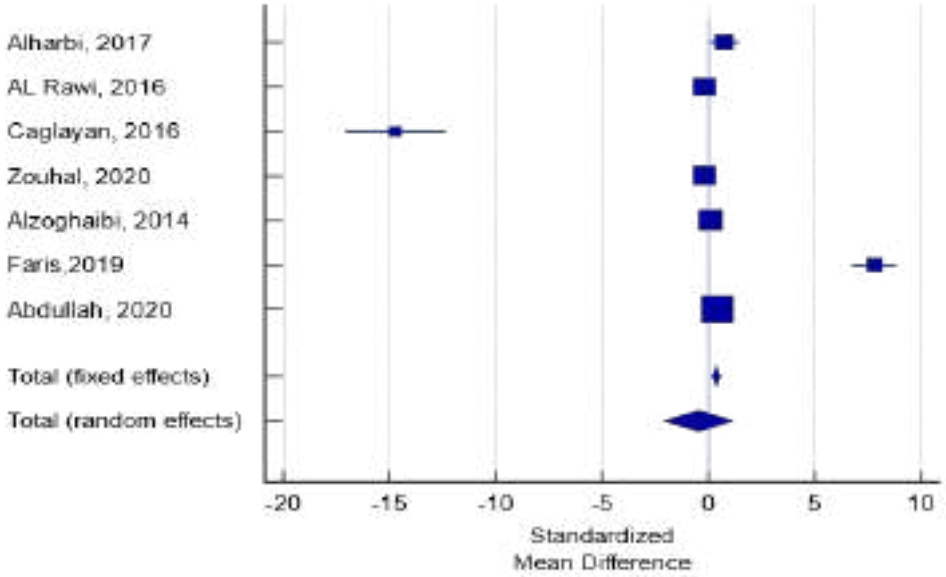
Table 1. Systematic review article results

No.	First author, years	Population (n) and country	Age	Fasting length	Sampling time	Results	Quality assessment
1.	Alharbi, 2017[20]	Overweight and obese type 2 DM patients (n=12) in Sydney Australia	31 ± 1 year	29-30 days	Before Ramadan and the End of Ramadan	Significant increase in ghrelin (p=0.045), and peptide YY (p=0.002).	High
2.	Al-Rawi, 2020[21]	Overweight and obese (n = 57) in the United Arab Emirates	38.4 ± 11.2 years	30 days	3 days before and End of Ramadan	Significant decrease in serum ghrelin and leptin (p<0.001). Melatonin changes are not significant	Moderate
3.	Caglayan, 2016[22]	Pregnant women (n=40) in Turkey	28.95 ± 6.21 years	30 days	Before and the 3rd week of Ramadan	Increased leptin and decreased adiponectin (p<0.001)	Low
4.	Zouhal, 2020[23]	Obese men (n=30) in Tunisia	24.2 ± 3.6 years	30 days	Before Ramadan and the 3rd week of Ramadan	There was a significant decrease in leptin, GLP-1, PYY, and CCK (p=0.004, 0.01, 0.004, and 0.001), but there was no significant change in ghrelin (p=0.08).	Moderate
5.	Alzoghaibi, 2014[24]	Healthy male(n=38) In the United Arab Emirates	26.6 ± 4.9 years	30 days	The week before Ramadan and the 4th week of Ramadan	Significant decrease in leptin (p=0.012), but no significant change in ghrelin (p=0.607)	Low
6.	Faris, 2019[25]	Overweight and obese (n=61) in the United Arab Emirates	36.2 ± 12.5 years	30 days	Before Ramadan and last week of Ramadan	Changes in appetite hormones in the form of a significant decrease in adiponectin and a significant increase in leptin (p<0.05)	Moderate
7.	Abdullah, 2020[26]	Men with Type 2 DM and their offspring (n=98) in Yemani	34.61 ± 4.31 years	30 days	Before Ramadan and the 4th week of Ramadan	There was a significant decrease in adiponectin and an increase in leptin (p<0.05).	Low

Table 2. Meta-analysis results

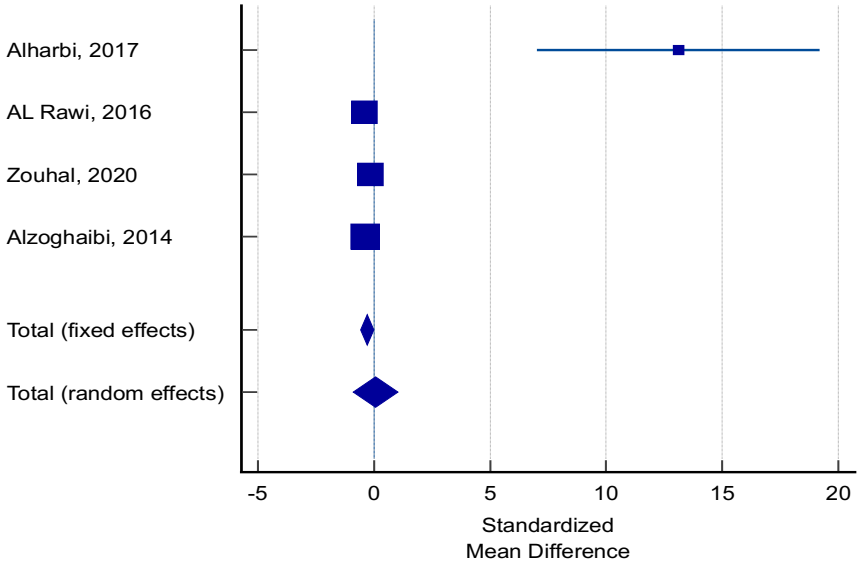
	I2	95% CI	p.s
Leptin	98.38	-2,069 to 1,163	<0.001*
Ghrelin	87.88%	-0.896 to 1.032	0.891

p* significance



Study	SMD	SE	Weight (%)
Alharbi, 2017	0.746	0.335	14.71
AL Rawi, 2016	-0.193	0.264	14.85
Caglayan, 2016	-14,723	1,185	11.49
Zouhal, 2020	-0.178	0.255	14.86
Alzoghaibi, 2014	0.0778	0.227	14.90
Farris, 2019	7,795	0.531	14.19
Abdullah, 2020	0.440	0.144	15.01
Total (fixed effects)	**0.372**	**0.0952**	**100.00**
Total (random effects)	**-0.453**	**0.823**	**100.00**

Figure 2. Leptin



	SMD	SE	95% CI	Weight (%)
Alharbi, 2017	13,113	2,731	7,029 to 19,197	2.94
AL Rawi, 2016	-0.416	0.266	-0.951 to 0.118	32.00
Zouhal, 2020	-0.168	0.255	-0.679 to 0.343	32.25
Alzoghaibi, 2014	-0.396	0.229	-0.853 to 0.0612	32.81
Total (fixed effects)	**-0.293**	**0.143**	**-0.576 to -0.00967**	**100.00**
Total (random effects)	**0.0679**	**0.489**	**-0.896 to 1.032**	**100.00**

Figure 3. Ghrelin

Peptide hormones in the digestive tract mediate food intake. Food intake is largely controlled by the hypothalamus, which integrates neural and hormonal signals of eating behavior, satiety, and calorie intake. [2] Several hormones that affect brain centers are synthesized and released from peripheral tissues, including intestinal and adipose tissue (adipocytes). The main hormones that regulate appetite and satiety are leptin, ghrelin, glucagon-like peptide-1 (GLP-1), tyrosine-tyrosine peptide (PYY), and cholecystokinin (CCK). [3,4] These hormones undergo changes after fasting during Ramadan.

Leptin, the 'satiety' hormone, is mainly produced by adipose tissue, signals from the hypothalamus that are passed on to inhibit hunger. Another appetite-regulating hormone is ghrelin, a 'hunger' hormone produced in the digestive tract, brain, and stomach secretions to stimulate appetite. [7] Obesity is accompanied by changes in the secretion of leptin, ghrelin, GLP-1, PYY, and CCK, which will be associated with accelerated weight gain.[12]

Several lifestyle changes that occur during Ramadan include meal times, sleep/wake schedules and sleep duration, light exposure, and physical activity or exercise as well as food composition [1,16–18]. These changes may affect plasma leptin and ghrelin levels. Previous research has shown important links between sleep and metabolic hormones including leptin and ghrelin. Researchers report that short sleep duration is associated with low leptin concentrations and high ghrelin. One study found that the plasma leptin concentration pattern showed a significant decrease at 22:00 and 02:00 during fasting and at 22:00 during the second week of Ramadan. [18] Moreover, changes in plasma leptin concentrations in this study were not associated with significant changes in plasma ghrelin concentrations. [21,27] A possible explanation for the differences in the results of this study may be that it did not control for behavioral habits such as eating habits and environmental conditions, including light exposure, which may have resulted in delayed shifts in the circadian rhythm cycle.[9]

In an experimental fasting study (fasting for 48 hours), reported an increase in leptin levels after breaking the evening fast. [28] These results differ from Bogdan et al.'s study, which reported no significant increase in nighttime leptin levels. [9]

Ghrelin is an endogenous ligand for the growth hormone receptor isolated from the stomach of humans and rats. Elevated plasma ghrelin levels before meals have been reported, which suggests a role for meal initiation. [14] In this systematic review, one study found a significant increase in serum ghrelin and PYY concentrations in obese women, but 4 other studies found no significant change in plasma ghrelin levels during intermittent Ramadan fasting. However, in the experimental fasting study by Natalucci et al, 6 volunteers fasted for 33 hours (from 00:00 on the 1st day to 00:90 on the 2nd day), and plasma ghrelin levels were at an average of every 20 minutes for 24 hours (from 08:00 on day 1 to 09:00 on day 2). The results reported a significant decrease in ghrelin levels over a 24-hour period.[29]

In another experimental fasting study, Chan et al asked six volunteers to fast for three days, and blood samples were collected every 15 minutes for 24 hours on day 3. Fasting for three days abolished the pattern of food-related ghrelin secretion. Leptin in physiological doses did not increase ghrelin levels compared to baseline in the same subjects. Other findings suggest that ghrelin levels do not increase during fasting and that decreased plasma leptin levels are not associated with increased ghrelin levels.[29]

The observed meal-related changes in ghrelin levels may be due primarily to a decrease in post-prandial ghrelin secretion, rather than a pre-prandial increase. Several studies have reported a lack of effect of combined intravenous glucose and insulin, hypoglycemia, or hyperglycemia on ghrelin secretion. [30,31] In addition, consumption of non-calorie fiber results in a decrease in ghrelin levels. Mechanisms other than food may be related to the control of ghrelin secretion. Natalucci et al demonstrated the characteristic pulsatility of ghrelin secretion and suggested that neural signals rather than gastrointestinal signals may modulate ghrelin secretion. [29] Differences between studies could also be due to regional variations in culture and/or eating habits [32], or longer duration of daytime fasting and longer duration of eating which may alter gastrointestinal responses and the body's nerve signals related to the secretion of the hormones leptin and ghrelin.[9]

The limitation of this research is that the samples taken in each study had different body mass indexes and, in some studies, fasting was accompanied by other interventions. In addition, the lack of a control group (for example, individuals who do not fast), can also cause considerable bias.

4. Conclusion

From the results of a literature review and meta-analysis of plasma leptin levels in several articles on fasting during and before Ramadan, it shows that there is a decrease in leptin after Ramadan fasting which is caused by changes in the amount of food and duration of eating during fasting. However, data analysis showed no change in ghrelin levels either during or before Ramadan. This may be due to changes in circadian rhythms during Ramadan.

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Literature Review

Role of magnesium in pregnancy

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Abstract: Magnesium is a cation that is useful in biological and cellular functions, such as protein synthesis, nucleotide metabolism, ATP production, neuromuscular transmission, increased vitamin D synthesis, and bone mineralization. Magnesium is important during pregnancy in formation of new tissues for both mother and fetus. Therecommended intake of magnesium during pregnancy is 350 mg/day. Approximately 48-60% of adults do not achieve the recommended average intake of magnesium. The needs for magnesium increase as gestational age while serum magnesium levelsdecrease during pregnancy. Hypomagnesemia in pregnancy is related to hemodilution,renal clearance, and consumption of minerals by the growing fetus. Magnesium deficiency in pregnancy is associated with adverse antenatal and perinatal outcomes. Hypomagnesemia causes neuromuscular hyperexcitability which results in uterine hyperactivity resulting in preterm labor. Administration of magnesium can increase glucose absorption and limit the accumulation of lactate in the skeletal muscles and reduce pain in leg cramps. Adequate magnesium intake in pregnant women has an important role in preventing leg cramps, preeclampsia, gestational diabetes, prematurebirth, IUGR, and low birth weight. Pregnant women must be educated to increase magnesium intake to prevent pregnancy and birth complications.

Keywords: leg cramps; magnesium; preeclampsia; pregnancy; premature

1. Introduction

Magnesium (Mg) is a cation that plays important role in more than 300 reactions involving metabolism. Magnesium required in various biological and cellular functions, such as protein synthesis, nucleotide metabolism, ATP production, neuromuscular transmission, vitamin D synthesis, and bone mineralization.[1] Magnesium deficiency can result from inadequate dietary magnesium, intestinal malabsorption, and increased excretion via the gastrointestinal or renal systems. The World Health Organization states that magnesium deficiency occurs in both developed and developing countries.[2]

In pregnancy, Mg required for the formation of new tissue, both mother and fetus. Therefore, pregnant women need higher intake of Mg than non-pregnant women of the same age.[3]

Physiologically, Mg serum levels decrease during pregnancy resulting in hypomagnesemia state. The hypomagnesemia in most pregnant women is related to hemodilution, renal clearance, and consumption of minerals by the growing fetuses.[4]

Evidence from several literatures shows that optimal Mg levels are very important for maternal and fetal health during pregnancy. Magnesium deficiency in pregnancy has been associated with adverse antenatal and perinatal outcomes. Severe magnesium deficiency can lead to leg cramps, preeclampsia, poor fetal growth, premature delivery and fetal death. Research shows that proper magnesium levels during pregnancy help keep the uterus from contracting prematurely, reduce fetal growth restriction, pre-eclampsia, and increase birth weight.[5] The aims of this literature review is to focus on the role of magnesium in pregnancy and the adverse effects of Mg deficiency in maternal, antenatal and perinatal outcome.

2. Magnesium

In Magnesium (Mg) is the most common metal ion involved in enzymatic function; acts as a cofactor in more than 300 enzymatic reactions and as an activator for others 200 enzymes. It is one of the essential minerals needed by humans in large quantities to regulate body temperature, synthesize nucleic acids and proteins, and maintain electrical potential in nerves and muscle membranes. It also known to have an important role in modulating vasomotor tone and cardiac excitability. In addition, Mg is useful in the utilization of glucose and the production of adenosine triphosphate, as well as bone formation.[6]

The adult body contains 25 g or 1,000 mmol of magnesium.[7] Almost 99% of magnesium in the body is stored intracellularly in bones, muscles and soft tissues, whereas 1% is found in serum.[8] Serum is the best possible way to measure Mg status, while it can only represent < 1% of total Mg level in our body, so generally no adequate measure to assess magnesium status. Normal serum magnesium levels are between 1.8 – 2.2 mg/dL, with levels below 1.8 mg/dL often referred to as hypomagnesemia.[1]

A normal diet should be enough to provide magnesium daily, but often it doesn't. Dietary surveys of people from Europe and the United States prove that the daily intake of magnesium is lower than the recommended dose.[6] This may be due to the purification and cooking of food which can cause the loss or reduction of Mg levels.[9] Magnesium is found in many foods such as nuts, seeds, fish, seafood, vegetables, legumes, berries, banana coffee and cocoa drinks. Even tap and bottled water can make a significant contribution to magnesium intake.[6]

Magnesium is absorbed in the small intestine by paracellular diffusion and transcellular active transport via TRPM6 and TRMP7. With normal magnesium intake, 30% of intestinal absorption of magnesium occurs by transcellular transport. When magnesium intake is lower, more magnesium is absorbed via transcellular transport. When magnesium intake is higher, more magnesium is absorbed

by paracellular diffusion. Magnesium is transported in the blood as free Mg2+ (60%), protein bound (30%), and complexed to citrate, phosphate, or sulfate (10%).[8] Magnesium homeostasis is regulated by the kidney, small intestine, and bones.[7] Approximately 70% of serum magnesium is filtered by the glomerulus, and 96% of filtered magnesium is reabsorbed in the kidney through several mechanisms in the proximal tubule, ascending tubule, and distal tubule, then the remainder is excreted in the urine.[8]

3. Magnesium in pregnant women

Approximately 48-60% of adults do not reach the average recommended daily intake of magnesium, and 15-42% of people who appear healthy have evidence of magnesium deficiency. Magnesium deficiency is more common in women than men. This may be partly influenced by the tissue-stimulating hormone estrogen in magnesium utilization, so that hormonal rhythms in women influence and modulate magnesium status.[10]

Magnesium has an important role in the body's homeostasis, especially during pregnancy. It is responsible for the formation of new tissue, both for mother and fetuses.[3] Women are advised to consume as much as 280 mg of magnesium/day, but the need increases during pregnancy.[11] The recommended intake of magnesium during pregnancy is 350 mg/day to maintain the function of more than 300 enzymes that utilize ATP. A review of six studies conducted in the United States found that Mg intake during pregnancy ranged from 30 – 50% of the recommended daily intake of 355 mg/day.[9]

Magnesium is also used as a tocolytic in preterm labor. Magnesium stimulates calcium reuptake by the sarcoplasmic reticulum, which promotes muscle relaxation and vasodilation. It also works in uterine smooth muscles. Therefore, it reduces uterine contraction by blocking calcium which can lead to cessation of labour progression.[1]

Physiologically, serum magnesium levels decrease during pregnancy. Magnesium levels decrease with increasing gestational age.[3] Deficiency of magnesium in pregnant women is mostly related to inadequate intake of magnesium, increased requirements, increased renal clearance and physiological hemodilution.[12] Research at Sree Balaji Medical College and Hospital Chennai showed that as many as 57% of pregnant women experienced a decrease in serum magnesium levels and a decrease in magnesium levels had a statistically significant correlation with a p-value <0.0001.[5] Early signs of magnesium deficiency in pregnancy are leg cramps, back pain, constipation, muscle tension, insomnia, migraines and headaches.[2] Deficiency of magnesium during pregnancy will affect the occurrence of pregnancy complications such as leg cramps, preeclampsia, gestational diabetes, premature birth, low birth weight (LBW), and intrauterine growth retardation (IUGR).

