BUKTI KORESPONDENSI ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul Artikel : Association of vitamin D with deoxyribonucleic acid (dna) damage: A systematic review of animal and human studies

Nama Jurnal : The Journal Of The Polish Biochemical Society and Of The Polish Academy Of Sciences

Penulis : Mayang Indah Lestari, Krisna Murti*, Iche Andriyani Liberty, Zen Hafy, Violantina Linardi, Muhammad Khoirudin, Tungki Pratama Umar

No	Perihal	Tanggal
1	Bukti Konfirmasi Submit Artikel	31 Januari 2023
2	Bukti Konfirmasi Review dan Hasil Revisi	15 Mei 2023
3	Bukti konfirmasi artikel accepted dan artikel lengkap	15 Mei 2023

Bukti Konfirmasi Submit Artikel (31 Januari 2023)

Unsri Google Apps

Krisna Murti unsri <krisna.arinafril@unsri.ac.id>

[ABP] Submission Acknowledgement

abp@ptbioch.edu.pl <abp@ptbioch.edu.pl> Tue, Jan 31, 2023 at 3:47 PM
Reply-To: Małgorzata Basaj <abp@ptbioch.edu.pl>
To: Krisna Murti <krisna.arinafril@unsri.ac.id>, Iche Andriyani Liberty <icheandriyaniliberty@fk.unsri.ac.id>, Zen Hafy <zenhafy@gmail.com>, Violantina Linardi <violantinalinardi10@gmail.com>, Muhammad Khoirudin <mkhoirudin32@gmail.com>, Tungki Pratama Umar <tungkipratama@gmail.com>

Hello,

Mayang Indah Lestari has submitted the manuscript, "Association of Vitamin D with Deoxyribonucleic Acid (DNA) Damage: A Systematic Review of Animal and Human Studies: Vitamin D and DNA Damage" to Acta Biochimica Polonica.

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Małgorzata Basaj

The following message is being delivered on behalf of Acta Biochimica Polonica.

[ABP] Submission Acknowledgement

External Inbox ×

abp@ptbioch.edu.pl Tue, Jan 31, 2023, 3:47 PM

to me, Iche, Zen, Violantina, Muhammad, Tungki

Hello,

Mayang Indah Lestari has submitted the manuscript, "Association of Vitamin D with Deoxyribonucleic Acid (DNA) Damage: A Systematic Review of Animal and Human Studies: Vitamin D and DNA Damage" to Acta Biochimica Polonica.

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Małgorzata Basaj

The following message is being delivered on behalf of Acta Biochimica Polonica.

Received, thank you. Thanks a lot. Noted with thanks.

Bukti Konfirmasi Review dan Hasil Revisi (15 Mei 2023)

Krisna Murti unsri <krisna.arinafril@unsri.ac.id>

[ABP] Editor Decision reference number 6641 Revision

abp@ptbioch.edu.pl <abp@ptbioch.edu.pl> Mon, May 15, 2023 at 12:06 AM
Reply-To: Dr Mikołaj Olejniczak <m.olejniczak@abp.ptbioch.edu.pl>
To: Mayang Indah Lestari <mayangindah@fk.unsri.ac.id>, Krisna Murti <krisna.arinafril@unsri.ac.id>, Iche Andriyani Liberty <icheandriyaniliberty@fk.unsri.ac.id>, Zen Hafy <zenhafy@gmail.com>, Violantina Linardi <violantinalinardi10@gmail.com>, Muhammad Khoirudin <mkhoirudin32@gmail.com>, Tungki Pratama Umar <tungkipratama@gmail.com>

reference number 6641 entitled: "Association of Vitamin D with Deoxyribonucleic Acid (DNA) Damage: A Systematic Review of Animal and Human Studies: Vitamin D and DNA Damage"

Dear Dr. Murti,

I would like to inform you that your paper can be accepted for publication in Acta Biochimica Polonica after major revisions.

Please find the comments below this letter. Please, note that the comments of the Reviewer D are in the attached word file, titled "review_Acta_Biochimica_Polonica_2023.docx" (if this file is not accessible, please, contact me to send it directly).

Please consider carefully the report and amend the manuscript accordingly.
You are also kindly requested to answer in detail to all comments and describe them
in the accompanying letter which should be attached as the first page of the revised manuscript.
When preparing the revised version of your manuscript please refer to the Instructions to Authors and to the latest issue of Acta Biochimica Polonica available on-line at https://ojs.ptbioch.edu.pl/index.php/abp/about/submissions to follow the style and requirements of the journal.

It is assumed that the final manuscript will be accepted by all the authors.

Please send the revised version of the article (with changes marked in color) and the cover letter describing the changes made to the manuscript by the system (under Revisions).

Thank you in advance for your prompt revision,
Sincerely

Dr Mikołaj Olejniczak
m.olejniczak@abp.ptbioch.edu.pl

Associate Editor

Acta Biochimica Polonica

Reviewer A:

The review is quite interestig, prepared carefully and with details. In general, I do not have any major comment. Besides, that I would change the order of paragraphs, as follows: paragraph Discussion describing the role of vitD should be first, before Study characteristics and Risk of bias. As an minor point I noticed that 'RCT' shortcut not exlained in the next and Table 2.

Recommendation: Accept Submission

Reviewer D:
Recommendation: Resubmit for Review

The following message is being delivered on behalf of Acta Biochimica Polonica.

D-review_Acta_Biochimica_Polonica_2023.docx
20K

EXPLANANATION OF STATEMENT, COMMENT AND RESULT OF REVISED PAPER FROM

REVIEWER A

The highest thanks to reviewer who have patiently and carefully examined and revised our article titled: **Association of Vitamin D with Deoxyribonucleic Acid (DNA) Damage: A Systematic Review of Animal and Human Studies**

We are very happy to receive your comment that you have presented in our article. We realize that there is a shortcoming in our article based on your review. Finally, we could construct better article with your substantially important issue addressed to us.

1. **The review is quite interestig, prepared carefully and with details. In general, I do not have any major comment. Besides, that I would change the order of paragraphs, as follows: paragraph Discussion describing the role of vitD should be first, before Study characteristics and Risk of bias. As an minor point I noticed that 'RCT' shortcut not exlained in the next and Table 2.**

   Adjustment: Thank you very much for your comment. We have corrected the manuscript ac cording to your input.

EXPLANANATION OF STATEMENT, COMMENT AND RESULT OF REVISED PAPER FROM

REVIEWER D

The highest thanks to reviewer who have patiently and carefully examined and revised our article titled: **Association of Vitamin D with Deoxyribonucleic Acid (DNA) Damage: A Systematic Review of Animal and Human Studies**

We are very happy to receive your comment that you have presented in our article. We realize that there is a shortcoming in our article based on your review. Finally, we could construct better article with your substantially important issue addressed to us.

For Author and Editor:

The paper is interesting and gives an overview of articles on the relationship between vitamin D and DNA damage. The methodology for selecting articles for the review is described in detail. I have some concerns that need to be addressed:

1. **Page 2 – List of abbreviations: it should be "25(OH)D = 25 hydroxy vitamin D" instead of "25(OH)2D = 25 dihydroxy vitamin D" because the molecule contains only one hydroxyl group at position 25.**
   Adjustment: Thank you for your comment. We have corrected the abbreviation as instructed.

2. **Page 4, Introduction: There is a problem with nomenclature. Vitamin D3 is known as cholecalciferol. Calcipotriol is a synthetic derivative of Vitamin D3 used to treat psoriasis. This should be corrected.**
   Adjustment: Thank you for your comment. We are sorry about this issue and have corrected it as instructed. It is also aligns with the third comment below.

3. **Page 4, Introduction: Add an appropriate reference after "…from human skin".**
   Adjustment: Thank you for your comment. We have added a new reference in the revised manuscript:
   Osmancevic A, Sandström K, Gillstedt M, Landin-Wilhelmsen K, Larkö O, Wennberg Larkö A-M, F. Holick M, Krogstad A-L (2015) Vitamin D production after UVB exposure – A comparison of exposed skin regions. J. Photochem. Photobiol. B Biol. 143: 38–43.

4. **Page 6: The following sentence, "Studies must detail how vitamin D solely (not in combination with other substances) affects DNA damage to be considered" is unclear and should be rephrased.**
   Adjustment: Thank you for your comment. We have edited the sentence:
   Studies must demonstrate the influence of a single vitamin D substance administration on DNA damage (rather than a formulation containing multiple compounds) to be considered.

5. **Page 7: DNA damage parameters should be specified**
   Adjustment: Thank you for your comment. It has been addressed in our revised manuscript as mentioned below:

"comet tail tail length, tail DNA, and tail moment; phosphorylated Histone H2AX (γH2AX), 8-hydroxy-2'-deoxyguanosine (8-OHdG), chromosomal aberration, DNA damage score, micronuclei formation, telomere length, Urinary cyclobutane thymine (T–T) dimer, and DNA repair indicator"

6. **Discussion: the first paragraph describing DNA damage parameters should be moved to the Introduction.**
   Adjustment: Thank you for your comment. We have moved the section containing chromosomal aberration and sister chromatid exchange to the introduction (in addition to other parameters like micronuclei and comet assay).

7. **25 dihydroxy vitamin D (25(OH)2D) – it is monohydroxy derivative with OH group at position 25, please note my previous remark**
   Adjustment: Thank you for your careful observation. Several phrases loacted at the page 12-14 has been revised.

8. **Please note that abbreviations should be explained only when used for the first time. T2DM is explained on pages 12 and 13.**
   Adjustment: Thank you for your careful observation. It has been revised.