Evidence from the literature indicates that optimal magnesium levels are critical for maternal and fetal health during pregnancy. Magnesium supplementation during pregnancy can improve the quality of pregnancy and the fetus by reducing the risk of preterm labor, IUGR, prevention and management of seizures in preeclampsia and eclampsia in pregnancy, and reduce the incidence of maternal and newborn care in hospital.[13]

4. Magnesium and leg cramps

Leg cramps are sudden and involuntary contractions of skeletal muscles accompanied by pain and often involve the gastrocnemius muscles. In most cases, muscle cramps last from seconds to minutes and often occur at night. In severe cases, muscle cramps can disrupt daily activities, disrupt sleep, and reduce quality of life. About 30-50% of pregnant women experience leg cramps in the second and third trimesters of pregnancy. The etiology and mechanism of leg cramps during pregnancy is still unclear, but mostly related to lack of activity or excessive exercise, metabolic disorders, changes in blood circulation, nutritional deficiencies (vitamins E and D) or electrolyte imbalances (magnesium, calcium and sodium). [14,15]

Magnesium plays an important role in regulating muscle function. When serum magnesium is reduced, the axonal stimulation threshold decreases and the nerve conduction velocity increases, resulting in increased muscle and nerve excitability. The results of experimental research show that giving magnesium can increase glucose absorption and limit the accumulation of lactate in skeletal muscles so that the feeling of pain during muscle cramps is reduced.[16] This is supported by the research of Barna, et al. which stated that there was a decrease in the frequency ($p<0.001$) and duration ($p<0.007$) of leg cramps after being given magnesium supplementation with 226 mg magnesium oxide monohydrate (MOMH) daily for 60 days in pregnant women compared to the placebo group.[16]

In addition, there are still many conflicting studies regarding the effectiveness of magnesium supplementation for leg cramps. Research by De Araujo, et al. stated that there was a decrease in the incidence of leg cramps by 28.4% in both the magnesium supplementation group (300 mg/day of oral Magnesium citrate) and the placebo group; and there was no significant reduction in the frequency and duration of muscle cramps ($p = 0.408$) in supplementation group.[14] This is also supported by the research of Liu, et al who said that magnesium supplementation was not effective in reducing leg cramps during pregnancy ($p = 0.094$).[17]

5. Magnesium and preeclampsia

Pre-eclampsia is a state of hypertension (systolic and diastolic blood pressure ≥ 140 and 90 mmHg in pregnant women, on two measurements, with urine protein ≥ 300 mg in a 24-hour urine sample, or

dipstick ≥ 2+), this condition occurs after 20 weeks gestation. [11] This disease encompasses 2 to 8% of pregnancy-related complications, greater than 50,000 maternal deaths, and over 500,000 fetal deaths worldwide. Studies shows that low magnesium levels in pregnant women can increase the risk of preeclampsia in the mother.

Magnesium is an intracellular ion that is important for cellular metabolism such as muscle contractility and neuronal activity. A good balance between magnesium and calcium is essential for regulating blood pressure, while calcium is needed for blood vessels to contract, magnesium is needed for blood vessels to relax those maintaining the homeostatic of muscles throughout the body. Magnesium acts as a calcium channel blocker thereby preventing the increase in intracellular calcium concentration which causes vasodilation. The vasodilating effect of magnesium, apart from increasing blood flow, has been shown to prevent pre-eclampsia/eclampsia by selectively dilating cerebral blood vessels and relieving the cerebral spasms associated with pre-eclampsia.[11]

Research by Atiba, et al. found that 27 out of 28 patients with severe systolic hypertension (≥160 mmHg) had low serum magnesium levels (<0.63 mmol/l) and all nine patients with mild systolic hypertension (140 - 159 mmHg) had low serum magnesium levels, although this was not statistically significant ($P > 0.05$).[18] These results are also supported by the research of Yulia, et al. that magnesium supplementation 365 mg/ day during pregnancy starting at 22 weeks to 36 weeks in pregnant women with hypomagnesemia helps prevent preeclampsia 6.51 times.[19]

6. Magnesium and gestasional diabetes

Low magnesium levels in the mother can also be associated with the incidence of gestational diabetes. This is supported by a meta-analytic study by Ren, et al. covering 17 studies and involving 2858 participants with 1404 cases of GDM and 1454 control cases that magnesium levels were significantly lower in GDM compared to control cases (SMD: − 0.35; 95% CI: − 0.62, − 0.07, $P = 0.013$).[20] Similar research was also conducted by Qu, et al. that magnesium supplementation resulted in significant reductions in glycemic control markers—fasting plasma glucose ($p<0.0001$) and insulin levels ($p<0.0001$).[21]

In addition, there is conflicting evidence regarding the effectiveness of magnesium supplementation during pregnancy in patients with gestational diabetes mellitus (GDM), so further research is needed to explain the possible benefits of preventing gestational diabetes through magnesium supplementation. Although there are still few studies regarding Mg in GDM, Mg supplementation during pregnancy is suggested to improve maternal and neonatal outcomes because of the beneficial effects observed in diabetic patients following oral Mg intake.[20,21]

7. Magnesium and Perinatal Outcomes

Magnesium deficiency status during pregnancy can interfere with the growth and development of the fetus, and trigger preterm labor.[22] Preterm labor is a state of regular uterine contractions and cervical changes in women with intact fetal membranes at <37 weeks gestation. Hypomagnesemia causes neuromuscular hyperexcitability which results in uterine hyperactivity resulting in preterm labor.[6]

Research by Atiba, et al. stated that there was a statistically significant relationship between preterm birth ($p = 0.001$) and low birth weight ($p = 0.002$) in infants of mothers with low serum magnesium. [18] This is also supported by the research of Anand, et al. that 66% of patients who experienced preterm labor had low magnesium levels (1.1 mg/dL) with a statistically significant difference ($p<0.0001$).[3] Similar results were also carried out by Citu, et al. that the proportion of preterm births was significantly higher in the group of pregnant women with COVID-19 who did not take magnesium supplements compared to those who took magnesium supplements (p-value = 0.038). As a result of the higher incidence of preterm birth in the group of patients without nutritional supplements, birth weight and APGAR scores were also significantly lower than newborns of mothers taking magnesium-based supplements.[22]

The hypothesis that magnesium deficiency may be the basis of IUGR has been confirmed in a study aimed at correlating maternal umbilical vein and peripheral blood magnesium levels with fetal weight at birth. In that study, infants born to pregnant women supplemented with magnesium sulfate (25% magnesium sulfate 20 ml in 5% glucose 500 ml i.v.) were found to have significantly higher birth weights than non-supplemented women with lower serum magnesium levels.[6]

8. Conclusion

Magnesium has an important role in the body's homeostasis, especially during pregnancy. During pregnancy, magnesium levels decrease physiologically, while the need for magnesium increases during pregnancy for the formation of new tissues in the mother and fetus. The recommended intake of magnesium during pregnancy is 350 mg/day. Adequate magnesium intake in pregnant women plays an important role in preventing leg cramps, preeclampsia, gestational diabetes, premature birth, IUGR, and LBW. Therefore, pregnant women should be educated to increase their intake of magnesium such as nuts, seeds, green vegetables, or magnesium supplements to prevent pregnancy and birth complications.

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Literature Review

The role of physiologists in supporting a healthy society

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Abstract: Physiologists have the knowledge, skills and attitudes necessary to support the continued growth of a healthy society. Research expertise allows physiologists to expand the knowledge base and evaluate the scientific studies that must inform health recommendations. Physiologists are also trained to communicate scientific findings to diverse audiences, including partnering with educators at all levels. Health literacy requires that individuals at all age levels better understand how their body functions and use this understanding to incorporate changes in lifestyle that promote health.

Keywords: eco-health; healthy society; physiologist;

1. Introduction

The intersection of ecological sciences and health sciences profoundly impacts our world. This intersection can best be understood by contrasting the impacts at the level of an individual person with broader societal impacts. In both cases, Physiologists are uniquely positioned to help shape these impacts because of our focus on the function of the healthy body and through research to guide evidence-based recommendations for society.

2. Eco-health for the individual

Individual wellness reflects the balance among genetics, lifestyle choices and knowledge access. For an individual the genetic book is fixed and encoded in DNA. Individuals can partially edit the book through epigenetic modifications and lifestyle choices have input into which pages from this book are read.[1] Physiologists have already generated a research base to better understand the impacts of nutrition and exercise on individual health.[2] This research base needs to be expanded to include sex and gender influences as well as differences across populations.[3,4] To be impactful, this research base must be clearly communicated.[5] Physiologists must collaborate with other educators to expand the access individuals to this knowledge, particularly marginalized individuals.

Technology has dramatically enhanced the ability of an individual to access detailed information about their individual physiology. One product developed in Finland is the “Oura” ring (Oura.com). This biosensor tracks activity, heart rate, oxygen saturation, and stages of sleep, allowing an individual

to make lifestyle choices based on their health goals. Another product, the Garmin Vivosmart 5 biosensor (Garmin.com), provides similar information in a watch platform, integrating information to provide a measure of individual stress and wellness. Individuals can make data-driven decisions about their wellness with accurate and useful physiological information.[6,7]

Individuals also can use commercially available assays to track reproductive hormone levels at home. The first of these was an immunoassay to detect the hormone human chorionic gonadotropin (HCG) in the urine as a marker for pregnancy, now using colorimetric indicators to allow easier interpretation of results.[8] Home assays have expanded to monitoring luteinizing hormone (LH), tracking the LH surge as an indicator of impending ovulation.[9] This approach allows an individual to identify the time of peak fertility and the greatest chance of conception.

Access to data about their individual physiology is likely to expand. Physiologists need to support this trend, using research to both validate and expand available data. Physiologists also need to partner with other education and health professionals to promote health literacy to assist individuals in making informed decisions about their lifestyle.

3. Eco-health for society

Trends impacting medical education over the past 4 decades are similarly playing out in promoting health. In the United States in the 1950s, medical care focused on acute health problems. The clinical interaction involved one physician and one patient, often in the clinic or private practice office. By 2020, medical care shifted to long-term interactions and goals. Interactions are now more often with a healthcare team and the outcome is focused on population health. Healthcare interactions now more commonly occur in community settings. Part of this is the shift in medicine from one of disease management to a stance of promoting health.[10]

Healthcare in society faces significant challenges from lifestyle choices, from the aging of the population, and from climate degradation. Physiologists must play a significant role in each of these arenas.

The keyword “lifestyle” appeared in over 45,000 review articles since 2019 (Google Scholar, August 30, 2023). Physiologists have a skill set in research, dissemination, and role modeling that allows them to be scientifically credible partners with existing programs promoting a healthy lifestyle, and a research focus allowing them to expand the evidence-base needed to generate scientifically valid recommendations.

Health across the lifespan is an even more active area of interest. A Google search for the keyword “aging” produced 1.3 billion hits in August 2023, a number which has doubled in the past two years. “Antiaging” alone produced 169 million hits over the same period, and Google Scholar shows 47,000

manuscript results. In the United States the NIH National Institute on Aging had a budget of $4.2 billion in 2022.[11] Much like lifestyle, the research focus of physiology provides a needed skill set to partner with existing problems and expand the evidence base.

Society is facing challenges as climate change alters the world in which we live. In "Climate Change and Health 2021", the World Health Organization asserts that climate change affects the social and environmental determinants of health, including clean-air, safe drinking water, sufficient food, and secure shelter.[12] Areas with weak health infrastructure – mostly in developing countries – will be the least able to cope without assistance to prepare and respond. Climate change represents a major challenge for healthcare professionals in that we will not be able to "treat" our way out of the increased disease burden resulting from climate change. Unlike lifestyle and aging, the science supporting environmental accountability lies outside of the normal science of physiology. In spite of this, physiologists as members of society have an obligation to work to attenuate and reverse the causes of climate change, and also to mitigate when possible the impact of climate change.

4. Conclusion

Physiologists must play a significant role in supporting a healthy society. Physiologists have the essential skills to accomplish this task. Research allows physiologists to expand the knowledge fund and help identify those interventions that truly impact health and wellness. Partnering with educators, physiologists need to help develop review and refine teaching tools that allow individuals at all age levels to better understand how their body functions. This understanding is essential to incorporate changes in lifestyle that promote health. Finally, physiologists by virtue of our understanding of body function have an obligation to help shape social and political discourse on topics of health and wellness.

5. Acknowledgments

This manuscript describes commercial fitness products. The authors have no research or commercial ties to these companies and have no conflict of interest to declare.

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Literature Review

Empowerment of healthy lifestyle in community 'malaysian experience'

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Abstract : The pursuit of a healthy lifestyle confronts challenges such as aging, dietary habits, and living conditions. To tackle these obstacles, community empowerment emerges as a crucial approach, granting influence and fostering improved health outcomes by nurturing opportunities, skills, and resources within communities. Community-based initiatives (CBI), like food banks and community centers, play a pivotal role in enhancing quality of life through small-scale projects tailored to specific areas. This holistic approach integrates health into community development, emphasizing proactive community roles, solidarity, and empowerment for sustainable progress. Social mobilization amplifies community development through awareness building and organizational empowerment. Sensitization and orientation are pivotal for communities to grasp their local context and how CBIs can effectively address their needs. Furthermore, motivation and social preparation serve as essential elements in fostering healthy lifestyles. Organizational development empowers communities by fostering organization, network building, and capacity enhancement, promoting democratic principles and collaborative efforts across diverse sectors for effective community development. The Communication for Behavioural Impact (COMBI) program serves as a vehicle for sustained communication and social mobilization, notably demonstrated in managing dengue outbreaks in Malaysia. This program employs integrated communication strategies, encompassing administrative mobilization, community engagement, advertising, interpersonal communication, and point-of-service promotion to combat dengue. Community empowerment necessitates dedicated coordination, comprehensive technical and moral backing, effective leadership and training, sustained financial support, acknowledgment, clear communication, and collaboration across multiple sectors.

Keywords: community empowerment; healthy lifestyle

1. Introduction

Healthy lifestyle adopted are impeded by multiple factors, included healthy aging, heathy diet, dan healthy living.[1] It depends on so many covariates, sometimes intangible and difficult to control. One of the efforts to improve a healthy lifestyle is through improvement of the community, it called Community empowerment.[2]

Power refers to unequal relationships among people. It is a ability of someone to influence the behavior of somebody else. Who state empowerment lead better health outcomes.[3] It is tool of effectiveness of empowerment to improve health and reduce health disparities. It help community and

individuals develop opportunities, capacities, and tools that benefit them. It ensure communities mobilize targeted populations to obtain needed health resources by fostering awareness of a common problem.[4]

One of the method to maintain healthy lifestyle is community based initiative (CBI). Community empowerment needs CBI to involve small scale projects/programs and benefit a specific geographic area. This is often used in public health and has been done in Singapore. Examples of such economic empowerment efforts include food banks, cooperative farms, and community centers, all which are aimed at providing direct assistances to local individuals and families.[5] The components in CBI aim to achieve health for all through improving quality of life. More holistic approaches to development, health is an integral part of the development process. This is an objective that should be pursued using all available means and influenced by all aspects of society. The communities proactive role, solidarity, and empowerment are the keys to sustainable development and addressing their needs.[6]

2. Social mobilization

Social mobilization is tool for enabling people to organize themselves for collective action, by pooling resources and building solidarity. Genuine participation of the community is essential for its development and sustainability.

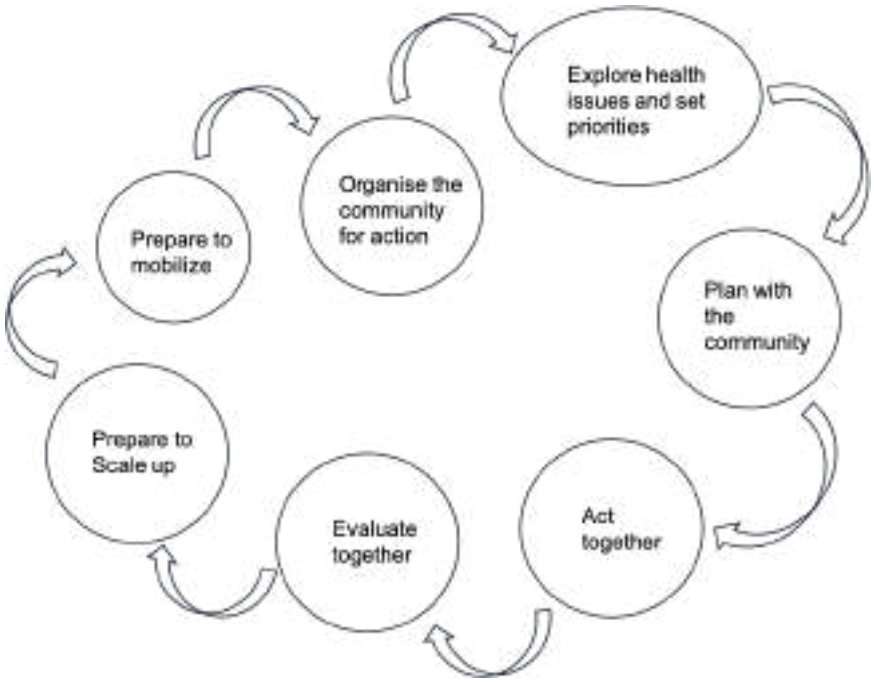

Figure 1. Social mobilization framework

3. Sensitization, orientation and development of awareness

In healthy lifestyle communities, it should be informed about local situation and how the CBI

approach can assist in solving the problems. This approach improve awareness regarding society's need, rights, potentials and resources as well, as their roles in the society where they live and work. Repeated interaction and communication with community members will sensitize people and generate interest.[5]

Heathy lifestyle also need motivation and social preparation. Motivation is a stimulus and response process that involves the inducements of the people to contribute effectively and efficiently towards the assigned tasks. Socially prepare the people for new roles and the implementation of unconventional approaches.[7]

4. Organizational development

The community helped to become organized, develop networks to formulate collective and coordinated actions. People work on democratic principles, based on the roles agreed by all members. An inclusive approach adopted by involving all or most community members in the social mobilization. It promote equal opportunities and prevent conflict. The capacity building ant training of the community leadership is a part of their empowerment to take up a new role, manage the program. Community encouraged to maximize their potentials. Community upgrade their existing skills and knowledge.[8] People from different sectors, various levels of society should be engaged in dialogue negotiation for collective and collaborative action. The various intersectoral departments, organizations, stakeholders, opinion makers and political leadership should be mobilized to foster partnerships. Collaborative action towards the goal of community development. Continuous communication, sharing of information about the developments in the programmed area will be vital for advocacy and social mobilization. Motivational incentives, material support essential to create interest among community members. Technical support continuous process to make the change effective and sustainable. The creation of capital through mobilization of community savings. Profit sharing and contributions (e.g. a community development fund) will facilitate the functions of the community organization and enhance its powers to realize its full potential. The community can only appreciate and own the actions if they are on an agreed track which is of benefit to them. Socioeconomic development initiatives are a great incentive for community members to organize themselves.[9]

5. Communication for behavioural impact (COMBI)

World Health Organization (WHO) promoted Communication for Behavioural Impact (COMBI) as a methodology for planning sustained actions in communication and social mobilization. The major issues are not the effectiveness of COMBI but how to achieve long-term sustainability**.** Based on behavioural change, communication and marketing theories. A comprehensive and flexible approach

in designing, implementing and monitoring social and communication mobilization actions that are modified according to the objectives of the chosen behaviour.[10]