9. **Page 13: The role of vitamin D hydroxylation in reducing insulin resistance is unclear when based on the following description: "Vitamin D also mediates Ca2+-dependent endopeptidase activity to convert pro-insulin to insulin, and cells have the ability to hydroxylate 25(OH)D to the active form 1,25(OH)2D3 via the 1-α hydroxylase enzyme (Bland et al., 2004). The above findings confirm that vitamin D can reduce insulin resistance in T2DM patients (Wenclewska et al., 2019)". Please, explain what you mean.**
   Adjustment: Thank you for your careful observation. It has been revised.

10. **Page 13: The following description of the synthesis and metabolism of vitamin D should be moved to the Introduction: "Endogenous synthesis of vitamin D begins with cholesterol oxidation, producing pro-vitamin D3. In the skin, UVB radiation converts pro-vitamin D3 to pre-vitamin D3. Pre-vitamin D3 isomerization to vitamin D3 is also referred to as cholecalciferol. Two hydroxylations by the enzymes vitamin D 25-hydroxylase (CYP27A1) and renal mitochondrial 1-hydroxylase (CYP27B1) are required to convert vitamin D3 to active 1α,25(OH)2D3 (calcitriol). Calcitriol binds to vitamin D receptors belonging to the nuclear receptor family and forms a complex with RXR to regulate gene expression (Deuster et al., 2017)."**
    Adjustment: Thank you for our valuable input. We have changed the position of these sentences to the introduction while maintaining its coherence with previously available phrases (also mentioned in comment 3). It is mentioned below:
    "Endogenous synthesis of vitamin D begins with cholesterol oxidation, producing pro-vitamin D3. In the skin, Ultraviolet B (UVB) from sunlight converts pro-vitamin D3 to pre-vitamin D3. Then, isomerization of pre-vitamin D3 is done, with vitamin D3

(cholecalciferol) as its main end product (Osmancevic et al., 2015). Two hydroxylations by the enzymes vitamin D 25-hydroxylase (CYP27A1) and renal mitochondrial 1-hydroxylase (CYP27B1) are required to convert vitamin D3 to active 1,25(OH)2D3 (calcitriol). Calcitriol binds to vitamin D receptors (VDRs) belonging to the nuclear receptor family and forms a complex with RXR to regulate gene expression (Deuster et al., 2017)."

11. **Page 14: "In this study, it was found that vitamin D deficiency is a risk factor for malignancy (cancer) and accelerate the invasion process." – reference for the study should be added.**
    Adjustment: Thank you for highlighting this issue. We have added the references for it.

12. **Page 14: the following sentence should be rephrased: "Consequently, sperm function by nongenomic mechanisms during spermatogenesis or fertilization (Jurutka et al., 2001)"**
    Adjustment: Thank you for your observation. We have deleted the sentence because it did not aligns with previous discussion.

13. **Page 15: provide appropriate references for the mentioned studies: "The results of studies in animal models (in vivo) are in line with those of studies in cells (in vitro)".**
    Adjustment: Thank you, we have added the references (Chen et al., 2018; Liu et al., 2019).

14. **Table 1: ensure that all abbreviations are explained when used for the first time.**
    Adjustment: Yes, we have checked all abbreviations in the table.

15. **The following recommendation made by the Authors should be more precise: "a diet with sufficient vitamin D content and supplementation is recommended to prevent DNA damage and oxidative stress in cells." Do the Authors mean any specific serum concentrations or a recommended daily dose of vitamin D?**
    Adjustment: Thank you, we have added the dose of >1000 IU (approximately 2000-5000 IU).

For Editor: The article may be of interest to readers. However, the Authors should thoroughly revise it before publication.

Association of Vitamin D with Deoxyribonucleic Acid (DNA) Damage: A Systematic Review of Animal and Human Studies

Mayang Indah Lestari[1,2], Krisna Murti[3*], Iche Andriyani Liberty[4], Zen Hafy,[1] Violantina Linardi[5], Muhammad Khoirudin[5], Tungki Pratama Umar[5]

[1]Doctoral Study Program in Biomedical Science, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia

[2]Department of Anesthesiology and Intensive Therapy, Faculty of Medicine, Sriwijaya University-Dr. Mohammad Hoesin General Hospital - Siti Fatimah General Hospital, Palembang, Indonesia

[3]Department of Anatomical Pathology, Faculty of Medicine, Sriwijaya University-Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia

[4]Department of Public Health, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia

[5]Research Assistant, Department of Anesthesiology and Intensive Therapy, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia

*Corresponding author:

Krisna Murti

Department of Anatomical Pathology, Faculty of Medicine, Sriwijaya University-Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia

E-mail: krisna.arinafril@unsri.ac.id

Keywords: Vitamin D, DNA damage, Observational studies, In vivo studies, Systematic review

Acknowledgements of financial support

None

Conflict of interest

None declared

Data availability statement

Data available upon request from the corresponding author

List of Abbreviations

γH2AX = phosphorylated histone H2AX

1,25(OH)2D = 1,25-dihydroxyvitamin D

8-OH-dG = 8-hydroxy-2'-deoxyguanosine

25(OH)D = 25 hydroxy vitamin D

DNA = Deoxyribonucleic acid

NF-κB Nuclear factor kappa-light-chain-enhancer of activated B cells

ROS = Reactive oxygen species

T2DM = Type 2 diabetes mellitus

UV = Ultraviolet

VDR = Vitamin D receptor

Abstract

Vitamin D has anti-proliferative, anti-inflammatory, and apoptotic abilities. Vitamin D deficiency can induce deoxyribonucleic acid (DNA) damage. The aim of the study was to create a systematic review to analyze the relationship between vitamin D and DNA damage in various populations. PubMed, Scopus, EbscoHost, Google Scholar, and Epistemonikos were used to identify literature regarding the relationship between vitamin D and DNA damage. Assessment of study quality was carried out by three independent reviewers individually. A total of 25 studies were assessed as eligible and included in the study. 12 studies were conducted in humans consisting of 2 studies with experimental design and 10 studies with observational pattern. Meanwhile, 13 studies were conducted in animals (in vivo). It is found that the majority of studies found that vitamin D prevents DNA damage and minimizes the impact of DNA damage that has occurred ($p<0.05$). However, two studies (8%) did not find such an association and one research only found a specific association in the cord blood, not in maternal blood. Vitamin D has a protective effect against DNA damage. The diet rich in vitamin D and vitamin D supplementation are recommended to prevent DNA damage.

Keywords: Vitamin D, DNA damage, Observational studies, In vivo studies, Systematic review

Introduction

Vitamin D and its receptors play an essential role in cancer development due to its anti-proliferative, anti-inflammatory, and apoptotic properties (Nair-Shalliker et al., 2012a; Deuster et al., 2017; Elhusseini et al., 2018). Endogenous synthesis of vitamin D begins with cholesterol oxidation, producing pro-vitamin D3. In the skin, Ultraviolet B (UVB) from sunlight converts pro-vitamin D3 to pre-vitamin D3. Then, isomerization of pre-vitamin D3 is done, with vitamin D3 (cholecalciferol) as its main end product (Osmancevic et al., 2015). Two hydroxylations by the enzymes vitamin D 25-hydroxylase (CYP27A1) and renal mitochondrial 1-hydroxylase (CYP27B1) are required to convert vitamin D3 to active 1,25(OH)2D3 (calcitriol). Calcitriol binds to vitamin D receptors (VDRs) belonging to the nuclear receptor family and forms a complex with RXR to regulate gene expression (Deuster et al., 2017). VDRs are nuclear receptor superfamily members expressed in tumours to regulate cell cycle-related proliferation and angiogenesis (Khrisnan et al., 2012; Christakos et al., 2015). In addition, vitamin D has been shown in several studies to be effective in stimulating Deoxyribonucleic acid (DNA) synthesis in mature alveolar cells, modulating epithelial cell proliferation, and repairing injury (Usman et al., 2021).

Furthermore, adequate vitamin D levels help to maintain DNA integrity. Vitamin D's role can be divided into two categories: primary functions that prevent DNA damage and secondary processes that regulate cell growth rate (Nair-Shalliker et al., 2012a; Wenclewska et al., 2019). Vitamin D is really crucial since its deficiency is associated with an increased frequency of chromosomal aberrations, sister chromatid exchanges, micronuclei formations, and alteration of comet assays (related to oxidative stress, hypoxic, and apoptotic process), the important indicators of DNA damage (Peng et al., 2010; Nair-Shalliker et al., 2012a; O'Callaghan-Gordo et al., 2017; Liu et al., 2019). The potential of vitamin D in reducing oxidative DNA damage in humans refers to clinical trials in which vitamin D supplementation reduced levels of 8-

hydroxy-2'-deoxyguanosine (8-OH-dG), an oxidative damage biomarker found in colorectal epithelial crypt cells (Nair-Shalliker et al., 2012a). Vitamin D administration has also been shown to reduce oxidative stress-induced damage and chromosomal aberrations, prevent telomere shortening, and inhibit telomerase activity in animal models and other cell types (Siebert et al., 2018). Vitamin D's secondary functions in preventing DNA damage include regulating poly-ADP-ribose polymerase activity on the DNA damage response pathway during the DNA lesion detection process. Vitamin D can also prevent the replication of damaged DNA and regulate apoptosis, which promotes cell death (Nair-Shalliker et al., 2012a).

Although vitamin D has long been discussed as one of the essential DNA protectors, a systematic review of the relationship between vitamin D and DNA damage has yet to be found. As a result, the purpose of this study is to use a systematic review approach to examine the association between vitamin D and DNA damage in different populations of human studies and animal models.

Methods

The manuscript was arranged using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) 2020 guidelines (Page et al., 2021). All members approved the review panel's study procedure before conducting the literature search. The protocol has been registered in the International prospective register of systematic reviews (PROSPERO) with the registration number: CRD42023393054.