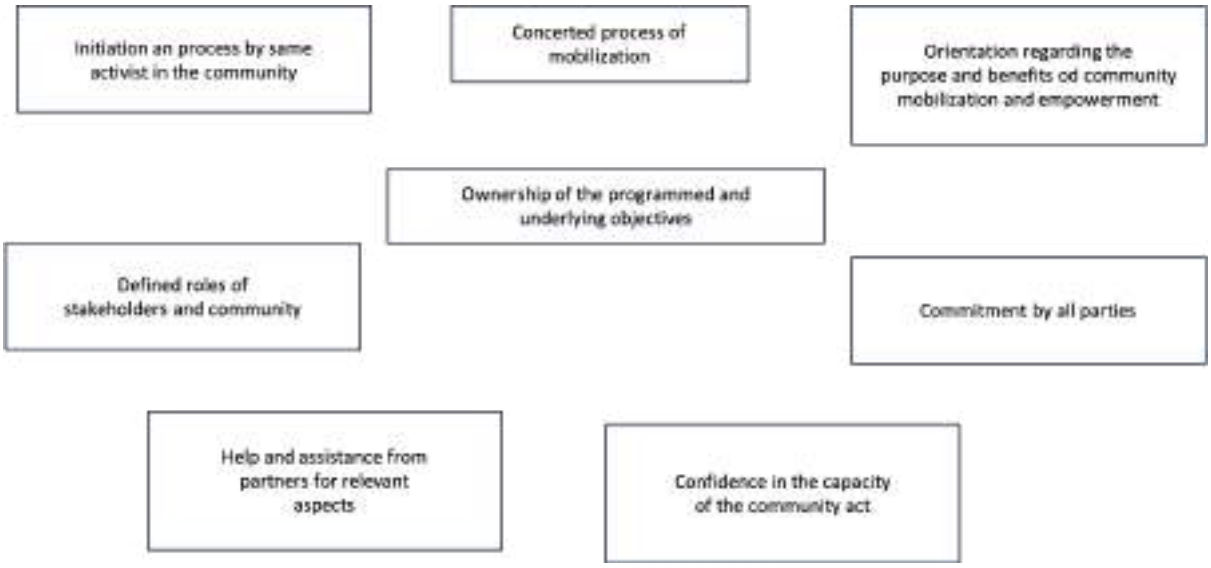

Figure 2. Community participation an empowerment framework

In 2000, World Health Organization (WHO) promoted Communication for behavioural Impact (COMBI) as a methodology for planning sustained actions in communications and social mobilization. In 2001, COMBI was piloted in Johor Bahru District, Johore State, Malaysia with assistance from the World Health Organization (WHO)**.** Result from Johor Bahru pilot project proved COMBI positively contributed towards behavioural outcomes

To implement the COMBI program, five (5) basic principles that use integrated communication strategies should be practices;

1. Administrative Mobilization/Public Relation/Advocacy
2. Community Mobilization
3. Advertising
4. Personal Presentation/Interpersonal Communication
5. Point-of-Service Promotion

6. COMBI programmed

Huge numbers of dengue cases in Malaysia has been concerning and lead to programs to manage dengue with community approach. This program called COMBI with activities such as Radio talk, Meetings with political influence people, committees meetings, "gotong royong" or communal work culture especially for environment cleansing project, carnival, walkabout COMBI in various events,

COMBI convention. The aim is to alert community about dengue, on where to look for larva breeding sites-search and destroy it, recycling of waste, how to store water and breeding of larvae predators. Target locality included repeated outbreaks area, uncontrolled outbreak or outbreak locality, locality with cases before, Locality with high Entomological Index, Locality that voluntarily establishes a COMBI project. In the rural area, on which the citizens are closer to each other this program is more likely to succeed with their "gotong royong" cultures compared to the urban area.[11]

There are still issues related to COMBI in Malaysia which is became obstacles that we need to adjust. COMBI chairman is appointed by health department/local council/automatic appointment by virtue of Village Development and Safety Committee (VDSC) leadership. Influential leaders in the community. Factors deterring a strong leadership of the chairman are migration, various portfolios, and opposing political view. COMBI coordinators are assistant environment Health Officers from Vector Unit/ District Health Education Officer (for technical and moral support).[12] Factors deterring a strong leadership of COMBI coordinators is they are overwhelmed with daily routine duties. Funding are some allocation from health departments/ allocation for programs which is unspecific and became factors regarding COMBI. COMBI committee was not well structured with fewer human resources because some were established or activated during outbreaks only. Only given a T-shirt, vest, cap, bag, or uniform and certificate of appreciation makes this program lack of recognition. Lack of the skills to empower the community form the leader or chairman also can be issues. Challenge in sustainability including lack of self-funding skills, quality leadership, partnership effort, a sense of ownership, and changes in the political structure. [11],[12] New technologies to combat dengue include dengue vaccines and Wolbachia mosquitoes need further works and research.

7. Conclusion

Community empowerment needs highly committed coordination, technical and moral support, leadership and training, financial sustain, recognition, good communication and multisectoral collaboration.

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Research Article

The effect of high intensity interval training on the prevention of skeletal muscle damage viewed from the role of P62 in rats with type-2 diabetes mellitus

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Abstract: Type-2 Diabetes Mellitus (Type-2 DM) is a chronic disease characterized by insulin resistance.One of the complications of type-2 DM is metabolic disorders in skeletal muscles. This disorderis characterized by excessive catabolic processes of skeletal muscles. The process of muscle catabolism includes a degradation process that has 2 regulatory systems, namely ubiquitin proteosomes and autophagy. The difference between these two mechanisms lies in the way and location of managing the damaged protein. Autophagy is a cell recycling process that occurs in lysosomes. One of the proteins that plays an important role in the process of autophagy is p62. P62 is a multifunctional protein and also acts as a cargo transporter. How the expressionof p62 on muscle damage in DM sufferers is unclear. To overcome type-2 DM, there is an intervention that is quite popular and much in demand, namely High Intensity Interval Training (HIIT). This workout is a workout that combines high-intensity exercise with alternating rest periods. There have been many studies that prove HIIT can activate regulation of the skeletalmuscle synthesis pathway so that it experiences repairs to skeletal muscle cell damage. However, the degradation path still needs to be studied further. Because researchers want to prove how HIIT affects the repair of skeletal muscle damage due to type-2 DM. In this case, what is investigated is how the expression of p62. The HIIT intervention was carried out for 6days per week with a total of 6 weeks of exercise in type-2 DM rat models. After exercise, the gastrocnemius muscle tissue is incised and then muscle mass is analyzed by weighing the tissueand using the western blot method to see p62 expression. The results of the analysis of musclemass data in the HIIT group tended to increase compared to the DM group, although it was not significant. The western blot analysis showed that the HIIT-treated DM group reduced p62 expression, although not significantly.

Keywords: high intensity interval training; p62; skeletal muscle; type-2 diabetes mellitus

1. Introduction

Diabetes Mellitus (DM) is a chronic metabolic disease that has become one of the biggest epidemics in the world of health.[1] In 2017, the Global Burden of Disease (GBD) estimates that around 465 million people worldwide suffer from diabetes mellitus and more than 1 million deaths are associated with DM.[2] In the same year, the International Diabetic Federation (IDF) estimated that 1 in 11 adults aged

20-79 years suffered from DM.[3] With this high prevalence rate, researchers and paramedics throughout the world continue to innovate in preventive and curative efforts to overcome the surge in cases. DM. The World Health Organization (WHO) defines Diabetes Mellitus as a disease characterized by increased glucose or called hyperglycemia which can be accompanied by damage to the heart, blood vessels, eyes, kidneys and nerves.[4] In type-1 DM there is an increase in blood sugar expression due to damage to pancreatic beta cells so that insulin production is reduced or absent altogether.[5] Meanwhile, type-2 DM is characterized by insulin resistance. Insulin resistance can occur due to disorders of carbohydrate, protein and fat metabolism.[6] Type-2 DM is the most common case in the world and is suffered by various groups from young people, adults to the elderly. [4] Complications that occur in type-2 DM can be are acute and chronic.[7] At the tissue level, type-2 diabetes not only causes damage to the epidermis but can also reduce skeletal muscle mass.[8] Skeletal muscle is known as the largest tissue in the body, and is the site of energy metabolism related to glucose and insulin.[9] In cases of insulin resistance, protein synthesis and degradation in muscle will also be disturbed, resulting in a decrease in muscle fiber size or excessive atrophy.[10] Changes in skeletal muscle mass can be proven by measuring muscle mass scales, histological examination by examining the size and number of muscle fibers.

The pathophysiology of muscle atrophy, especially in type-2 DM, is not fully understood.[11] In normal conditions, there are various cellular and molecular complexes that play a role in muscle anabolism and catabolism pathways.[12] The interaction between hormones, proteins and transcription factors is very important in the growth of muscle mass.[13] Induction of the insulin hormone has an important influence on energy burning and protein turnover in skeletal muscle. In this case, there are two systems that play a role in muscle protein turnover, namely the ubiquitin proteosome (UPS) and autophagy.

The autophagic muscle degradation system is responsible for removing cellular components such as protein aggregates and organelles that are excessive and damaged in muscle cells.[14] The results of protein degradation are encapsulated in autophagosomes and then conjugated into active forms LC3B-I, LC3B-II and p62.[15] P62 or SQSTM1 is a protein that is used as a center for integration of various functions, namely the formation of autophagosomes, delivery of ubiquitin to proteasomes, and formation of aggregates for the elimination of autophagy.[16] P62 acts as a receptor or reporter of autophagy activity and is also known as a protein that regulates mitochondrial biogenesis.[17] At the subcellular level, p62 is found not only in the cytoplasm, but also in the nucleus, mitochondria, autophagosomes, and lysosomes.[15]

Measured and controlled exercises are effective ways that are known to maintain skeletal muscle mass. Exercise is known to have an influence on changes in skeletal muscle mass and strength.[18] The exercise that can be used as an alternative and is currently popular is High Intensity Interval Training (HIIT).[19] HIIT training generally combines high intensity training with interspersed rest periods accompanied by changes in training intensity.[20] HIIT training is proven to improve aerobic and anaerobic fitness, increase insulin sensitivity, decrease cholesterol expression, abdominal fat, body weight and maintain muscle mass.[19] HIIT can be easily modified and can be done in all types of exercise including cycling, walking, swimming, etc.[19] HIIT training can induce the process of protein synthesis due to stimulation of muscle fibers as a site for anabolic signaling. In catabolic pathways, the effects of HIIT can be seen on biomarkers of muscle degradation. There have been many studies proving the effect of HIIT on various muscle metabolic signaling pathways. In the degradation pathway, namely in the autophagy process, HIIT is known to control excessive p62 expression, but there are different opinions regarding the accumulation of p62 in muscles under atrophic conditions.[15] In conditions of type-2 DM, there have not been many studies related to HIIT that have looked at P62 protein expression as a specific biomarker in muscles experiencing degradation damage. Therefore, this study wants to further analyze the effect of HIIT on the muscle protein degradation pathway which will be proven by observing the P62 marker on damage to skeletal muscle mass degradation in Type-2 Diabetes Mellitus mice after being given HIIT treatment.

Mechanism of Skeletal Muscle Degradation In Type-2 DM

Insulin resistance due to metabolic disorders in Type-2 DM and obesity are the main contributors to muscle atrophy signaling. The specific activity of Akt kinase in response to insulin was reduced by 34% in type-2 DM patients compared with healthy patients. Decreased PI3K-Akt pathway was implicated in reduced insulin-mediated glucose uptake and protein synthesis in experiments with mice and type-2 DM patients. In addition, there is a main regulator of the main synthesis, namely the mammalian target of rapamycin (mTOR), which is activated by Akt through insulin and insulin-like growth factor 1 (IGF-1) and mechanical stimulation. However, the Akt-mTOR pathway also interacts with the ubiquitin-proteosome and autophagy-lysosomal pathways. With reduced Akt activity, it will decrease the phosphorylation of the FoxO transcription factor which leads to nuclear translocation and further increases the transcription of Murf-1 and Atrogin-1. It is known that in obesity and DM in rats there is muscle atrophy and increased muscle degradation up to 43% accompanied by insulin resistance.[11]

Definition of P62

The P62 protein (also known as Sequestosome1/SQTSTM1) is present throughout eukaryotes and is associated with the main process of autophagy. At the subcellular level, p62 is found not only in the cytoplasm but also in the nucleus, mitochondria, autophagosomes and lysosomes. P62 is expressed in all tissues in the body. P62 is known as an autophagy substrate that is used as a receptor or reporter of autophagy activity. P62 also functions to bind cargo to deliver proteins to be degraded to the proteasome. Additionally, P62 can shuttle between the nucleus and cytoplasm to bind cargo and facilitate quality control of nuclear and cytosolic proteins. In humans, P62 is located on chromosome 5 and consists of eight exons spanning 16 kb.[21]

The P62 protein is a signaling adapter that contains the N-terminal Phox1 and Bem1p (PB1), zinc finger type -Z domain (ZZ), nuclear localization signal (NLS), TRAF6 binding domain (TB), nuclear export signal (NES), LC3- interacting region (LIR), Keap1-interacting region (KIR) and C-terminal ubiquitin-associated domain (UBA). 16. P62 is not only degraded by proteasomes or endosomal autophagy, but also degraded through selective autophagy. There are several studies that use P62 level analysis as a general method for monitoring autophagic flux. The function of p62 as SQSTM1 is also to decipher patterns and map post-translational modifications. P62 delivers the protein cargo for autophagic degradation via the C-terminal UBA domain or the LIR domain, and then the PB1 domain promotes the process. In some cell types, Expressed p62 increases protein aggregation and has a protective effect on cell survival. In addition, deletion of p62 causes disruption of LC3-II formation, interferes with the autophagosome mechanism, exacerbates cell injury and decreases cell viability under conditions of basal and stressed proteins that fail to fold in cardiomyocytes.[22]

Definition of Physical Training (HIIT)

Systematic and continuous physical exercise carried out in an exercise program will significantly increase an individual's physical abilities. Meanwhile, a person's physical abilities will decrease if exercise is not done regularly. In addition, exercises that are carried out regularly and continuously with sufficient intensity and for a certain period of time will cause changes in physiology. The process of contraction in muscles occurs due to stimulation causing the activation of actin filaments and myosin filaments. The faster the stimulus received and the faster the reaction given by the two filaments, the faster the muscle contraction will be. so that the explosive power generated due to the combination of speed and power becomes greater. The effect that occurs as a result of training with a gradual increase in load is an increase in the percentage of muscle mass so that hypertrophy increases by 30-60%. Meanwhile, speed training will cause fast-twitch muscle fibers to experience hypertrophy, the

occurrence of hypertrophy is caused by changes in skeletal muscle, an increase in the number of actin and myosin filaments in each muscle fiber, causing enlargement of each muscle. By increasing the number and size of mitochondria in muscle cells, the function of the mitochondria will be more effective. Furthermore, there is an increase in the number of mitochondria in muscle cells, which physiologically stimulates improved oxygen uptake.[19]

A physical activity is categorized as aerobic or anaerobic depending on its intensity and duration. Most physical exercises are characterized by static and dynamic contractions and aerobic and anaerobic metabolism.Aerobic exercise includes any type of exercise, usually one that is performed at a moderate level of intensity for a long period of time and maintains an elevated heart rate. Activities such as cycling, swimming, jogging, rowing and aerobics require oxygen to produce ATP.[23] Regular aerobic exercise increases maximal oxygen consumption and overall endurance performance. Anaerobic training can increase strength, power and speed. Generally, anaerobic exercise has a short duration and high intensity activity. Unlike aerobic exercise, it does not depend on exogenous oxygen. Activities such as lifting weights, any type of sprint (running, cycling or swimming) or other vigorous exercise require anaerobic metabolism. During high-intensity (anaerobic) exercise, almost all of the metabolic fuel source is glucose, whereas during low-intensity (aerobic) exercise, fat utilization increases and glucose oxidation decreases. Both types of exercise improve the mechanical efficiency of the heart (cardiac adaptation), morphological changes, and left ventricular function.[24,25]

Many exercises have been developed and proven to have a significant effect on skeletal muscle metabolism. Examples of exercises that are gaining popularity and are becoming an option in overcoming skeletal muscle weakness*is* High Intensity Interval Training (HIIT) High Intensity Interval Training (HIIT). HIIT workouts generally combine high-intensity workouts with periods of rest or reduced intensity. In some fitness training classes, these exercises are often combined with aerobic exercise and resistance training. However, most of the interval training the researchers studied focused on aerobic exercise only. Periods of intense exercise can range from 5 seconds to 8 minutes, and are performed at 80% to 95% of a person's estimated maximum heart rate, i.e. the maximum number of times the heart will beat in one minute without overexerting itself. The recovery period lasts the same as during the work period and is usually carried out at 40% to 50% of the estimated maximum heart rate of someone who is active. The workout continues for a total period of 20 to 60 minutes.[26]

HIIT training has been shown to improve aerobic and anaerobic fitness, lower blood pressure, maintain heart health, insulin sensitivity (which helps training muscles use glucose for fuel to more easily produce energy), decreased expression of cholesterol, belly fat and body weight while maintaining muscle mass.[27] HIIT training can be easily modified for people of all fitness levels and

special conditions such as overweight and diabetes. HIIT can be performed on all types of exercise including cycling, walking, swimming, etc. HIIT provides the same benefits as CT and resistance training, but for shorter periods. This is because HIIT workouts tend to burn more calories than regular workouts, especially after exercise.[18]

Effect of HIIT On Skeletal Muscle Signaling Pathways

The mechanical stimulus of exercise has the potential to activate muscle mTOR complex 1 (mTORC1) through phosphorylation and lysosomal exclusion and subsequent mTORC repressor tuberous sclerosis complex. Furthermore, extracellular signal regulated kinase 1/2 (ERK1/2) and the PI3K–Akt-mTOR pathway also upregulate mTORC1 activity. Upregulation of mTORC1 phosphorylates several proteins important for protein synthesis and hypertrophy, such as ribosomal S6 kinase 1 (p70S6K1) and 4E–BP1. Patients with type-2 DM show a decrease in protein synthesis capacity which may be due to a decrease in 4E-BP1 phosphorylation in muscle in response to protein and insulin treatment.[11]

Exercise is an effective therapy against anabolic resistance in type-2 DM, because mTOR can be activated independently of insulin or growth factors such as IGF-1 with exercise, it can increase vastus lateralis type I and II muscle fibers by 18-21% in DM patients type-2. However, whether resistance training or high-intensity interval-based training reduces muscle atrophy through preventing skeletal muscle degradation remains unclear, but presents a potential mechanism that requires further investigation. In addition to the direct effect of mTORC1 activation on protein synthesis, upregulation of mTORC2 through exercise can also attenuate muscle atrophy. mTORC2 by activating Akt, causes downregulation of the ubiquitin-proteasome system. To support this premise, eight weeks of exercise decreased muscle FoxO1 while increasing Akt and mTOR phosphorylation in healthy humans, while detraining decreased Akt phosphorylation and increased FoxO1. Furthermore, muscle Atrogin-1 mRNA in muscle was reduced 8 hours after a single bout of resistance exercise. The decrease in Atrogin-1 mRNA reported by Mascher et al., was accompanied by an increase in MuRF1 mRNA immediately after exercise and in a separate study, MuRF1 mRNA increased after intense interval training. Different signaling responses to ubiquitin E3 ligase suggest the mode and intensity of exercise are important factors. in the activation of the ubiquitin-proteasome system after exercise; with intense exercise potentially transiently increasing atrogen signaling, while training may decrease ubiquitin proteasome signaling. These different data further suggest that in healthy populations acute activation of the ubiquitin-proteasome system in response to exercise may be necessary for the removal of damaged proteins and does not have a prominent role in muscle atrophy or hypertrophy and is likely due to a substantial concomitant increase in protein synthesis. However, in inflammatory conditions, such as

type-2 DM, downregulation of the ubiquitin-proteasome system with training, potentially through upregulation of inflammatory pathways, may be more important. For example, long-term resistance training (12 months), increased thigh muscle mass by 4.5% in elderly patients with type-2 DM, and increased muscle mass were associated with lower circulation.[11]

2. Methods

In this study using stored biological material in the form of gastrocnemius muscle tissue of experimental animals. Previously, the research had been approved by the Health Research Ethics Commission, Faculty of Medicine, University of Indonesia-RSUPN Dr. Cipto Mangunkusumo with number: KET-97a/UN2.F1/ETIK/PPM.00.02/2022. The sample in this study is stored muscle tissue obtained from previous studies. This research was an in vivo experimental study using experimental animals, namely 18 adult male Wistar rats (Rattus Novergicus) aged 8 weeks, healthy and with initial body weight ranging from 180-250gr. Before and during the treatment, the health of the rats was maintained so they would not be attacked by disease. Mice were given standard food and water ad libitum. The cage is kept clean and set 12 hours of light and 12 hours of darkness. The ambient temperature was maintained at 23 ± 1oC. Prior to the experiment, mice were adapted to a laboratory diet for 7 days. Exclusion criteria: unable to participate in the exercise during the acclimatization period, unable to participate in the study after the acclimatization period is complete, illness and death. Other matters in the experiment are adjusted to the code of ethics of the commission for handling and using experimental animals. The experimental animals were divided into three groups, namely the healthy rats as the control group, the group of type-2 DM rats that were not given any treatment at all, and the group of type-2 DM which were given HIIT treatment. After that the HIIT rat group underwent training for 6 days per week for a total of 6 weeks based on the formula in Alfzafour's study (2015). After that, the mice were decapitated and the gastrocnemius muscle tissue was taken and then weighed. Then the tissue is kept at -80oC.