Literature retrieval

A comprehensive literature search was conducted across five databases to identify manuscripts on the relationship between vitamin D levels and DNA damage in human and animal subjects. The articles were discovered using Scopus, PubMed, EBSCOHost, Google Scholar, and Epistemonikos. Hand-searching was also conducted based on the included study bibliography to identify relevant publications that were not indexed in the previously reported databases (Umar et al., 2022). There will be no restrictions on geographical region or gender. However, we restrict our search to studies published between January 2012 and January 2023. The investigation was completed by January 19th, 2023. To ensure its validity, the study must be published in English. The search terms for this study is available as Supplementary Table 1.

Study selection

We sought animal and human studies investigating the link between vitamin D levels and DNA damage. Studies must demonstrate the influence of a single vitamin D substance administration on DNA damage (rather than a formulation containing multiple compounds) to be considered. The study was deemed ineligible if its design consisted of a literature review (e.g., systematic review, narrative review, scoping review) and opinion or editorial pieces. Meanwhile, studies must have access to the full text. As a result, we excluded conference abstracts, posters, and

unretrieved complete records. Duplicates were removed from the literature retrieval. Three independent reviewers (VL, MK, and ZH) assessed titles and abstracts (primary screening) using a semi-automated process aided by Rayyan QCRI software (Ouzzani et al., 2016; Umar & Siburian, 2022). Following the completion of the first screening stage, the full text was assessed by two reviewers (IAL and TPU) to determine its eligibility for inclusion in the review. Any disagreements were discussed and resolved by a senior author (MIL) at any stage of manuscript evaluation.

Data extraction

The following information was extracted from the data: authorship, country of study, research participant data in the form of age, sex, and comorbidities (human), as well as experimental research data on animal type and age (animal studies), DNA damage parameters (comet tail tail length, tail DNA, and tail moment; phosphorylated Histone H2AX (γH2AX), 8-hydroxy-2'-deoxyguanosine (8-OHdG), chromosomal aberration, DNA damage score, micronuclei formation, telomere length, Urinary cyclobutane thymine (T–T) dimer, and DNA repair indicator), and main findings. The information was recorded on the extraction sheet using Microsoft Office Excel 2019. Because of the vast diversity among included studies, the findings were presented as a qualitative synthesis rather than a meta-analysis.

Risk of bias analysis

The risk of bias (ROB) analysis was conducted for the animal studies using The Systematic Review Center for Laboratory Animal Experimentation's risk of bias (SYRCLE's RoB) tool. The RoB tool from SYRCLE contains ten items related to selection bias, performance bias, detection bias, reporting bias, and other biases (Hooijmans et al., 2014). Meanwhile, we used three different scales for human studies: the Newcastle-Ottawa Scale (for observational

studies), the Risk Of Bias In Non-randomized Studies - of Interventions (ROBINS-I), and Version 2 of the Cochrane risk-of-bias tool for randomized trials (ROB-2). The Newcastle Ottawa Scale is divided into three major domains (selection, comparability, and outcome) (Liana et al., 2022). In contrast, the ROBINS-I (Sterne et al., 2016) and ROB-2 (Sterne et al., 2019) are divided into several parts within the pre-intervention, intervention, and post-intervention stages. The evaluation was carried out independently by two authors (IAL and TPU). In the event of a disagreement, the decision is made by a senior author (KM).

Results

The search strategy identified a total of 942 studies. The search query found 551 studies on SCOPUS, 140 on Google Scholar, 211 on PubMed, 28 on EbscoHost, and 12 on Epistemonikos. Following duplicate detection, 243 studies were excluded. Then, 699 studies entered title and abstract screening, where 56 studies were deemed eligible for full-text screening, which resulted in 24 studies being included in the final analysis. Meanwhile, six studies were identified from the citation search, and only one was finally contained. This process resulted in 25 selected studies (13 animal studies/in vivo and 12 human studies (two experimental studies and ten observational research)) considered in the final process of manuscript evaluation (Figure 1).

Study characteristics

All of the included studies evaluated the association of vitamin D with DNA damage. Animal studies (table 1) were done on the vitamin D-deficient diet (Chen et al., 2018; Elhusseini et al., 2018; Merino et al., 2018), hypertension (Machado et al., 2016, 2019), oxidative stress (Haq et al., 2019; Liu et al., 2019), and neurological disorder (Alfawaz et al., 2014; Mehri et al., 2020). Meanwhile, all of following parameters were assessed only in one study: diabetes mellitus (Meerza et al., 2012), high-fat diet (Merino et al., 2018), kidney disease (Mohammed et al., 2019), aging (Qiao et al., 2020), and ovariectomy (Siebert et al., 2018) model. There are four main DNA damage detection methods, including immunohistochemistry (Chen et al., 2018; Elhusseini et al., 2018; Mohammed et al., 2019), comet assay (alkaline comet assay) (Alfawaz et al., 2014; Liu et al., 2019; Machado et al., 2016, 2019; Siebert et al., 2018), Enzyme-linked immunosorbent assay (ELISA) (Haq et al., 2019; Mehri et al., 2020), and micronuclei detection (Liu et al., 2019; Machado et al., 2016). Meanwhile, other detection methods are flow cytometry (Merino et al., 2018), gel electrophoresis (Haq et al., 2019),

Western blot (Qiao et al., 2020) and Reverse transcription polymerase chain reaction (RT-PCR) (Siebert et al., 2018). All studies used rodent as the animal model, either as mice or rat. It was found that all DNA damage parameters are associated with vitamin D levels, except in one study which did not found significant difference in the percentage of DNA in comet tails in the vitamin D deficiency group when compared to the control group (Machado et al., 2019). Another study also found that preventive impact of vitamin D supplementation is better than its treatment effect to ameliorate DNA damage (Alfawaz et al., 2014).