Next, p62 analysis was carried out using the western blot (WB) method with stages starting from making homogenates, electrophoresis stage, transfer and detection stages. Making homogenates was carried out before examining p62 expression with western blot from stored gastrocnemius tissue and then measuring the sample protein concentration. with a standard curve. The electrophoresis procedure is carried out at 100V-150V, 300 mA with a duration of 30 minutes-2 hours. The transfer stage is carried out by making a sandwich for transfer and running at 100v for 1 hour. The transferred membrane is blocked in buffer (BSA/skim milk). The next stage of detection is by incubating the primary antibody first. Previously, primary antibodies were diluted in a ratio of 1:500 for P62 and 1:1000 for GAPDH. The transferred membrane was incubated in the antibody solution at room temperature

for 1 hour. The HRP secondary antibody was diluted in a ratio of 1:10.000 and incubated at room temperature for 1 hour. Then the membrane can then be analyzed using chemilluminance techniques. The resulting image can then be read with the ImageJ application.

Statistical analysis was performed using the Graphpad Prism Version 8.0.2 program. The analysis begins with a normality test. If the data is normally distributed and homogeneous ($p \geq 0.05$), then a parametric test is performed using One Way Anova and if it is not normally distributed using non-parametric. Significant value if $p < 0.05$.

3. Results and Discussions

This research is an experimental study that aims to determine the effect of HIIT on P62 expression in experimental animals with hyperglycemia (type-2 DM).

3.1. Effect of HIIT on gastrocnemius muscle mass in type-2 DM rats

The Shapiro Wilk normality test showed that the data was normally distributed ($p > 0.05$). Therefore, a parametric test was carried out, namely the One Way Anova test, and it showed that there was no significant difference with a p value = 0.722 ($p > 0.05$).

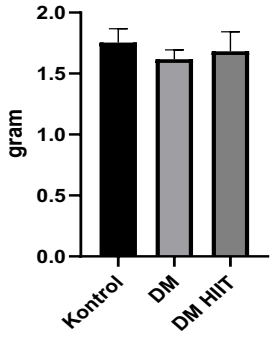

Figure 1. Analysis of rat skeletal muscle mass presented in the form of mean + SEM. There were no significant differences between groups. K: Normal control. DM: Type-2 DM group without treatment, DM-HIIT: Type-2 DM group given HIIT treatment

The Tuckey' multiple comparison test showed no significant difference between the DM group to the control group (p=0.700), DM-HIIT to the control group (p=0.894) and the DM-HIIT group to the DM group with a value of (0.930). it was concluded that there was no significant effect of HIIT treatment on the muscle mass of type-2 DM rats.

3.2. Effect of HIIT on P62 expression in the skeletal muscle of type-2 DM rats

In the test using western blot to detect the presence of p62 protein. The results of p62 western blot measurements were read using image J and statistically tested with GraphPad 8.0.2. Figure 3 shows the protein bands seen in the DM and HIIT control group for both primary antibody (p62) and housekeeping antibody (GAPDH). In general, the HIIT band looks fainter than the DM band and the control band. This indicates that there was a decrease in P62 expression in the DM group treated with HIIT.

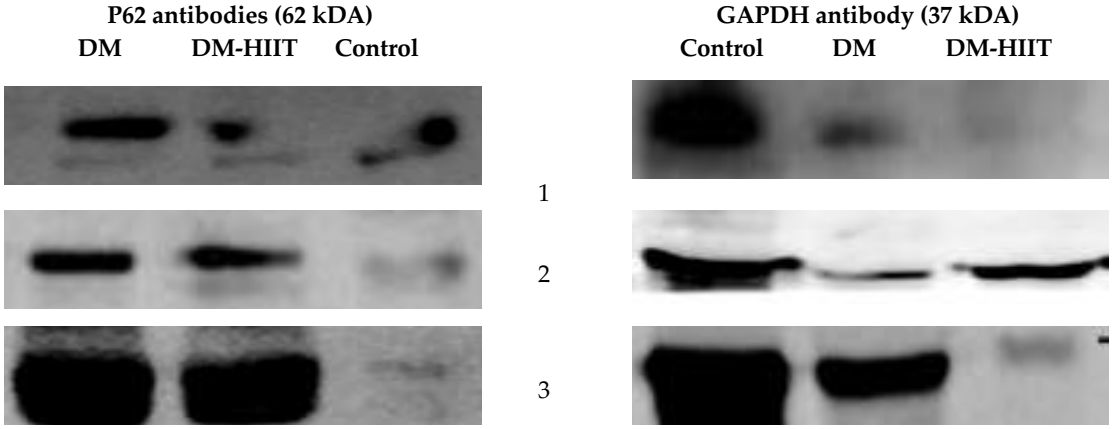

Figure 2. Results of WB readings on P62 (primary Ab) and GAPDH (comparison Ab) membranes

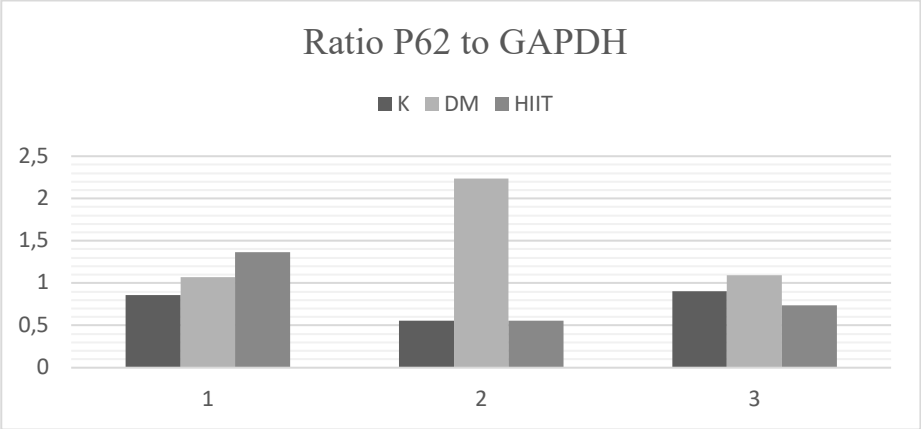

Figure 3. Comparison of the ratio of P62 to GAPDH in each membrane

After the value from imageJ is entered into MS. Excel to process the data, then the P62 value is rationed to GAPDH. The results are shown in Figure 3. To see the significance of the data, statistical tests were then carried out using GrapPhad.

The Shapiro Wilk normality test showed that the data was normally distributed ($p>0.05$). Therefore, a parametric test was carried out, namely One Way Anova, which showed that there was no significant difference with a p value = 0.196 ($p> 0.05$).

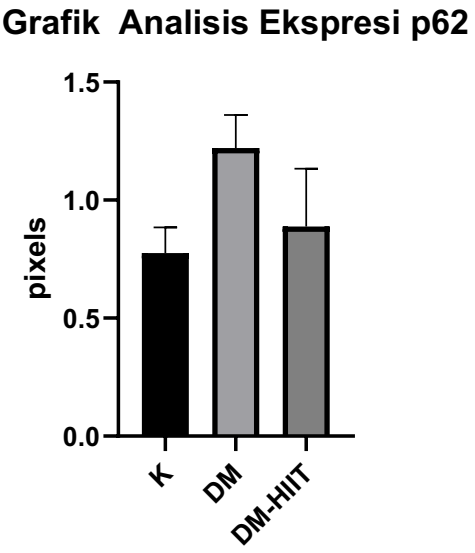

Figure 4. Results of p62 protein expression. Data are presented in the form of mean + SEM. There is no significant difference. K: Normal control. DM: Type-2 DM group without treatment, DM-HIIT: Type-2 DM group given HIIT treatment

As shown in Figure 4, the Tuckey multiple comparison's test showed an increase in p62 protein expression in the DM group compared to the control group with a value (p=0.247) but not significantly different (p=0.893). HIIT treatment in DM conditions (DM- HIIT) compared to controls also showed no significant difference in p62 expression with a value (p=0.424), then a comparison of the DM-HIIT group with the DM group did not show any significant difference with a lower value (p=0.539) for DM. Thus it can be concluded that there was no significant difference from the HIIT treatment on p62 expression in mice with type-2 DM.

3.3. Effect of HIIT on skeletal muscle mass of type-2 DM rats

In figure 1, a comparison graph of the average values of the gastrocnemius muscle mass of rats, there is an increase in muscle mass in the HIIT group compared to the DM group, even though the statistical analysis chart states that there is no significant difference between groups. HIIT has similarities with resistance training as a training modality to maintain muscle mass. There is some scientific literature that has evaluated the anabolic effects of HIIT alone or in combination with optimal nutritional support (increased protein availability) on skeletal muscle adaptation. Emerging evidence from human studies suggests that HIIT can modulate gene and protein expression in skeletal muscle. Noted for regulation of muscle mass, HIIT increases muscle protein synthesis and activates muscle satellite cells.[26]

At the myocellular level, HIIT can increase protein consumption and phosphorylation of the Akt/mTOR pathway and stimulate myofibril protein synthesis. HIIT increases sarcoplasmic and mitochondrial protein synthesis gradually until muscle mass increases. Increased expression of the myogenic regulatory factor MYOD1, amino acid transporter SNAT2 and transmembrane Wnt signaling receptor FZD7 as well as decreased expression of the muscle negative MTSN represent several protein gene coding processes that may contribute to the pathways that regulate muscle fiber size with HIIT and increased protein availability. CARNS1 and MYLK4 protein expression can increase calcium handling to support muscle contractions with higher intensity in the sarcomere.[28]

In other research, it is stated that there is a relationship between AMPK and changes in muscle mass related to physical exercise treatment.[19]In line with the research of Rusli et al (2022) in phase 1 research that there was an increase in total AMPK and AMPK phosphorylation in the HIIT treatment of the DM group. 5'AMP-activated protein kinase (AMPK) is a central regulator of cellular metabolism and energy homeostasis in mammalian tissues. AMPK (5'-adenosine monophosphate-activated protein kinase) is highly involved in the control of skeletal muscle metabolism through multi-target regulation of anabolic and catabolic cellular processes. The effect of AMPK activity on satellite cell-mediated muscle growth and regeneration after injury has been reviewed. AMPKα1 plays an important role in stimulating anabolism and in regulating satellite cell dynamics during regeneration whereas AMPKα2 plays a potentially more important role in regulating muscle degradation during atrophy.[29] Egawa et al (2015) proved that there was no difference in muscle mass regrowth between mice after 7 days of reloading, and simultaneously there was a decrease in AMPKα2 activity, but AMPK was higher in AMPK mice after 14 days, which means AMPK mediated increased atrophy.[9,19]

From various literature it is stated that there can be an increase in muscle mass with HIIT, even though the HIIT used as treatment in this study is aerobic HIIT, not HIIT which specifically uses weights. This can also be proven in previous research, namely Alfzafour (2015), which found an increase in muscle mass after DM rats were given aerobic HIIT treatment for 6 weeks, although the increase in muscle mass was not significantly different.

3.4. Effect of HIIT on P62 protein expression

In the results of the P62 western blot test, although the results of the statistical data did not show any significant changes, however, in Figure 5 there was a decrease in the group that was given the HIIT treatment and visually in Figure 3 the band on the membrane of the HIIT group looks fainter than the control group and the DM group without treatment which indicated that the amount of p62 in the HIIT treatment decreased. However, in the statistical test the data was not significantly different. There have been several previous studies that tried to see the expression of p62 when given exercise.

In a study by Pagano et al (2014) found that there was a decrease in the amount of p62 indicating the presence of autophagy flux in high-intensity exercise conditions with a duration of 120 minutes.[30] Then, research with an exercise duration of 110 minutes also showed a decrease in p62. Additionally in other experiments, p62 protein levels have been shown to decrease after 6 hours of recovery from low to moderate intensity exercise indicating a delay in autophagosome lysosomal degradation. Meanwhile, research by Botella et al (2022) showed that the protein expression of the autophagy receptor p62 remained unchanged at all time points in mice. Grumati et al reported an increase during exercise in autophagy flux as seen from the induced expression of LC3II/LC3I accompanied by a decrease in p62.[31] Experiments conducted by Cho et al (2017) on the muscles of mice with obesity induced by a high-fat diet showed a decrease in p62 expression after being given treadmill training for 10 weeks.[32] Experiments on humans by Schwalm et al (2015) with a training duration of 2 hours with 70% Vo2peak showed that there was a decrease in the p62 protein level one hour after doing high-intensity exercise. This shows that differences in exercise duration can have different effects on changes in protein expression.

In Schwalm's study (2015) also stated that there was a relationship between AMPK activation and autophagy activation related to differences in exercise intensity. In addition to being regulated by the insulin hormone, autophagy is also controlled by AMPK which is the main regulator of cellular energy homeostasis and is activated when there is a lack of nutrition and exercise. Then there is ULK1 which has several phosphorylation sites and depends on AMPK, including Ser317 and also requires the initiation of autophagy. Exercise increases ULK1Ser317 phosphorylation in fed and fasted subjects). ULK1Ser317 induced by exercise was higher and more persistent at high intensity exercise than at low intensity, because 1 hour after exercise ULK1Ser317 was still more phosphorylated than before exercise. The combination of HI with fasting potentiates ULK1Ser317 phosphorylation due to a 2-fold increase compared to the 50% increase in the fed state observed immediately after exercise. These findings collectively indicate that endurance training regulates AMPK-dependent autophagy pathways in human skeletal muscle, especially when performed in HI, without the additional effect of fasting.[33] This is in line with previous research, namely Rusli et al (2022), which has shown an increase in AMPK in DM rats treated with HIIT.[34] This study also proved that there was a significant difference in phosphorylation between the group given physical exercise compared to the control and diabetes groups.[34]

The non-significant results on p62 are also related to the treatment in WB which only uses representatives from each sample. From 10 WB experiments, only 3 doc gels were successfully identified. This is caused by proteins that are sometimes not read and indicates decreased sample

quality. Therefore, minimal data can lead to a tendency for meaningless results. However, the tendency for a decrease in P62 was in line with expectations based xperiments in previous studies.

4. Conclusion

The treatment of High Intensity Interval Training in Type-2 Diabetes Mellitus showsThere was a tendency to increase muscle mass although it was not significantly different. This can be attributed to the aerobic type of HIIT training which is known to regulate the reduction in fat mass more than the increase in skeletal muscle mass. There was a tendency for p62 to decrease in type-2 DM when given HIIT, although it was not significantly different. This is related to the difference in duration of HIIT treatment which causes adaptations in skeletal muscles to reduce excessive degradation processes.

5. Acknowledgments

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Research Article

Relationship between HbA1c levels and macrovascular complications in type 2 diabetes mellitus patients at Dr. Ramelan Surabaya

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Abstract: Diabetes mellitus (DM) is a serious chronic condition in which the body is unable to produce sufficient amounts of insulin or the insulin is unable to work effectively. Long-term hyperglycemia can cause macrovascular and microvascular complications if there is no control of significant risk factors. Macrovascular complications that often occur in DM patients are coronary heart disease, stroke, hypertension, and diabetic gangrene. Diagnostic criteria for diabetes according to HbA1c levels are when HbA1c levels are > 6.5% (48 mmol/mol). A 1% increase in HbA1c concentration is associatedwith a 30% increase in all-cause disease and a 40% increase in cardiovascular disease or ischemic heart disease in someone with diabetes. This study was conducted to determine the relationship between HbA1c levels and the incidence of macrovascular complications in type 2 DM patients. This study was an observational analytic study with a cross-sectional approach. The study sample was 182 type 2 DM patients at RSPAL Dr. Ramelan 2020-2022 which has met the inclusion and exclusion criteria. The data collection technique is purposive sampling. The type of data used in this study is secondary data in the form of medical records. The data analyzed using the SPSS computer program. The prevalence of macrovascular complications in type 2 DM patients was (73.1%) with the most complications being diabetic gangrene (48.1%), stroke (23.2%), CHD (15.8%), and hypertension (12.8%). %). Bivariate correlation tests showed that HbA1c levels were significantly associated with the incidence of macrovascular complications in type 2 DM patients (p=0.001). Statistically, there is a significant relationship between HbA1c levels and the incidence of macrovascular complications in type 2 DM patients.

Keywords: HbA1c levels; macrovascular complications; type 2 DM

1. Introduction

Diabetes mellitus (DM) is a serious chronic condition when the body is unable to produce sufficient amounts of insulin or insulin cannot work effectively. In the 21st century, DM has become a world health problem due to a significant increase in prevalence rates in both developed and developing countries. Around 537 million people in the world suffer from DM. According to the International Diabetes Federation (IDF), this figure is predicted to rise to 783 million people

(46%) in 2045.[1,2]

The region where Indonesia originates, Southeast Asia, is ranked third in the region with the highest DM rate in adults (20-79 years). The results of Riskesdas in 2018 showed an increase in the prevalence of DM in Indonesia from 1.5% to 2%. Even though the number of Indonesians who are not diagnosed with DM exceeds half of the population (73.7%), Indonesia is included in the top 10 countries with the most DM sufferers in the world (19.5 million), being the only country from Southeast Asia. This shows the magnitude of Indonesia's impact on the high prevalence of DM in Southeast Asia.[1,3]

The increase in the incidence of DM is in line with the high proportion (45%) of diabetes patients who have not been diagnosed with DM throughout the world with cases dominated by type 2 diabetes mellitus (T2DM). This happens because compared to type 1 DM, the symptoms of type 2 DM are not very significant and may be asymptomatic. As a result, the pre-diagnostic period becomes longer, causing many T2DM patients to go undiagnosed (International Diabetes Federation, 2017; Organization, 2018). Unhealthy lifestyles support the high death rate (1.6 million) before the age of 70 years due to T2DM which has a prevalence of 7% in Indonesia.[4,5] Hyperglycemia is one of the significant symptoms of DM. Long-term hyperglycemia can cause macrovascular and microvascular complications if there is no significant control of risk factors.[6]

In 2016, T2DM and its complications were responsible for 6% of deaths in Indonesia.[4] Diabetes mellitus can develop into vascular complications, both microvascular and macrovascular. Microvascular complications include nephropathy, neuropathy, and retinopathy, both peripheral and autonomic. Macrovascular complications affect the coronary, cerebral and peripheral blood vessels.[7] In Indonesia, more than half of T2DM sufferers experience complications. Macrovascular complications occur more frequently than microvascular. According to National Health Insurance data, the most common complication is cardiovascular disease (24%), followed by neuropathy (14%), nephropathy (7%), cerebrovascular disease (6%), retinopathy (5%), and peripheral vascular disease (2%).[8]

Glycated hemoglobin (HbA1c) is hemoglobin that binds to glucose so high levels of HbA1c are a sign of patient compliance with DM control.[1] Elevated HbA1c levels in DM patients are associated with an increase in more serious complications.[9] Every 1% increase in HbA1c results in a 10-20% increase in cardiovascular risk and death.[10] In diabetic foot ulcers, a Wagner score of 3-5 has the highest HbA1c level compared to 1 or 2.[11] Based on the research conducted by Rima Maulina Haniya et al (2016) regarding the relationship between HbA1c levels and the incidence of macrovascular complications, shows that there is an influence of increasing HbA1C

levels on increasing macrovascular complications.[12]

Something slightly different was found in a cohort study conducted by Guido et al (2015), which found that in all patients with T2DM, higher HbA1c levels were not significantly associated with cardiovascular events. Similar results were also found in patients with cerebrovascular, peripheral arterial, coronary artery, or vascular disease in various locations.[13] An increase in HbA1c levels is associated with an increased risk of death but is not associated with an increased risk of cardiovascular events.[14] In research conducted by Shen et al., not only did increasing HbA1c levels cause a higher risk of stroke, but low HbA1c levels also had a high incidence of stroke.[9] Based on the studies above, it is important to investigate the relationship between glycated hemoglobin levels (HbA1c) and the incidence of macrovascular complications in T2DM patients.

2. Methods

This research is an observational analytical study with a cross-sectional approach. Data were obtained from secondary data by looking at the medical records of patients with diabetes mellitus in the outpatient and inpatient departments of the internal medicine department of RSPAL Dr. Ramelan Surabaya for the period January 2020 - May 2022 which was taken using the purposive sampling method so that a total of 182 respondents were obtained.

In this section, you are asked to describe the method, model, design, subject and location of your research. Pleaseput the procedure of your research clearly so that it is easy to read. Make sure that you employ appropriate research methods in line with the research problem and the purpose of your research.

3. Results and Discussion

3.1. Results

Characteristics of study subjects based on gender, age, and HbA1c levels of type 2 DM patients can be seen in Table 1. Based on Table 1, it was found that more than half of the respondents were aged 51-75 years (69.8%). Based on gender, there were more female respondents (53.8%) than males. Based on HbA1c levels, there was a significant difference where the majority of respondents had HbA1c levels >7% (85.7%). Based on macrovascular complications, the majority of respondents had macrovascular complications (73.1%).

Table 1. Subject characteristics based on age, gender, HbA1c levels, and macrovascular complications

Variable	n	%
Age (years-old)		
26 – 50	38	20.9
51 – 75	127	69.8
76 – 100	17	9.3
Sex		
Man	84	46.2
Woman	98	53.8
HbA1c levels		
<7%	26	14.3
≥7%	152	85.7
Macrovascular complications		
Yes	133	73.1
No	49	26.9
Total	182	100

Table 2. Overview of macrovascular complications in type 2 DM patients

Macrovascular Complications	n	%
Coronary Heart Disease (CHD)	21	15.8
Stroke	31	23.3
Hypertension	17	12.8
Gangrene	64	48.1
Total	133	100

Based on Table 2, the most common macrovascular complication in type 2 DM patients is gangrene (48.1%) followed by stroke, CHD and hypertension.

Table 3. Chi-square test

	Value	df	Asymptotic significance (2-sided)
Person Chi-Square	4.917[a]	1	.027
Continuity corrections[b]	3.969	1	.046
N of valid	182		

a. 0 cells (0.0%) have an expected count of less than 5. The minimum expected count is 8.08
b. Computed only for a 2x2 table

Based on Table 3, it is known that the Asymp value. Sig. (2-sided) in the Pearson Chi-Square test is 0.027. Because the Asymp. Sig, (2-sided) 0.027 < 0.05, then based on the basis for decision making above, it can be concluded that H0 is rejected and H1 is accepted. Thus, it can be interpreted that there is a relationship between HbA1c levels and macrovascular complications at RSPAL Dr. Ramelan Surabaya. At the bottom of the Chi-Square Tests output Table there is the statement "0 cells (0.0%) have expected count less than 5. The minimum expected count is 8.08" which means that the assumption of using the chi-square test in this study has met the requirements because there are no cells that have an expected frequency below 5 and the largest expected frequency is 8.08.

Table 4. Contingency coefficient test

		Value	Approximate significance
Nominal by nominal	contingency coefficient	.162	.027
	n of valid cases	182	

Based on the contingency coefficient test above, the correlation value obtained is 0.162, which means that the strength of the relationship between HbA1c levels and macrovascular complications is very weak.

3.2. Discussion

Based on the distribution of patient characteristics based on age, it shows that the majority of research subjects were in the 51-75 year age group, namely 127 patients (69.8%) with the youngest age being 34 years and the oldest being 93 years. Based on HbA1c levels aged 51-75 years, the highest number of patients with HbA1c levels >7% with the highest number of macrovascular complications. These results are in accordance with research conducted by Guido et al (2015) which found that DM sufferers were on average 60.2 years old (SD 10.2 years). Utami Maulina et al. (2018) also found that DM sufferers with the most complications were over 60 years old.[13,15]

According to Sonta (2019), the older you are, the more likely the incidence of insulin resistance will be. According to Haryati (2013), after 30 years the body will experience changes both anatomically, physiologically and biochemically. This causes changes as we age to occur from the cell level to the organ level which can affect homeostatic function. One of the functions affected is reduced cell sensitivity and activity of pancreatic beta cells in producing insulin.[13,15] Age-related increases in insulin resistance are also caused by several factors such as decreased mitochondrial function, increased intramyocellular lipids, increased levels of inflammation, increased oxidative and endoplasmic reticulum stress, weakened enzyme activity, decreased muscle mass, and an overactive renin-angiotensin system. [16]

The distribution of samples based on gender showed that most DM sufferers were female, namely 98 patients (53.8%). Cross-tabulation data also shows that more women experience increased HbA1c levels and also experience macrovascular complications. This is in line with research conducted by Imelda (2019) which found that 61% of DM sufferers who were research patients were women.[17] Another study conducted by Maulina et al (2018) also found that 59% of DM sufferers were women. Women tend to suffer from diabetes more easily due to the influence of reproductive hormone fluctuations that occur during menstruation, pregnancy or menopause. A study conducted by Meka (2015) found that women who experienced shorter menstrual cycles had higher blood glucose levels. This is influenced by the secretion of the hormone estrogen. The

estrogen hormone is antagonistic to blood glucose levels because estrogen hormone receptors on pancreatic β cells cause the release of insulin which is the most important hormone in blood glucose homeostasis. Meanwhile, the hormone progesterone can cause cells to become less sensitive to insulin resulting in insulin resistance in the body. This causes the pancreas to work more to excrete insulin. Diabetes is more likely to occur in older women due to menopause. Menopause causes estrogen and progesterone levels to drop quickly and significantly. This will affect how the body's cells respond to insulin because the hormone changes significantly so that blood sugar levels are more difficult to predict than before menopause.[15,18]

The characteristics of HbA1c levels in this study sample showed that the majority were at levels more than equal to 7%, namely 156 patients (85.7%). These results are directly proportional to research conducted by Nathasia Omega in 2019 at Tk Hospital. II Putri Hijau, Medan where it was found that 70.6% of patients had HbA1c levels more than equal to 7%. High levels of HbA1c in sufferers are caused by poor glycemic control factors. Then for type 2 DM sufferers with HbA1c levels more than equal to 7%, 116 people (87.2%) had macrovascular complications. This is also in line with research conducted by Nathasia Omega where 42 people with type 2 DM experienced macrovascular complications (82.4%). In research conducted by Sonta Imelda (2018), there were several significant factors related to HbA1c levels, namely age, gender, parental disease history, knowledge, occupation, physical activity and diet patterns. Medication adherence, distance to health facilities, duration of diabetes, medical history, and distance to health facilities also influence glycemic control.[17,19]

In this study, there were 133 patients with type 2 DM who had macrovascular complications (73.1%). The most common description of macrovascular complications in this study was diabetic gangrene in 64 people (48.1%), followed by stroke in 31 people (23.3%), coronary heart disease in 21 people (15.8%), and hypertension in 17 people (12.8%). %). This is in line with research conducted by Haniya et al (2017) at AMC Hospital in 2016, namely that the most common macrovascular complication in type 2 DM patients was diabetic gangrene, namely 28 people (45.1%). Different results were found in Natashia Omega's research where the most common macrovascular complication in type 2 DM was hypertension (45.7%). [12,19]

Physiologically, when glucose is in the blood it will diffuse passively into the tissues through the endothelial membrane. In T2DM, excess glucose is metabolized in the sorbitol pathway to sorbitol and fructose by aldose reductase. This metabolism coincides with the oxidation of NADPH to NADP+ and the reduction of NAD+ to NADH. Reduced NADPH and increased NADH/NAD+ cause a change in redox potential that accelerates glycolysis and increases the

synthesis of Diacyl Glycerol (DAG). As a result, protein kinase C is activated and nitric oxide (NO) is reduced. This effect causes vascular permeability to increase contractility. So simultaneously, an increase in the NADH/NAD+ ratio which also results in ROS, higher LDL oxidation, a cytotoxic effect on endothelial cells, and reduced NO availability can cause endothelial dysfunction.[20]

Atherosclerosis begins with damage to the vascular endothelium that occurs due to endothelial dysfunction. This condition increases the adhesion of molecules with endothelial cells and decreases the release of nitric oxide and other substances that help prevent the adhesion of macromolecules, platelets, and monocytes to the endothelium. As a result, there will be a buildup of lipids and monocytes at the injury site where lipids are dominated by LDL. Monocytes will cross the endothelium to enter the intima layer of the vascular wall and then differentiate into macrophages which will engulf the accumulated lipoproteins. This situation will create an appearance called cell foam. This cell foam will later aggregate in the vasculature to form fatty streaks. As the disease progresses, the growing fatty streak coupled with the enlargement of the fibrous tissue and surrounding smooth muscle due to the effects of macrophages will make the plaque protrude into the interior of the lumen. This protrusion can reduce or even inhibit blood flow if it is completely blocked. Then, the stiffness of blood vessels comes from the deposition of calcium salts, cholesterol and lipids.[21]

In this study, there was a statistically significant relationship between HbA1c levels and macrovascular complications in type 2 DM patients (p-value = 0.027). This is in line with what Haniya et al and Natashia Omega did. However, there are differences in the strength of the relationship test. In the research conducted by Natashia Omega, there was a positive and strong correlation in her research, while in this study the correlation value was considered quite weak.[12,19] According to PERKENI, in certain conditions such as anemia, hemoglobinopathy, history of blood transfusions in the last 2-3 months, conditions that affect the age of erythrocytes, and impaired kidney function, HbA1c cannot be used as an evaluation and diagnostic tool.[22] In research conducted by Ford et al, a positive correlation was found between Hb levels and HbA1c levels. So when there is a decrease in Hb levels due to anemia, there is a decrease in HbA1c levels due to a decrease in the number of red blood cells.[23] Anemia patients (low hemoglobin) or patients with shorter red blood cell survival (sickle cell disease, G6PD deficiency, etc.) will show HbA1c in a false "good" result.[24] However, different results are found in iron deficiency anemia where increased red blood cell turnover can increase Hb glycation leading to higher HbA1c values. Thus, the lower the iron level, the higher the glycation HbA1c leading to a falsely high value in diabetic and non-diabetic patient.[25]

4. Conclusion

Based on the results of research using an observational analytical research design using a cross-sectional approach, it was found that there was a significant relationship between HbA1c levels and macrovascular complications in patients with type 2 Diabetes Mellitus at RSPAL dr. Ramelan Surabaya for the period January 2020 – May 2022.

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Research Article

Unraveling the relationship between overtraining and varicocele recurrence among Indonesian Army Soldiers

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Abstract: Varicocele is an abnormal dilation and enlargement of the pampiniform plexus and testicular veins with continuous or intermittent venous blood reflux. Varicocele can lead to male infertility. The aim of this study was to investigate the correlation between physical activity (overtraining) and varicocele recurrence among Indonesian Army soldiers in Yonkav 1/BCC. Method: This study employed a quantitative research design using a Cross-sectional study method. The research involved 30 respondents of Indonesian Army soldiers stationed at Yonkav 1/BCC, selected through total sampling. The research instruments used were the Baecke index and a varicocele recurrence observation sheet. The data were analyzed using the chi-squaretest. Results: Hypothesis testing with the Chi-Square test indicated that the p-value was smaller than 0.05, which means that Ha (alternative hypothesis) was accepted, and H0 (null hypothesis) was rejected. Based on this interpretation, it can be concluded that thereis a significant correlation between physical activity and varicocele recurrence among Indonesian Army soldiers in Yonkav 1/BCC. Conclusion: Fifty percent of the IndonesianArmy soldiers in Yonkav 1/BCC engaged in heavy physical activity (overtraining), and there was a 66% recurrence of varicocele at a high level. Therefore, soldiers need to pay attention to the frequency and type of exercises to avoid overtraining, especially those with a history of varicocele.

Keywords: Overtraining; Physical Activity; Varicocele Recurrence

1. Introduction

The Indonesian Army National soldiers (TNI-AD) play a pivotal role in establishing and maintaining peace, ensuring the nation's safety, and safeguarding its sovereignty from foreign threats. In the current context, each soldier is expected to possess individual and group capabilities to be responsive, resilient, and resourceful. These diverse skill sets demanded of every soldier as part of their technical expertise are fundamentally rooted in their physical fitness in prime condition. Therefore, Indonesian Army National soldiers are mandated to engage in daily physical activities.[1]

Physical activity is any body movement produced by skeletal muscles that increases energy expenditure. According to the *World Health Organization* (2018), physical activity is defined as any

bodily movement produced by skeletal muscles that requires energy expenditure. Regular physical activity has numerous health benefits, and lack of physical activity is one of the global risk factors for mortality. To become a member of the Indonesian National Armed Forces (TNI), individuals must meet specific training standards, which largely involve strenuous physical activities. Common physical activities performed by TNI personnel during both training and active service include running, push-ups, pull-ups, cross-country, combat training, and military swimming.[2]

Overtraining can lead to increased pressure in the groin area, which may compress the muscles around the testes. This muscle compression in the testicular region can result in swelling of the pampiniform plexus in the scrotal veins, which carry blood from each testicle. This condition can lead to varicocele. Varicocele is a male reproductive system disorder that occurs in the testicles.[3] Varicocele is a condition that describes the dilation, tortuosity, and elongation of the spermatic cord veins or the pampiniform plexus. Varicocele is defined as a vascular abnormality that results in the enlargement and elongation of the pampiniform venous plexus within the spermatic cord and can be palpable.[4,5]

Radojetiv et al. (2016) suggests that varicoceles resulting from exercise in adolescents may have a positive prognosis. Sperm parameters measured show improvement in fertility indicators after exercise is paused and its intensity reduced. However, considering all the other benefits of physical activity, men with a history of varicocele are advised to switch to light physical activities.[6]

The author conducted interviews as a preliminary study with members of the Indonesian Army (TNI AD) and found that 70% of the TNI AD members in Yonkav 1/BCC had a history of varicocele, with most having undergone treatment, including surgery, pharmacological, and traditional methods. Additionally, 20% of them experienced recurrent varicocele. On the other hand, 30% of TNI AD members did not have a history of varicocele, but they were aware of the risk of strenuous exercise causing varicocele.

The total number of soldiers in Yonkav 1/BCC is 380, with approximately 40% of them having a history of varicocele. There is a pattern of excessive physical training without adequate rest. From the interviews conducted with the TNI members, it was reported that they engage in physical training for 16-17 hours a day. From the interview results, it was found that individuals who engage in intense-level training have a history of varicocele, although the exact cause of varicocele is not yet known. Therefore, this research is crucial to investigate one of the potential causes of varicocele. It is important to further explore whether there is a relationship between overtraining conducted by TNI AD soldiers and a history of varicocele. This is significant because varicocele is one of the male reproductive system disorders that can lead to reduced fertility in men. Hence, this study can serve as a reference for TNI AD leadership to pay closer attention, closely monitor, and provide education on the importance of

balanced physical training. Additionally, this research aims to provide a wise and comprehensive perspective and contribute to the development of nursing knowledge regarding the significance of understanding the impact of strenuous exercise as a potential cause or risk factor for varicocele.

2. Methods

The type of research used in this study is descriptive with a quantitative approach. The research design employed is cross-sectional. This research aims to describe the relationship between heavy training and the recurrence of varicocele among TNI AD soldiers in Yonkav 1/Badak Ceta Cakti.

The population in this study consists of 380 TNI AD soldiers serving in Yonkav 1/BCC. The sample calculation in this research is done through total sampling, where the number of samples is equal to the population.[7] The sample size for this study on varicocele recurrence due to physical activity is 30 respondents. The inclusion criteria include having a history of varicocele, being unmarried, having a rank from Prada to Letda, being post-operation, willing to participate as respondents and who have obtained permission from the commander.

The instrument used in this research is a questionnaire consisting of three parts. The first part contains demographic data (name, age, service history, rank), the second part contains a physical activity questionnaire using the Baecke index, divided into three categories of questions: scheduled physical training, sports, and leisure time, totaling 22 questions with interpretations of moderate and heavy physical activity. The final part is an observation sheet regarding varicocele recurrence. The author conducted validity and reliability tests on 20 respondents in Yonkav 1/BCC Company 12. There were 22 valid questions and 2 questions that were not valid, with interpretations of moderate recurrence and severe recurrence. Statistical analysis in this study uses the chi-square test to determine the presence or absence of a relationship between variables with a 95% confidence level.

3. Results and Discussion

3.1. Univariate analysis

Univariate analysis was conducted to examine the distribution of 30 TNI AD respondents serving in Yonkav 1/BCC based on respondent characteristics. The data collected by the researcher are primary data obtained through the completion of the instrument. This univariate frequency distribution includes age, service history, and rank. Univariate analysis helps provide a clear overview of the characteristics of the respondents in the study.