Human research (table 2) were conducted in experimental (randomized and non-randomized clinical trial) (Wenclewska et al., 2019; Gungor et al., 2022) and observational (cross-sectional and cohort) (Ladeira et al., 2015; Lan et al., 2014; Nair-Shalliker et al., 2012b; Najeeb et al., 2020; Ng et al., 2021; O'Callaghan-Gordo et al., 2017; Petersen et al., 2014; Usman et al., 2021; Wang et al., 2016; Fagundes et al., 2019) manner. In human studies, the most commonly employed parameter is comet assay (comet tail length, DNA damage score, and percentage of DNA in comet tail) (Fagundes et al., 2019; Lan et al., 2014; Najeeb et al., 2020; Ng et al., 2021; Wang et al., 2016; Wenclewska et al., 2019), and micronuclei formation (buccal or lymphocyte) (Fagundes et al., 2019; Ladeira et al., 2015; Nair-Shalliker et al., 2012b; O'Callaghan-Gordo et al., 2017; Usman et al., 2021). Other parameters are aniline blue staining (sperm DNA damage) (Gungor et al., 2022), thymine dimer (Petersen et al., 2014), and ELISA (urinary 8-OHdG) (Usman et al., 2021). Several conditions are observed in the included studies, such as diabetes mellitus, cancer, obesity, vitamin D-deficient state, and infertility. Similar finding was also observed as in animal studies, when majority of the investigations revealed a significant association between vitamin D and DNA damage status. However, a study in workers occupationaly exposed to formaldehyde (Ladeira et al., 2015) and on general population (Nair-Shalliker et al., 2012b) did not showed significant association between vitamin D level and micronuclei formation, while another study only found the association in

cord blood but not in maternal blood (O'Callaghan-Gordo et al., 2017). Another research also did not presented any association between vitamin D level and comet assay result as the DNA damage marker in general population (Wang et al., 2016).

Risk of bias

All of the animal studies included in the analysis followed a similar pattern (figures 2 and 4), with a low risk of bias on baseline characteristics, random outcome assessment, selective outcome reporting, and other bias. However, it is noteworthy that allocation concealment, random housing, and intervention blinding were not met by all studies, resulting in a high risk of bias. Meanwhile, only four studies (Liu et al., 2019; Mehri et al., 2020; Mohammed et al., 2019; Siebert et al., 2018) have a low risk of bias for outcome assessor blinding, and only two studies (Mehri et al., 2020; Siebert et al., 2018) have a low risk of bias for incomplete outcome data analysis.

We assessed the risk of bias in human studies (figure 3) using three scales: NOS, ROBINS-I, and ROB-2. Most studies (10/12; 83.33%) have moderate/some concerns about bias. A non-randomized study, on the other hand, runs the risk of bias due to insufficient intervention classification. Meanwhile, there is only one study (O'Callaghan-Gordo et al., 2017) that has a low overall risk of bias. According to the summary graph (figure 4) on observational studies, 70% have a high risk of bias on comparability due to a lack of explanation on confounding control. However, regarding selection, 70% of the studies have a low risk of bias, while 20% of the included research has a high risk of bias. Most studies have a moderate risk of bias in outcome assessment, primarily due to non-blinding outcome assessment.

Discussion

DNA damage can be divided into two types, endogenous and exogenous. Endogenous DNA damage stems from chemically active DNA involved in hydrolytic and oxidative reactions with air and reactive oxygen species (ROS), which are naturally present in cells. In contrary, exogenous DNA damage occurs due to the involvement of environmental, physical, and chemical substances such as UV and ionizing radiation, alkylating agents, and cross-linking agents (Chatterjee & Walker, 2018). Vitamin D is regarded as an essential factor in the status of DNA damage (Najeeb et al., 2020). Vitamin D deficiency (plasma 25(OH)D <50 nmol/l) and severe deficiency (<30 nmol/l) have been associated with elevated oxidative stress, DNA damage promotion, and overall mortality (Wang et al., 2016).The impact of vitamin D on DNA damage is prominent several disorders, including hyperglycemia and cancer (Gabryanczyk et al., 2021).