Based on Table 1, it can be concluded that the distribution of respondent characteristics shows that the majority of respondents are aged 20-23 years (73.4%) with the most common service history being

1-3 years (70%), and the most prevalent rank is Prada at 60%.

Table 1. Description of respondent characteristics

Variable		Respondents	Percentage
Age	20-23 years	22	73,4%
	24-25 years	6	20%
	27-30 years	2	6,6%
Total		30	100%
Service	1-3 years	21	70%
History	4-6 years	7	23,4%
	7-10 years	2	6,6%
Total		30	100%
Rank Private	Prada	18	60%
	Pratu	5	16,7%
	Praka	1	3,3%
	Serda	5	16,7%
	Letda	1	3,3%
Total		30	100%

Table 2. Overview of physical activity and varicocele recurrence

	Variable	Respondents	Percentage
Physical Activity	Light Physical Activity	-	-
	Moderate Physical Activity	15	50%
	Heavy Physical Activity (Overtraining)	15	50%
Total		30	100%
Varicocele	Minor Recurrence	-	-
Recurrence	Moderate Recurrence	10	33,3%
	Severe Recurrence	20	66,7%
Total		30	100%

Table 2 shows that the average TNI AD soldiers in Yonkav 1/BCC have a physical training intensity that falls into the category of overtraining. The value of moderate physical activity is reported by 15 soldiers (50%), and heavy physical activity is also reported by 15 soldiers (50%).

Table 2 also shows that the respondents predominantly have a high recurrence of varicocele, with 20 soldiers (66.7%) out of 30 respondents. In the second position, there are 10 soldiers (33.3%) with a moderate recurrence. There are no respondents with a minor recurrence.

3.2. Bivariate analysis

From Table 3, it can be concluded that the correlation analysis between overtraining and varicocele recurrence is significantly associated. The two-sided significance value of 0.000 is less than 0.05, thus, the alternative hypothesis is accepted. In the case of moderate physical training and moderate varicocele recurrence, there are 10 soldiers (33.3%), and for moderate physical training with high varicocele recurrence, there are 5 soldiers (16.7%). Meanwhile, soldiers with heavy physical training

overall experienced a high varicocele recurrence, totaling 15 soldiers (50%).

Table 3. The relationship between physical training and varicocele recurrence in TNI AD soldiers of Yonkav 1/BBC

Physical activity	Varicocele recurrence								p-value
	Minor recurrence		Moderate recurrence		Severe recurrence		Total		
	N	%	N	%	N	%	N	%	
Light Physical Activity	-	-	-	-	-	-	-	-	
Moderate Physical Activity	-	-	10	33,3	5	16,7	15	50	0,000
Heavy Physical Activity	-	-	-	-	15	50	15	50	
Total	-	-	10	33,3	20	66,7	30	100	

4. Discussion

4.1. Characteristics of respondents

Based on the characteristics of the respondents, the results are as follows: the majority of respondents are aged 20-23 years (73.4%). The subjects in this study are male and unmarried. Respondents have a history of varicocele prior to joining the TNI AD and have undergone treatment (post-op).

In the study by Alsaikhan et al. (2016), they observed that the prevalence of varicocele increases by approximately 10% for each decade of life. The prevalence is 75%, and varicocele occurs primarily in adolescents aged 17-23 years ($p > 0.05$).[8] Varicocele is a condition that can either occur at birth or during childhood, with its incidence increasing during the development of puberty when testicular endocrine and exocrine functions increase dramatically.[9] Therefore, age is one of the factors associated with the occurrence of varicocele. Another study revealed that varicocele occurs during puberty, with the highest incidence rate found in the age range of 16-25 years.[10]

According to the author's assumption, adolescence is a productive age for trying new things. It is a period filled with curiosity, where many individuals are eager to discover their identity and pursue their aspirations. Additionally, during this time, there is rapid maturation of the body's organs. Therefore, when someone does not maintain a healthy lifestyle, it can have negative effects on the body. Hence, it is crucial for teenagers to prioritize their health, including engaging in moderate physical activities to reduce the risk of varicocele.

4.2. Overview of physical training

From the univariate analysis conducted by the author, it was found that the majority of respondents engage in strenuous physical exercise, totaling 20 individuals (66.7%). These research findings are

consistent with Vaamonde's study (2016) titled 'Impact of physical activity and exercise on male reproductive potential: a new assessment questionnaire.' In their research, it was noted that athletes regularly engage in strenuous physical activities to maintain and enhance physical endurance ($p < 0.05$).[11] Physical exercise refers to any bodily movement generated by skeletal muscle contractions that elevate energy expenditure above the basal level and pertains to movements that promote health.[12]

Anwar (2021) states that physical activity plays a crucial role for a soldier in its strategic implementation to enhance the performance of soldiers in activities that require physical engagement, such as technical skill, communication skill, and job-hunting skill training.[1] The physical performance of an individual undergoing endurance training can be influenced by their ability to adapt to increased training loads. As a result, soldiers and trainers are constantly challenged to find the optimal balance between training and recovery to enhance their performance.

This maladaptation to training can manifest as a state of non-functional overreaching (NFOR), and in extreme and prolonged cases, the accumulation of this fatigue can ultimately develop into the overtraining syndrome (OTS).[13] Overtraining syndrome involves changes in parasympathetic and sympathetic effects, such as fatigue, depression, low motivation, bradycardia, insomnia, irritability, agitation, and anorexia. Overtraining is dependent on individual differences and capabilities, making it challenging for trainers to accurately determine its occurrence, delaying immediate response and causing long-term performance decline. In the military, soldiers are trained to endure high levels of physical and psychological stress, which can lead to the development of overtraining syndrome either during training courses or military operations.[14]

The American College of Sports Medicine (ACSM) recommends avoiding excessive exercise to achieve maximum benefits from endurance training. Endurance training for the same muscle group should be separated by a 48-hour period to limit the frequency of physical activity.[14] According to the researcher's assumptions, implementing physical training for the Indonesian National Army soldiers should take into account tolerance and physical endurance standards, as excessive physical exercise can have adverse effects on health. Preventing the overtraining syndrome can reduce the number of injuries and illnesses, leading to more available manpower and extended operational careers, which is a valuable investment.

Preventing the overtraining syndrome can reduce the number of injuries and illnesses, leading to a larger available workforce and extended operational careers, which is a costly investment. Regular physical training with appropriate intensity and duration can reduce physical stress, leading to adaptation. These adaptive effects can promote overall health benefits. Proper exercise intensity can

enhance athletic capacity through physiological adaptation, but excessive exercise without adequate rest can disrupt health maintenance and athletic performance. Besides adequate rest, there is no effective intervention to combat the overtraining syndrome.

4.3. Overview of varicocele recurrence

The results of the univariate analysis conducted by the author revealed that a higher percentage of respondents experienced a high recurrence of varicocele, accounting for 66.7%, while those with a moderate recurrence constituted 33.3%. These findings align with a study conducted by Alkhamees et al. (2020), which noted that varicocele recurrence is associated with the surgical technique employed. However, further research revealed that the condition of the veins became one of the causes of varicocele recurrence in their study. Recurrence occurred in 2.9% after 3 and 6 months (P=0.002).[15]

Varicocele forms from a mass that results from the convolution of dilated veins within the plexus venosus cordis. Patients commonly experience symptoms such as testicular pain and changes in the size of the testicles and scrotum. In his study, S. Chen (2014) stated that the most frequent complication of varicocele is varicocele recurrence, primarily due to the persistent compression of the veins, which can be exacerbated by physical activity.[16]

In their study, Eisenberg et al. (2020) stated that varicocele recurrence is caused by the persistence of small spermatic branches being bypassed under high venous pressure. Increased venous pressure affects the blood supply to the testes by reducing arterial blood flow to maintain intratesticular pressure homeostasis, potentially disrupting the testicular nutrient supply, ultimately impacting spermatogenesis.[17]

According to the researcher's assumptions, in addition to understanding that varicocele is one of the causes of infertility as a complication of varicocele, respondents should also be aware of varicocele recurrence and its contributing factors. One of the contributing factors is repeated compression of the veins, which is a result of repetitive physical activities and exercises, preventing the veins from experiencing pressure relief. Varicocele recurrence typically does not occur in the same location but often in different areas, so individuals with a history of varicocele should maintain physical activity to avoid its recurrence. Furthermore, varicocele is one of the factors that disrupt the process of spermatogenesis, so when varicocele recurrence occurs, it has the potential to retrigger disturbances in spermatogenesis.

4.4. Relationship between overtraining and varicocele recurrence

The hypothesis test conducted on 30 Indonesian Army soldiers revealed a significant relationship between heavy physical training (overtraining) and varicocele recurrence (p-value: 0.000). Among the

soldiers, those who engaged in moderate-intensity physical training experienced a moderate varicocele recurrence, with 10 soldiers (33.3%), and a high varicocele recurrence, with 5 soldiers (16.7%). Additionally, it was found that soldiers who engaged in heavy physical training had a high varicocele recurrence rate, totaling 15 soldiers (50%).

The findings of this study align with research conducted by Radojevic (2015), which indicated that physical activity and sports participation among a group of athletes contributed to varicocele prevalence. In Radojevic's study, participants were engaged in sports for an average of three and a half years before the research was conducted. The results showed a significantly higher varicocele percentage in the first group compared to the control group ($p<0.49$), while the percentage of young men diagnosed with varicocele in the second group was lower than that in the control group (9.09% vs. 12.35%). After a 6-month period of cessation and abstaining from all sports activities, every parameter of seminal fluid analysis improved in the first group, with statistical significance found for both sperm concentration ($p<0.001$) and sperm motility ($p<0.023$). Testicular volume was not found to significantly increase in either group ($p>0.05$).[6]

According to the researcher's assumptions, maintaining consistent physical activity is generally beneficial, but it is essential to be aware of one's physical capacity when engaging in physical activities. Moderate to heavy-intensity physical activities should be reduced, especially in individuals who have not yet developed varicocele, as they can be a contributing factor to the occurrence of varicocele due to persistent vein pressure. Even in individuals with a history of varicocele, recurrences can occur in different locations. Furthermore, varicocele is one of the causes of male infertility, so varicocele recurrence resulting from excessive physical activity has the potential to lead to a decline in sperm quality.

In this study, respondents actively engaged in long-term moderate to heavy-intensity physical training, both those with varicocele and soldiers with a history of varicocele who had undergone treatment before joining the Indonesian National Army. The Indonesian National Army command should consider all the other benefits of physical training. Active sports involvement in men with varicocele should ideally shift towards sports that do not pose an increased risk of varicocele recurrence, coupled with the maintenance of a healthy lifestyle.

5. Conclusion

Based on the results of the research conducted on the "Relationship between Overtraining and Varicocele Recurrence in TNI AD Soldiers of Battalion Kavaleri 1/Badak Ceta Cakti in 2023," which was carried out from January to March 2023, it can be concluded that the soldiers of Yonkav 1/BCC have a moderate to heavy level of physical training, with 15 individuals each (50%). Soldiers of

Yonkav 1/BCC experience a high recurrence of varicocele, which is 20 individuals (66.7%), and a moderate recurrence rate of 10 individuals (33.3%).

Based on the hypothesis testing analysis conducted, it can be concluded that there is a significant relationship between overtraining and varicocele recurrence in the soldiers of TNI Yonkav 1/BCC.

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Literature Review

Formation of a Healthy Life Attitude with Emotional Intelligence

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Abstract: Healthy is influenced by various factors, such as diet physical activity pattern and non physical, mindset, and behavior pattern. Healthy behavior is determined by the way a person behaves. Some of the factors that influence the formation of a healthylifestyle include individual insights, environment, culture, beliefs, information media, institutions, religion, residence and emotions. Ability in control is determined by the condition of emotional intelligence. Emotional intelligence is believed to be the main basis in realizing the ability of self-management. Good self-management encourages to determine the formation of a healthy attitude Based on the above background it is important to examine the formation of a healthy lifestyle with emotional intelligence. This study aims to determine the formation of a healthy life attitude with emotional intelligence. Respondents in this study were grouped into control group and treatment group, each group of 10 employees. The control group and the treatment group tested the emotional intelligence as well as the healthy life attitude with the questionnaire. This measurement is carried out before and after the treatment. Treatment groups were givenemotional intelligence training for 4 stages. Each stages 3 face-to-face sessions in the meeting room. The result of One Sample Kolmogorov-Smirnov Test and Pair Sample Test showed that after training treatment there was a significant increase of emotionalintelligence (p = 0,002) and healthy life attitude (p = 0,010). The result of linear regressionanalysis showed very strong correlation (R = 0,800) and there was significant correlation correlation (p = 0,005) between emotional intelligence to the increasing of healthy life attitude in factory employees. A healthy life attitude on factory employees can be formed with emotional intelligence.

Keywords*:* attitude of healthy living; emotional intelligence

1. Introduction

Healthy conditions can be realized properly if you have a healthy attitude to life. A healthy life attitude as a self-controlling motor for a healthy life. The ability to control oneself can direct action in meeting the needs of balanced nutrition, physical activity, rest and behavior with other healthy lifestyles.[1] Unhealthy life attitudes can result in low attention to the necessities of life (balanced nutrition, rest, physical activity and others). Serious attention to the needs of a healthy life is determined by the ability to control oneself. The success of self-management can be supported by the quality of emotional intelligence.[2]

The results of research by Linley, et.al., 2011 stated that emotional intelligence can reduce the level of emotional expression and self-dependence on others.[3] The ability to reduce emotional expression is supported by the level of emotional intelligence in overcoming challenges of emotional turmoil.[4] Leo (2011) explains that emotional intelligence plays a role in providing the ability for self-knowledge, self-awareness, social awareness, empathy and good communication with others.[5] Laila et.al. , 2012 said that increasing emotional intelligence in patients can reduce personality disorders and depression.[6] Emotional intelligence also functions to control pleasure, impulse, trigger self-motivation, prevent frustration, foster a sense of empathy, direct one's thoughts and actions (Goleman, 2007).[7] The results of research Yalcin, et. al. (2008) concluded that programs to improve emotional intelligence in people with type 2 diabetes mellitus had a positive effect on improving their quality of life.[8] Another study was conducted by Li, et.al. (2009) in high school students in Taiwan who were given training in emotional intelligence programs positively correlated with increased encouragement for physical activity, mental health and ability in social interaction.[9]

Social interaction in a work environment that is of sufficient quality contributes to achieving a healthy life. Achieving a healthy life requires the ability to be patient which is supported by the level of emotional.[10] This healthy life for employees is useful for reducing the risk of work accidents.[11] Based on this description, it is important to research the formation of healthy living attitudes with emotional intelligence in employees.

2. Methods

This type of research is in the form of an experimental study, which is carried out by providing training to improve emotional intelligence. The research design with pre-test and post-test accompanied by a control group (without emotional intelligence training).

The research subjects (respondents) for the control group consisted of 10 people and for the treatment group (given education and training) a total of 10 people. Determination of research subjects using purposive sampling technique, namely by considering the inclusion and exclusion criteria. The inclusion criteria were in the form of permanent employee status at a leather glove factory in Yogyakarta with a minimum working period of 1 year. The exclusion criteria were permanent employees of a leather glove factory in Yogyakarta who do not suffer from mental disorders or are not currently suffering from illness under a doctor's care.

The variables of this study consist of dependent variables and independent variables. The dependent variable (dependent) is the healthy life attitude of leather glove factory employees in Yogyakarta and the independent variable (independent) is the emotional intelligence of leather glove factory employees in Yogyakarta.

Data collection was carried out by filling out a questionnaire as a measure of emotional intelligence and a healthy lifestyle questionnaire. Filling in this questionnaire was carried out before and after the treatment of emotional intelligence training. This questionnaire measuring tool consists of 25 question items that have previously been tested for validity with product moment and reliability testing with Alpha Cronbach. The results of testing the emotional intelligence questionnaire have an r count value greater than r table for n = 20 and α = 5%, namely 0.444 so that the question items are declared valid. The reliability test results with Cronbach's Alpha of 0.981 (meaning 0.981> 0.60) indicate that the emotional intelligence variable is stated to be reliable. The condition for testing the validity of the healthy attitude questionnaire with r count is 0.444 so that it is said to be valid and the reliability test for the healthy attitude questionnaire with Alpha Conbrach is 0.763 (> 0.60) this condition indicates that this questionnaire is reliable.

The research was conducted in September-December 2019 in a leather glove factory meeting room in Yogyakarta. Before being given the training, all respondents (control group and treatment group) were measured for their emotional intelligence and healthy life attitude using a questionnaire. The training implementation process is carried out periodically with 4 levels. Each period requires 3 meeting sessions with each session lasting 100 minutes. After completing the provision of education and training, then all respondents (control group and treatment group) were measured for their emotional intelligence and healthy attitude to life. The data obtained was then tabulated and analyzed using the pair sample test.

3. Results and Discussion

Table 1. Analysis of the paired sample test for emotional intelligence and healthy lifestyle

		N	Correlation	Sig
Pair 1	EI_Before & After	10	,858	,002
Pair 2	HLA_Before & After	10	,786	,010

Information :
EI = Emotional Intelligence
HLA = Healthy Life Attitude

The results of linear regression analysis (Table 2) emotional intelligence on healthy life attitudes show a very strong correlation (R=0.800).

Table 2. Linear regression analysis of healthy living attitudes on emotional intelligence

Model	R	R square	Adjusted R square	Std. error of the estimate
1	,800 (a)	,641	,596	5,61837

Information : a *Predictors (Constant*), EI_After

The correlation between the effect of emotional intelligence on healthy life attitudes (Table 3) shows a significant relationship (P = 0.005). This linear regression analysis shows that increasing emotional intelligence has a significant effect on increasing healthy life attitudes.

Table 3. Correlation of emotional intelligence to healthy life attitudes

Model	Sum of square	Df	Mean square	F	Sig
Regression	449,971	1	449,971	14,255	,005 (a)
Residual	252,529	8	18,567		
Total	702,50	9			

Information : a Predictor (constan), HLA _After, EI_After

The condition of the respondents (employees) after the training (Table 1) showed a significant increase in the score of emotional intelligence (p=0.002) and attitude to life (p=0.010). This situation shows that employees in the leather glove factory environment in Yogyakarta have developed emotional intelligence and healthy attitudes in a certain period of time. The development of the quality of emotional intelligence and healthy living attitudes of employees is due to the provision of insightful input of knowledge and enlightenment of thinking patterns with training provided on an ongoing and systematic basis.[10]

The results of the linear regression analysis (table 2) make it clear that there is an increase in emotional intelligence that has a very strong correlation (R=0.800) to an increase in healthy life attitudes. This correlation condition is strengthened by table 3 data which shows that the emotional intelligence correlation is significant (P = 0.005) on the formation of a healthy life attitude for leather glove factory employees in Yogyakarta. This increase in emotional intelligence provides positive abilities [12] for employees of a leather glove factory in Yogyakarta to know themselves and increase their self-awareness so that they are able to fulfill the needs for a healthy life. The ability to regulate the turmoil of self-desires is due to the important role of emotional intelligence. [13]

The condition of self-awareness of these employees is in accordance with Reeves' statement (2005) that self-awareness can be achieved if a person's emotional intelligence is high enough so that it can lead to the ability to control oneself against all forms of pleasures that can be self-destructive and awareness to direct choices towards self-safety. This ability to self-awareness encourages employees of a leather glove factory in Yogyakarta to work towards forming attitudes to choose to meet the needs of achieving a healthy life. The establishment of a healthy life attitude contributes to action for a healthy life. The results of this study are supported by the results of Yalcin, et. al. (2008) that programs to improve emotional intelligence in patients with type 2 diabetes mellitus have a positive effect on improving their quality of life.[8]

4. Conclusion

Based on the description above, it can be concluded that employees' healthy living attitudes can be shaped by emotional intelligence. The emotional intelligence of employees after being given training has increased significantly.