Hyperglycemia increases the production of free radicals and also induces DNA damage (Giacco & Brownlee, 2010). Studies conducted in patients with type 2 diabetes mellitus (T2DM) showed that vitamin D significantly prevented DNA damage and oxidative stress in patients with T2DM ($p<0.05$). A vitamin D-responsive element has been identified in the promotion region of the insulin receptor gene in humans (Gikas et al., 2009). Pancreatic cells express the nuclear receptor for 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), which modulates insulin action (Bland et al., 2004). Furthermore, vitamin D minimizes insulin resistance by its effect on calcium and phosphorus metabolism along with the upregulation of the insulin receptor gene, as well as suppresses the synthesis of proinflammatory cytokines that contribute to insulin resistance, including interleukins and TNF-a due to its antioxidative properties (Wenclewska et al., 2019; Maestro et al., 2002; Talaei et al., 2013).

In malignancy, vitamin D has positive functions as anti-proliferative, proapoptotic, anti-inflammatory, anti-angiogenesis, anti-metastatic and anti-invasion as well as inhibiting estrogen signaling (Vuolo et al., 2012; Deuster et al., 2017; Wacker & Holiack, 2013). Calcitriol

(active form of vitamin D) inhibits the proliferation of many malignant cells by inducing cell cycle arrest and cell accumulation in the G0/G1 phase of the cell cycle. In cells, calcitriol causes G1/G0 arrest in a p53-dependent manner by increasing the expression of the cyclin-dependent kinase inhibitors p21Waf/Cip1 and p27Kip1, decreasing the activity of cyclin-dependent kinase 2 (CDK2), and causing hypo-phosphorylation. Calcitriol also increases the expression of p73, a homologue of p53, which which is associated with the induction of apoptosis in several human and murine tumor systems. Suppression of p73 abrogates calcitriol-induced apoptosis and reduces the ability of calcitriol to enhance the cytotoxic effect of agents such as gemcitabine and cisplatin in a squamous cell carcinoma (SCC) model (Krishnan & Feldman, 2010; Khrisnan et al., 2012). In the previous study, it was found that vitamin D deficiency is a risk factor for malignancy (cancer) and accelerate the invasion process (Najeeb et al., 2020; Migliaccio et al., 2022).The populations of the 25 studies in this systematic review are diverse. Twelve studies conducted in humans analyzed DNA damage in patients with comorbid diseases such as T2DM, obesity, infertility, cancer patients, and the general population. Meanwhile, thirteen in vivo studies analyzed DNA damage using animal models with hypertension, ovariectomy, nephrotoxicity, vitamin D deficiency, and oxidative stress.

Several studies on vitamin D indicated that the vitamin has a beneficial impact on all organ systems of the human body. Both 25(OH)D and its hormonally active form, 1,25(OH)2D are vital for physiological functions, especially to reduce inflammation and excessive cellular oxidative stress. The 1,25(OH)2D hormone or calcitriol modulates cell proliferation through direct and indirect pathways, such as by the inhibition of the transcription factor, Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) which is associated with an elevation of oxidative stress and cellular response to inflammation and injury (Tilstra et al., 2012). Due to suppression of NF-κB activation, calcitriol helps to reduce chronic inflammation (Myszka

& Klinger, 2014). However, more research into the relationship between DNA damage, oxidative stress, and vitamin D is required.

Vitamin D receptor (VDR) is found in testicular tissue, prostate and spermatozoa. In addition, intense metabolism of vitamin D in the male reproductive system and increased expression of VDR in the neck of the sperm cause males to require vitamin D for functionally active sperm (Jensen et al., 2011). Incubation of semen samples with vitamin D for 30 minutes led to a significant increase in sperm velocity parameters. This progressive increase in motility is due to vitamin D-dependent calcium release and subsequent cyclic AMP/protein kinase A (cAMP/PKA) activation and Adenosine triphosphate (ATP) production (Gunter et al., 2004). There was a significant negative correlation ($p<0.05$) between vitamin D and sperm DNA damage in this systematic review. After binding to the VDR receptor, vitamin D initiates slow genomic effects by stimulating the release of ligand-activated transcription factors in the nucleus. In unexplained infertile patients with vitamin D deficiency, sperm DNA damage may occur due to delayed genomic effects (Jurutka et al., 2001).

Studies in various animal models show that vitamin D exerts a protective effect on DNA. Vitamin D can reduce the DNA damage index (percentage of DNA in comet tails assessed from comet tests). In addition, in animal models induced by cyclophosphamide, vitamin D can reduce the frequency of micronuclei which is a marker of DNA damage. Beside percentage of DNA in comet tails and the frequency of micronuclei, DNA damage can also be assessed by the levels of 8-OHdG, a marker of oxidative DNA damage (Smith et al., 2005). Elevated levels of 8-OHdG in rats animal models are also associated with a complete loss of VDR expression. Because the expression of VDR depends on the availability of 1,25(OH)2D, the loss of VDR suggests that there may be a role for 1,25(OH)2D in protecting cells against hyperproliferation and oxidative DNA damage (Kállay et al., 2002; Nair-Shalliker et al., 2012a). Decreased levels of 8-OHdG after vitamin D supplementation was proven in the animal

model studies (Haq et al., 2019; Mohammed et al., 2019). The results of studies in animal models (in vivo) are in line with those of studies in cells (in vitro) (Chen et al., 2018; Liu et al., 2019). These outcomes can confirm that vitamin D has a protective effect on DNA.