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Literature Review

Ramadan fasting: Is it really affecting inflammation?

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Abstract: It has been known that intermittent fasting is safe and may helpreduce markers of metabolic syndrome. Intermittent fasting itself is one ofthe latest health trends with goals to reduce weight or conducted as a part of religious rituals. But, the effect of intermittent fasting on inflammation is less clear. This paper aims to review relevant literatures on beneficial effects of fasting on inflammatory markers. This review was conducted asnarrative literature review based on previously published researches and reviews to summarize the effect of intermittent fasting on inflammation, with specific focus on Ramadhan fasting. Some studies have found decreases in inflammatory markers, including TNF-alpha, C-reactive protein (CRP), or IL-6, but other studies has failed to produce similar results. It is probable that Ramadhan fasting may help improve well-being and safe to conduct in wide range of people with metabolic diseases, but the overall benefit on inflammation is still questionable. Further studies arerequired to prove the effect of Ramadhan fasting on inflammatory markers.

Keywords: inflammatory markers; ramadan fasting; systemic inflammation

1. Introduction

Ramadan fasting, conducted by Moslems around the world in Islamic month Ramadan, lasts for 29-30 days [1]. Ramadan fasting is mandatory for every adult Moslems, except those who are ill, pregnant females, elderly with chronic conditions, those who mentally infirm, and people on travel [1].

Fasting allows the religious to reflect, to be involved in community, and increase self-control [2]. Fasting also helps decrease blood sugar and body fat composition without changing lean mass. Fasting also increase lipid utilization as energy source [3]. Meanwhile, fasting may provide anti-inflammatory effect due to increased C-leptin expression and reduced expression of TNF-α and IL-6, [4]. Less clear though, whether this effect is observable across different studies or limited to subset of populations. Therefore, we aimed to review researches in Ramadan fasting and present as literature review.

2. Metabolism in fasting

Insulin secretion decreases and glucagon secretion increases in fasting period to maintain blood sugar during the time of lack of glucose intake. Glucagon secretion stimulates glycogenolysis and gluconeogenesis. In particularly long fasting period, fatty acid metabolism occurs to provide energy for cellular functions [1].

Time-restricted eating (TRE), such as in Ramadan fasting, shifts hepatic circadian rhythm and mediated by AgRP (agouti-related neuropeptides) and AMPK. The TRE fasting induces AgRP, which in turn increases per, Cry, Clock, and Bmal1 expression. The TRE also activates AMPK and changes circadian rhythm through phosphorylation of CRY1 and degradation of FBXL3 [5]. Increased AMPK activity also activates pathways involved in catabolism, involving increase of AMP and ADP while decreasing intracellular ATP [6].

Reduction of fat deposit in fasting occurs due to utilization of fat and triglycerides as alternate energy source in low-calorie intake condition [7]. Thus, fasting may improve BMI and total body fat composition [8]. Fasting also increases growth hormone (GH) secretion and decrease insulin-like growth factor-1 (IGF-1) activity. Increased GH secretion is thought to occur due to decreased IGF-1 activity in fasting from increased IGFBP-1 secretion as compensatory mechanism. Increased GH secretion may stimulate lipolysis, increase hepatic insulin resistance to maintain normal blood sugar, and maintain protein concentration in blood [7].

3. Inflammatory markers in fasting

A meta-analysis found that Ramadan intermittent fasting may help reducing IL-1 (g = -0.016, 95%CI: -0.970 to 0.939; p = 0.975), CRP (g = -0.407, 95%CI: -0.676 to 0.439; p = 0.677), and malondialdehyde (g = -0.219, 95%CI: -0.576 to 0.139; p = 0.230). Said meta-analysis also showed reduction in TNF-α (g = 0.371, 95%CI: -0.999 to 0.258; p = -0.248) and IL-6 (g = -0.407, 95%CI: -0.597 to -0.216; p < 0.001). From all markers, only IL-6 showed statistically significant reduction, although the effect is small [9]. A systematic review found that time-restricted eating did not change CRP concentration, although weight loss was evident. Similarly, TNF-α concentration did not change either in different eating windows. Same story was found when IL-6 was measured [10]. The systematic review should be taken with caution though; the review relied on very limited data set and larger studies are required to corroborate the findings. A meta-analysis found that intermittent fasting may non-significantly lower TNF-α (WMD: -0.16, 95%CI: -0.68 to 0.35), IL-6 (WMD: -0.54, 95%CI: -1.15 to 0.06), and CRP (WMD: -0.02, 95%CI: -0.04 to 0.00). Intermittent fasting may slightly help reduce CRP, particularly in obese patients and for intervention of eight weeks and more [11].

In asthmatic population, erythrocyte sedimentation rate non-significantly reduced after Ramadan fasting (17.08 ± 16.67 mm/hr vs 10.62 ± 9.76 mm/hr), similar with control population (18.73 ± 15.61 mm/hr vs 18.27 ± 13.91 mm/hr). meanwhile, serum hs-CRP showed significant reduction after fasting in either normal (2.08 ± 0.52 ng/mL vs 1.24 ± 0.29 ng/mL; p < 0.001) or asthmatic population (2.86 ± 0.82 ng/mL vs 1.71 ± 0.63 ng/mL; p < 0.001). Although the difference of hs-CRP was significant, the variation

between both groups itself was not (-1.14 ± 0.008 ng/mL vs -0.083 ± 0.337 ng/mL). Also, no significant differences in asthma symptoms were observed [12].

Another study, when adults (20-85 years old) fast from daybreak to dusk, showed significant reduction of TNF-α during and after fasting (-26.3%, $p = 0.001$ and -9.6%, $p = 0.003$ respectively). Further analysis on data showed fasting increased IL-6 (coefficient: +0.77; 95%CI: 0.06-1.49) and IL-8, but reduced CRP (-1.14 mg/L) [13]. The data should be interpreted cautiously and further analysis is warranted. Meanwhile, another study found that IL-6 and hs-CRP concentration were significantly lower at Ramadan fasting (all $p < 0.05$) [14]. The study argued that prolonged intermittent fasting, such as in month of Ramadan, may reduce inflammation and protect against cardiovascular disease.

On the other hand, Ramadan fasting was found to worsen ulcerative colitis on partial Mayo score (median 1, IQR 0-3 vs median 3, IQR 0-5; $p = 0.02$) in patients. Nevertheless, Ramadan fasting did little impact on CRP (median 0.53, IQR 0.18-1.56 vs median 0.50, IQR 0.15-1.22; $p = 0.27$) and stool calprotectin (median 163, IQR 35-418 vs median 218, IQR 63-426; $p = 0.62$). Thus, Ramadan fasting should be taken with caution in ulcerative colitis patients but did not affect inflammatory markers in those population [15]. Interestingly, significant difference was observed in CKD patients underwent Ramadan fasting. In CKD patient, hs-CRP was significantly higher before Ramadan (85.9 ± 18.22 mg/dL vs 54.6 ± 22.72 mg/dL; $p < 0.001$) [16].

4. Discussion

Intermittent fasting may play significant role in religious life. Although fasting itself is not harmful when done to each own's capabilities, evidences suggest that intermittent fasting might not result in objective reductions of inflammatory markers (including TNF-α, CRP, and IL-6). Some studies found that in different populations marked with chronic inflammation may benefit from intermittent fasting, but in general population the inflammatory marker reduction is modest.

Fasting theoretically may provide anti-inflammatory effect due to increased C-leptin expression and reduced expression of TNF-α and IL-6, [4]. Inflammation itself is implicated to be related with metabolic syndrome and poor outcome in patients due to systemic disruption of homeostasis [17]. Unfortunately, researches measuring biomarkers during and after intermittent fasting found no significant impact except in CKD patients [16] and asthmatic [12]. Although asthmatic patient showed improvements in inflammatory markers after fasting, the opposite is true in ulcerative colitis patient [15].

In light of the findings, Ramadan fasting should not be restricted in people with chronic inflammatory conditions, as long as fasting is monitored closely and patient should be advised of

potential harm and its mitigations. Purported benefits of Ramadan fasting should be considered in light of the available evidences and clinical recommendations should always be based on best judgements to ensure benefits and safety of patients and population at large. Further studies should preferably be conducted as population-wide study according to scientific and unbiased manner to ensure meaningful conclusions.

5. Conclusions

In conclusion, we argue that the beneficial effect of fasting on inflammation is modest, if any. Meanwhile, meta-analyses showed no appreciable increase of proinflammatory cytokines either. We therefore argue that while fasting may not serve to lower inflammatory markers, fasting itself is not harmful as long as fasting is conducted to each own ability. Further researches involving different populations are therefore necessary to answer the pressing question: is prolonged intermittent fasting beneficial?

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Literature Review

Skin aging: is it skin booster really work?

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Abstract: Skin aging is a heterogeneous process that involves intrinsic and extrinsic mechanisms that lead to various structural and physiological changes in the skin. Aging induces a gradual decrease in levels of collagen and elastin which are main proteins responsible for maintaining skin firmness and elasticity. An effort to combat the symptoms of aging, various topical agents and clinical procedures such as chemical peels, injectables, and energy-based devices have been developed. Skin boosters are biological materials or bio-actives that encourage the skin to increase or improve skin aging. The aim of skin booster is to maintain skin hydration, delaying aging process. The concept of "skin boosters" originally pertains to the use of hyaluronic acid with the small particle size. Now, the various ingredients such as botulinum toxin, Poly-L-Lactic acid, Polymer D-Lactic acid, Polydeoxyribonucleotide (PDRNA) salmon, exosome, growth factors, peptide are currently being used as skin boosters. The crucial aspect of enhancing the effectiveness of skin boosters are particle size and delivery of the ingredients through the skin barrier. Various methods can be employed to achieve the delivery of the ingredients such as injection into the lesions, micro needling, electrophoresis, laser, and the others. The method of delivery is as important as the type of skin boosters choose and still needed the further research to enhance the effectiveness.

Keywords: aging; effectiveness; skin booster

1. Introduction

Globally, most people nowadays can anticipate living well into their sixties and beyond. Both the number and percentage of older people in the population are rising in every nation on the planet. At this point, there will be 1.4 billion people over the age of 60, up from 1 billion in 2020. The number of individuals in the world who are 60 years of age or older is expected to double (to 2.1 billion) by 2050. It is anticipated that between 2020 and 2050, the number of people 80 years of age or older will treble, reaching 426 million.[*)]

Skin aging is a heterogeneous process that involves intrinsic and extrinsic mechanisms that lead to various structural and physiological changes in the skin. Aging induces a gradual decrease in levels of collagen and elastin which are the main proteins responsible for maintaining skin firmness and elasticity.[1] Fine lines and a thinned epidermis are signs of intrinsic aging, which happens as people age. Conversely, extrinsic aging is primarily brought on by prolonged sun exposure and is typified by

deep wrinkles, skin laxity, and hyperpigmentation.[2] Glogau developed a photoaging scale to help determine the extent of skin photodamage. The Glogau photoaging scale was originally designed to characterize white skin, but with a modification, it can also be very helpful in the analysis of Asian skin and has the benefit of practical clinical use.[1]

The targeted therapeutic anti-aging effect of the skin is a continuous, step-by-step process, which combines various methods of skin rejuvenation, augmentation, restoration of each skin layer individually and in consideration of numerous other factors, such as lifestyle choices, immune system, heredity, emotional state, and general health One of the therapeutic modalities for skin aging is a skin booster.[1,3]

An effort to combat the symptoms of aging, various topical agents and clinical procedures such as chemical peels, injectables, and energy-based devices have been developed. Skin boosters are biological materials or bio-actives that encourage the skin to increase or improve skin aging. The aim of skin booster is to maintain skin hydration, delaying aging process.[3]

The concept of “skin boosters” originally pertains to the use of hyaluronic acid with the small particle size. The crucial aspect of enhancing the effectiveness of skin boosters are particle size and delivery of the ingredients through the skin barrier. Various methods can be employed to achieve the delivery of the ingredients such as injection into the lesions, micro needling, electrophoresis, laser, and the others. The method of delivery is as important as the type of skin boosters choose and still needed the further research to enhance the effectiveness.[4]

2. Skin aging

Skin aging is a component of the "aging mosaic" that occurs naturally in humans and takes different forms over time in various organs, tissues, and cells. Skin aging is a multifaceted biological process that results in progressive changes to each layer of the skin, cumulative changes to its structure and physiology, and changes to the appearance of the skin, particularly in areas exposed to light.[3]

Skin physiologic changes that seem inevitable and are influenced by hormonal and genetic factors over time are referred to as intrinsic or chronologic skin aging. Reticulated ridges disappear, blood flow is diminished, lipid levels are decreased, and collagen production is suppressed. Dry, pale skin that is less elastic, has fine wrinkles, and is less able to heal itself is the end result. The emergence of a variety of benign neoplasms, which are brought on by a disruption in the control of cell proliferation, is another feature of intrinsically aged skin.[5]

The physiologic and histologic alterations brought on by environmental factors are referred to as extrinsic skin aging. UV light is by far the most potent extrinsic aging factor. Photoaging is the term for the structural and functional changes in the skin caused by UV radiation. In addition to air pollutants,

smoking cigarettes, diet, chemical exposure, trauma, and other exogenous factors also contribute to extrinsic skin aging. Indeed, there has been a surge in interest in the effects of air pollution on skin physiology recently, which has led to a number of epidemiologic and mechanistic studies.[2]

Extrinsic aging is more responsive to intervention and preventive measures than intrinsic aging. Clinical signs of extrinsically aged skin include multiple telangiectases, deep wrinkles, laxity, coarseness, and increased fragility. These signs are primarily caused by UV radiation. Additionally, the pigmentation of photodamaged skin may appear mottled and darker. Skin that has aged externally is more likely to develop growths that are benign and malignant.[1,5] The histologic and clinical characteristics of intrinsically and extrinsically aged skin are summarized in **Table 1**.

Table 1. The typical histologic and clinical features of instrinsic and extrinsic skin aging[1,5]

	Instrinsic aging	Extrinsic aging
Histologic features	Epidermal thinning	Solar elastosis
	Loss of rete ridges	Reduced number of fibroblasts
	Decreased number of collagen and elastin fibers	Reduced amount of extracellular matrix
Clinical features	Xerosis	Xerosis
	Pallor	Multiple telangiectases
	Fine wrinkles	Deep wrinkles
	Decreased elasticity	Decreased elasticity
	Fragility	Fragility
		Dyspigmentation

The Glogau photoaging scale was originally designed to characterize white skin, but with a modification, it can also be very helpful in the analysis of Asian skin and has the benefit of practical clinical use[1].

Table 2. Glogau's classification of photoaging[5]

Type	Severity	Age group (years)	Features
I	Mild	Usually 28-35	Mild pigmentary change
			No keratoses
			Minimal wrinkles
II	Moderate	Usually 36-50	Early senile lentigines
			Keratoses palpable but not visible
			Parallel smile lines beginning
III	Advanced	Usually 51-65	Obvious dyschromia,
			Telangiectasias
			Visible keratoses
			Wrinkles even when not moving facial muscles
IV	Severe	Usually 66-75	Yellow-gray color of skin
			Prior skin malignancies
			Wrinkled throughout with no normal skin

The Glogau Scale is a four-level scale that is used to evaluate the severity of wrinkles and fine lines

on the face. Level 1 indicates minimal wrinkles, while Level 4 indicates severe wrinkles and deep folds in the skin.[5] This scale is an important tool in the evaluation of photoaging, as it provides a visual representation of the severity of the aging process (**Figure 1**) **)

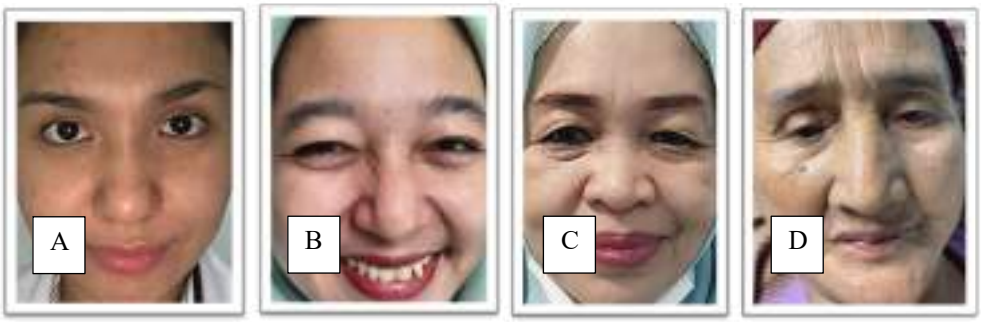

Figure 1. Type I photoaging (a), type II photoaging (b), type III photoaging (c), type IV photoaging (d) **)

**) Documentation of Dermatology Venereology and Aesthetic Department

3. Intervention in skin aging

Intervention of skin aging categorized into non-invasive and invasive procedures. The non-invasife skin treatment comes in the form of cosmetics, chemical peels, and phototherapy. In invasive therapy, micro-needling have gained attention in skin therapy which allows drug molecules to penetrate the skin. Other invasive procedures include injectables. These treatments target various signs of aging, but a complete understanding of the mechanisms underlying skin aging is necessary to develop a successful anti-aging treatment plan.[2]

Ultraviolet rays are a major factor in the aging of cells and skin. For this reason, the most fundamental and important form of sun protection is wearing sunscreen or protective clothing and staying in the shade. Some topical therapies that can be used for skin aging are retinoids, hydroxy acids, kinetin, antioxidants, and copper peptides. Improvement of skin aging can be seen after using topical therapy for 2-3 months.[2,6]

Endogenous antioxidant defenses are both nonenzymatic (eg, uric acid, glutathione, bilirubin, thiols, albumin, and nutritional factors, including vitamins and phenols) and enzymatic (eg, superoxide dismutases, glutathione peroxidases [GSHPx], and catalase. The most well-known systemic antioxidants include carotenoids, vitamin C, vitamin E, and the trace minerals selenium and copper. Studies have also shown that ferulic acid and the vitamins C and E provide both an antioxidant and a sunscreen effect.[3]

Various energy-based devices, such as lasers, high-intensity focused ultrasound (HFU), and radiofrequency (RF) devices, have grown increasingly common to address aging phenotypes. These

devices deliver thermal energy to the reticular dermis and subcutaneous tissue, which subsequently causes tissue contraction and stimulates neocollagenesis, leading to improvement in skin laxity and rhytides. An ablative laser, such as a CO2 laser or an Erbium:YAG laser, which requires re-epithelialization, has been used in the past, but recently, a non-ablative fractional laser has been used mainly to reduce the downtime and risk of adverse events including postinflammatory hyperpigmentation or scarring.[2]

4. Skin booster and mechanism

Skin booster is biological material or bio-actives that can increase or improve skin function. Repair the loss in skin's important component is the concept in skin booster.[7] Skin booster work by injecting a substance into the skin. The substance will improve the function of the skin naturally. Clinical studies show that skin booster hydration fillers can improve the elasticity of the face.[8]

Skin booster has many variation that has been used. Growth factor (GF) is the oldest type of skin booster. Growth factor can stimulate cells regeneration, but normal cell has less cell receptors that accept growth factor. Hyaluronic acid (HA) is the most common type os kin booster that can maintain and absorb water. Hyaluronic acid can induced collagen production and improve hydration of the skin. However, HA has some weak point like its longevity, pain during injection and as a filler, HA must be injected in right place. [4]

Botulinum toxin (botox) used in wrinkle and muscle hypertrophy treatment. Botulinum toxin work by suppressing of neurogenic inflammation, botox can also improve elasticity by increasing skin hydration. Poly-L-Lactic Acid (PLLA) and Polymer D-Lactic Acid (PDLA) being used as skin boosters especially in korea. It has strong point that volume retention in PLLA and PDLA is more than HA. This polymer also can increase collagen synthesis. However, long tem observation in side effect of PLLA and PDLA required. Polydeoxyribonucleotide (PDRN) are widely used in the korean markets because it does not make lump after injection. Our body use deoxyribonucleic acid (DNA) fragment for wound healing. Deoxyribonucleic acid also show similar result with PRP in skin rejuvenation procedures. Exosome is the prominent skin booster. Exosome is extracellular vehicles that produced inside or outside cell membrane that can used for facial rejuvenation. However, the production of exosome need more costs. [4]

5. Hyaluronic acid

Hyaluronic acid is widely used ingredients in cosmetic formulation. Hyaluronic acid can enchance viscosity and become skin conditioning agent. Hyaluronic acid divide to low molecular weight hyaluronic acid (LMW-HA) and high molecular weight hyaluronic acid (HMW-HA). High molecular

weight hyaluronic acid (up to 2,4X10^4 kDa) cannot penetrate epidermis, its function to decrease transepidermal water loss (TEWL) and make skin hydrated. Low molecular weight hyaluronic acid can penetrate to dermis and has anti aging function to the skin.[9]

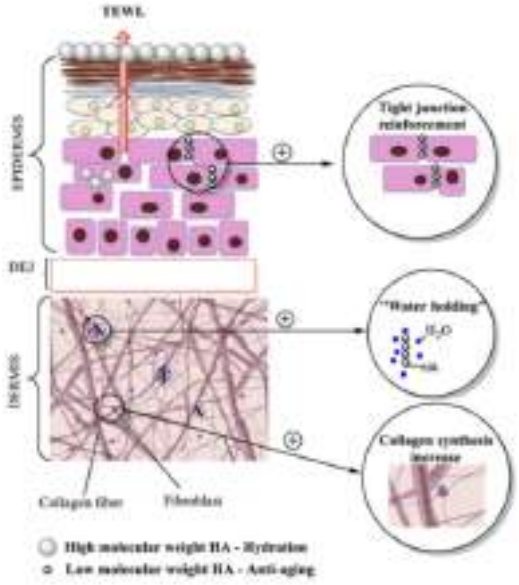

Figure 2. Hyaluronic acid activity[9]

Hyaluronic acid aslo has other function, such as collagen stimulation, anti aging, anti nasolabial folds, soft tissue augmentation, face rejuvenation, increase skin elasticity and tightness, anti-wrinkle, anti-erythema, skin hydration and skin augmentation.[9]

Hyaluronic acid found in the body especially in skin. Hyaluronic acid produced by fibroblast and keratinocytes. However, HA easily degraded but constantly renewed. Hyaluronic acid can be injected to dermis, make the skin bind more water and increase mechanical tension in extracellular matrix. Hyaluronc acid also stretch protein fibers, stimulate fibroblast, and increase collagen synthesis especially collagen type 1. Hyaluronic acid acitivity depend with the molecular weight and stability. Both cross link and non cross-link HA effective to increase skin hydration, improve elasticity and reduce roughness, but cross-link has longer periode than non-cross link HA.[8]

6. How to enhance the effectiveness of skin booster?

Hyaluronic acid as skin booster need to penetrate to the right place to get optimal effect.[4] Topical application is not too effective because no proper penetration to dermis. There are three ways that drugs can penetrate to dermis, intracellular pathway, intercellular pathway and follicular pathway.[10]

A RCT study shows that 50 and 130 kDa molecular weight of HA reduce wrinkle depth significantly. Low molecular weight of HA has more penetration potential.[11] Intradermal AH injection showed

improve skin texture (Kim, 2014).[12] Laser device can help HA penetrate to dermis. Combination fractional CO2 laser with AH cream show lower inflammation, infection, and wrinkle reduction. Meta analysis research with 2.363 subject show that HA product after fractional CO2 laser or peeling can improve wound healing repair. [11]

7. Conclusion

Skin aging induces a decrease in levels of collagen and elastin which are responsible for maintaining skin firmness and elasticity. Skin booster is a new concept of treatment that promotes a global improvement of skin aging, and to enhance the effectiveness of skin boosters are particle size and delivery of the ingredients through the skin barrier. The method of delivery is as important as the type of skin boosters chosen and still needed further research

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Literature Review

Reproductive function in female patients with Congenital Adrenal Hyperplasia (CAH)

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Abstract: Congenital adrenal hyperplasia (CAH) alludes to an uncommon endocrine disorders group inherited in an autosomal recessive manner. Most cases of CAH are caused by a lack of 21-hydroxylase enzyme deficiency, resulting in a decrease of mineralocorticoids but an increase of androgens. Affected individuals are classified into classic and non-classic CAH (NCCAH), with the first type associated with less 21-OH activity, resulting in severe clinical manifestations. Females with classic CAH often experience salt wasting and genital ambiguity in the early yearsof their life continuing with further health problems such as impaired fertility later in life. Females with CAH experience significant changes in their reproductive function. It primarily affects menstruation and fertility. Female patients affected by DSD will experience increased adrenal androgens leading to anovulation (oligomenorrhea, amenorrhea, or menorrhagia), dyspareunia, and polycystic ovary syndrome (PCOS).

Keywords: congenital adrenal hyperplasia; infertility; reproductive function

1. Introduction

Congenital adrenal hyperplasia (CAH) is a group of adrenal enzymes deficiencies that passed down in autosomal recessive manner. It is characterized by disorder of various key enzymes including 11β-hydroxylase (11βOH), 17α-hydroxylase (17OH; also known as 17, 20-lyase), 21-hydroxylase (21OH), steroidogenic acute regulatory protein (StAR), 3β hydroxysteroid dehydrogenase type 2 (3βHSD2), P450 oxidoreductase (POR) and P450 cholesterol side-chain cleavage (P450scc), which resulted in altered aldosterone and cortisol synthesis.[1–3] The most common enzyme deficiency is the 21-hydroxylase (21-OH) deficiency, comprising more than 95% of cases [4]. CAH are subdivided into classic (CCAH) and non-classic CAH (NCCAH), with the classical form is further separated into the simple-virilizing (SV) and salt-wasting (SW) form.[4–6]

Based on the remaining enzymatic activity, the clinical presentation can be minimal to severe.[2,5]

Classic CAH typically exhibits more severe clinical symptoms in infancy. Early clinical symptoms include varying degrees of abnormal genitalia and a salt-wasting crisis. Women with CAH, particularly those with the classic form, will have issues with menstruation and fertility later in life.[7] This characteristic features results from the altered production of glucocorticoids and mineralocorticoids. Along with other crucial tests like the serum 17-hydroxyprogesterone, testosterone level, and genetic testing, it will also aid in making the diagnosis of CAH.[2,5]

Females affected by the high adrenal androgen events and other relevant circumstances may experience numerous reproductive issues as adults as a result. They are more likely to develop polycystic ovarian syndrome (PCOS), dyspareunia, and anovulation (oligomenorrhea, amenorrhea, or menorrhagia), all of which can impair fertility. Additionally, a poor surgical reconstruction could make it difficult for them to engage in sexual activity. A further factor is whether affected children are given too many or too few glucocorticoids, which can affect their ability to conceive in the future. [2,5,7] This literature review intended to give a quick overview of the reproductive function in females with CAH because there are several potential causes for the reduced fertility in females with CAH.

2. Congenital adrenal hyperplasia (CAH)

2.1. Epidemiology

The prevalence of CAH is around 1:10,000, and ranges from 1: 5000 to 1: 20,000 annually.[8] The prevalence of the typical types of CAH ranges from 1:5000 to 1:15,000, but it may differ by ethnic/racial origin.[9] The non-classical form occurs in approximately 0.2% percent of the general white populations.[10] From two million newborns screened in New York, 105 confirmed instances of classical CAH were found; the prevalence was roughly one in 24,840 for black infants, one in 17,450 for Hispanic infants, one in 15,500 for Asian infants, and one in 15,500 for white children.[11] According to the 2018 Endocrine Society CAH guideline, which used a sample size of 30.000 cases, the incidence of CAH in China was around 1/6 064.[12] In Turkey, 38,935 infants were tested, and the results showed that 2265 (5.82%) needed more testing, and 212 (0.54%) were referred for clinical evaluation, during which six infants were found to have CAH. Following that, SW 21-hydroxylase deficiency (21-OHD) was discovered in four individuals (two males, two females). One male baby had 11-OHD CAH and one male newborn had simple virilizing 21-OHD. 1:7,787 people in the tested population had classical 21-OHD.[13] From 2009 to 2019, the Indonesian Pediatric Society documented 326 cases of CAH and 644 pediatric patients with CAH.[14]

2.2. Pathophysiology

Congenital adrenal hyperplasia (CAH) is an enzyme mutation disorder that affects one of the following enzymes: 11OH (11OH), 17OH (also known as 17, 20-lyase), 21OH (21OH), StAR (steroidogenic acute regulatory protein), 3HSD2 (three hydroxysteroid dehydrogenase type 2), P450 oxidoreductase (POR), and P450 cholesterol side-chain cleavage (P450scc but 21-hydroxylase (21OH) insufficiency is the main contributing factor (95%).[1–3]

When it is about to controlling the creation of CRH and ACTH, the hypothalamus receives feedback from cortisol secretion. The mechanism, which is encoded by CYP11A1 within the adrenal gland, starts with the conversion of cholesterol to pregnenolone by the P450 side chain cleavage enzyme in the conventional pathway. With progressively elevated 17-hydroxyprogesterone (17-OHP) and progesterone concentrations (consequences of cortisol deficit in final outcomes), decreased enzymatic activity of 21-hydroxylase hinders cortisol production. Impaired cardiac function, increased anti-diuretic hormone release, and poor vascular reactivity to catecholamines are all effects of cortisol insufficiency. A synthesis of aldosterone is hindered by loss of function mutations in full forms, which causes decreased urinary salt reabsorption and hyponatremia. Hypovolemia and high plasma renin levels follow if the mutations are not rapidly identified and treated. Because potassium cannot be eliminated effectively without aldosterone, hyperkalemia results. Due to their anti-mineralocorticoid actions and interference with aldosterone-mediated mineralocorticoid receptor transactivation in vitro, the higher 17-OHP and progesterone concentrations worsen the mineralocorticoid shortage. Reduced prenatal cortisol exposure hinders the growth of the adrenomedullary system and may be linked to hypoglycemia and epinephrine insufficiency.[9,14]

2.3. Diagnosis

The two subtypes of CAH that are of clinical importance are classic (CCAH) and non-classic (NCCAH), and the classical form is further split into salt-wasting (SW) and simple-virilizing (SV) CAH [4-6]. Males are diagnosed far less frequently than females, which may be related to the fact that males are less inclined to seek medical attention for symptoms associated with androgen excess.[13] Other causes of higher mortality include adrenal insufficiency, metabolic impairment, cardiovascular illness, and children who are incorrectly diagnosed with sepsis.[15]

The clinical symptoms (varying degrees of atypical genitalia, hirsutism, oligomenorrhea, and infertility in females), obesity, tumor, osteoporosis, serum 17-hydroxyprogesterone testing, and genetic testing are taken into consideration for making the first diagnosis. [2] Premature pubarche, also known as NCCAH or simple-virilizing CAH in children, is the early development of pubic hair. It is the

condition of pubic and axillary hair or apocrine odor existing before the ages of 8 for females and 9 for boys (before hitting puberty). The boys exhibit penile enlargement with prepubertal-sized testes and some reported gynecomastia, while the girls exhibit clitoromegaly. They reach puberty earlier, which has an impact on eventual height and causes short stature.[9,16,17]

A high level of 17-OHP (>240 nmol/L) is a sign for CAH, hence it is crucial to collect the sample in the morning and during the follicular phase in women who are menstruation. A value of less than 2.5 nmol/L in children and less than 6.0 nmol/L in adults has been suggested as the threshold to rule out CAH.[17] It is the primary substrate for the 21-hydroxylase and the biochemical sign of 21-hydroxylase deficiency. The ACTH stimulation test, which involves administering 250 mg of cosyntropin intravenously and measuring 17OHP after 60 minutes, is the following step and is regarded as the gold standard for the diagnosis. Basal and stimulated 17OHP levels in CCAH patients will be higher than 300 nmol/L. In both males and females during the follicular phase, a baseline 17OHP of greater than 15 nmol/L and/or an ACTH-stimulated 17OHP of greater than 30 nmol/L are regarded as diagnostic for NCAH.[17] When ambiguous genitalia first occurred, chromosomal analysis was taken into account.[18] Although genetic testing is required for diagnosis confirmation and genetic counseling, it is not thought to be a main diagnostic tool for NCCAH.[19] For accurate genetic counseling and carrier discovery, CYP21A2 genotyping is crucial.[20]

2.4. Treatment

Maintaining fertility, lowering hyperandrogenism, reducing long-term side effects of glucocorticoid medication, avoiding adrenal or testicular adrenal rest tumors, and improving quality of life are the key care objectives for people with 21-hydroxylase deficiency.[2,5] Mineralocorticoids and glucocorticoids are utilized as replacement therapies in classical CAH. With a starting dose of 0.1 mg per day and a dosage range of 0.05-0.2 mg per day administered in 1-2 doses, fludrocortisone is employed.[21] Adults should prefer prednisolone 1–5 mg/day divided into two doses and should completely avoid dexamethasone due to concerns about a poorer metabolic process and bone profile. Growth rate, weight, and bone age monitoring are always done concurrently with treatment. It is advised to assess hormone levels.[13] Although fludrocortisone is rarely used in NCAH, it has been used, perhaps to reduce the doses of glucocorticoids. Other treatment options include employing antiandrogens to prevent the effects of androgens or GnRH agonists or oral contraceptives to reduce ovarian androgen output.[17]

3. Reproductive function in female patients with Congenital Adrenal Hyperplasia (CAH)

Both men and women are affected by infertility, but it is always correlated with the severity of the illness.[17] Women's sexual function is influenced by a number of variables, such as gender identity, body image, sexual identity, lower urinary tract function, overall perception of health capacity, and ability to carry out everyday activities.[22–25]

In women with CAH, reproductive processes are drastically altered. Fertility and menstruation are seen as the key outcomes. Women who are affected by increased adrenal androgens may experience anovulation (oligomenorrhea, amenorrhea, or menorrhagia), dyspareunia, and polycystic ovary syndrome (PCOS).[2,4,7] Continuous progesterone elevation has contraceptive effects, and glucocorticoid therapy may help things along and make it possible to conceive.[17]

According to reports, the lowered fertility rates range from 60–80% in women with typical SV to 7–60% in those with classic SW CAH.[3] Several contributing variables, such as adrenal progesterone hypersecretion, excess androgen, the effects of genital reconstructive surgery, secondary polycystic ovary syndrome (PCOS), ovarian adrenal rest tumors, or even psychosexual issues, can cause subfertility in females with classic CAH. [3] The gold standard of care has been early genital reconstructive surgery for patients with the most severe CAH and who were raised as girls, restoring normal genital appearance and function.[26]

One of the main causes of poor reproductive outcomes in females with CAH is androgen excess. Anovulation or ovulation disorders are brought on by androgen over-secretions (partially aromatized to estrogens), which cause ongoing steroidal feedback and additional gonadotropin cyclicity loss. Adrenal androgens may also directly affect the process of folliculogenesis by having a negative impact on the activity of the aromatase enzyme in granulosa cells. Nearly all NC 21-OHD patients can resume regular menstrual cycles with appropriate adrenal androgen suppression, while women with a classic variation do not benefit from treatment. [3]

The endometrium may have a minipill effect as a result of the increased progesterone levels in the follicular phase, which will cause anovulatory cycles. There were some negative effects of progesterone hypersecretion taken into account. It may have negative effects on the quality of cervical mucus and sperm penetration, as well as on endometrial maturation, endometrial receptivity, implantation, and oocyte quality. The inhibition of 17-a-hydroxyprogesterone does not necessarily result in an increase in progesterone. In a few circumstances, adrenalectomy has successfully normalized progesterone levels and led to spontaneous pregnancy. The risks of this surgery, however, include anesthesia and surgical problems. Additionally, the patient will become totally adrenal deficient. Sexual distress may follow genital surgery. 46% of the women in the CAH adult research executive follow-up expressed dissatisfaction with their sexual lives.[3]

Psychosexual issues are an additional crucial element. Girls with CAH exhibit more aggressive physical conduct and less feminine interests in toys, sports, and playmates as children. They are also more inclined to use physical force to resolve disputes. The effects of prenatal exposure to high testosterone levels, which resulted in the masculinization of the brain of female fetal tissue, have been linked to this masculinized behavior, which is most evident in SW CAH. Females with 21-OHD frequently have a troubled body image throughout their adolescent years. They also engage in less marriage, partnership, and sexual activity. The most frequent cause of ambiguous genitalia is Congenital Adrenal Hyperplasia (CAH), which may also be a factor in poor sexual characteristics.[3,27]

Less research has been done on women with NCAH, but it has been claimed that 10–30% of these women report having fertility issues, which may be caused by hormonal imbalances. Ovulation inducement produces successful results. In addition, 53-68% of women with NCAH can conceive on their own even before diagnosis and treatment, and the likelihood increases to 78% once hydrocortisone treatment begins. Treatment with glucocorticoids is beneficial for family planning. It reduced the gestation period from almost a year to less than six months.[3] The women's glucocorticoid dosage may need to be increased throughout pregnancy, and routine clinical follow-ups are advised, including blood pressure control and gestational diabetes management, which are on the list. [3,17] Additionally, improper surgical repair hinders sexual activity. Future infertility may be affected by the use of glucocorticoids inappropriately or excessively in pediatric medicine.[2,4,7,21]

4. Conclusion

Females with CAH experience significant changes in their reproductive function. It primarily affects menstruation and fertility. Women who are affected by increased adrenal androgens may experience anovulation (oligomenorrhea, amenorrhea, or menorrhagia), dyspareunia, and polycystic ovary syndrome (PCOS). Additionally, improper surgical repair hinders sexual activity.

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