Various parameters of oxidative stress are also presented in this systematic review. In human studies, vitamin D supplementation led to a significant decrease in NO and total thiols and an increase in the concentration of reduced glutathione (GSH) leading to a decrease in oxidative processes in cells (Fagundes et al., 2019). In a study of animal models with hypertension, vitamin D was not significantly associated with DNA damage. However, vitamin D3 deficiency alters the level of Thiobarbituric Acid Reactive Substance (TBARS) in a mouse model of spontaneous hypertension, which is an indicator of Reactive Oxygen Species (ROS)-initiated peroxidation of unsaturated fatty acids in membrane lipids and alters the permeability, fluidity, and integrity of the plasma membrane (Potter et al., 2011). Lipid peroxidation predisposes patients to conditions such as hypertension and thromboembolic (Yavuzer et al., 2016). Vitamin D can reduce oxidative stress that occurs in cells thereby reducing DNA damage.

This systematic review proved that vitamin D protects against DNA damage. However, there are some limitations to this systematic review. First, the study populations are largely heterogeneous with different diseases and DNA damage parameters; thus, the results can be biased. In addition, human studies are still sparse (only one randomized controlled trial/RCT) and mainly with a moderate risk of bias. Consequently, the application of the results to humans still needs to be considered. Further studies with randomized controlled trial designs are expected in the future to increase the strength of evidence.

Acknowledgements

None

Conclusion

There is a significant association between vitamin D and DNA damage. However, although the majority of studies have found that vitamin D has a protective effect against DNA damage, other research found contradictory findings. Thus, the need of further investigations with stricter criteria must be commenced. Nevertheless, it is safe to conclude that a diet with sufficient vitamin D content and supplementation (more than 1000 IU/day, preferably about 2000-5000 IU/day) is recommended to prevent DNA damage and oxidative stress in cells.

References

Alfawaz HA, Bhat RS, Al-Ayadhi L, El-Ansary AK (2014) Protective and restorative potency of Vitamin D on persistent biochemical autistic features induced in propionic acid-intoxicated rat pups. *BMC Complement. Altern. Med.* **14**: 416. https://doi.org/10.1186/1472-6882-14-416

Bland R, Markovic D, Hills CE, Hughes S V., Chan SL, Squires PE, Hewison M (2004) Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. *J. Steroid Biochem*121–125.

Chatterjee N, Walker GC (2018) Mechanisms of DNA damage, repair, and mutagenesis. *Environ. Mol. Mutagen.* **58**: 235–263. https://doi.org/10.1002/em.22087.Mechanisms

Chen L, Yang R, Qiao W, Yuan X, Wang S, Goltzman D, Miao D (2018) 1,25-Dihydroxy vitamin D prevents tumorigenesis by inhibiting oxidative stress and inducing tumor cellular senescence in mice. *Int. J. cancer* **143**: 368–382. https://doi.org/10.1002/ijc.31317

Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G (2015) Vitamin D: Metabolism, molecular mechanism of action, and pleiotropic effects. *Physiol. Rev.* **96**:

365–408. https://doi.org/10.1152/physrev.00014.2015

Deuster E, Jeschke U, Ye Y, Mahner S, Czogalla B (2017) Vitamin D and VDR in gynecological cancers-A systematic review. *Int. J. Mol. Sci.* **18**: 2328. https://doi.org/10.3390/ijms18112328

Elhusseini H, Elkafas H, Abdelaziz M, Halder S, Atabiekov I, Eziba N, Ismail N, El Andaloussi A, Al-Hendy A (2018) Diet-induced Vitamin D Deficiency Triggers Inflammation and DNA Damage Profile in Murine Myometrium. *Int. J. Womens. Health* **10**: 503–514. https://doi.org/10.2147/IJWH.S163961

Fagundes G, Macan T, Rohr P, Damiani A, Rocha F Da, Pereira M, Longaretti L, Vilela T, Ceretta L, Mendes C, Silveira P, Teixeira J, Andrade V de (2019) Vitamin D3 as adjuvant in the treatment of type 2 diabetes mellitus: modulation of genomic and biochemical instability. *Mutagenesis* **34**: 135–145.

Gabryanczyk A, Klimczak S, Szymczak-Pajor I, Śliwińska A (2021) Is Vitamin D Deficiency Related to Increased Cancer Risk in Patients with Type 2 Diabetes Mellitus? *Int. J. Mol. Sci.* **22**: 6444. https://doi.org/10.3390/ijms22126444

Giacco F, Brownlee M (2010) Oxidative stress and diabetic complications. *Circ. Res*1058–1070.

Gikas A, Sotiropoulos A, Pastromas V, Papazafiropoulou A, Apostolou O, Pappas S (2009) Seasonal variation in fasting glucose and HbA1c in patients with type 2 diabetes. *Prim. Care Diabetes*111–114.

Gungor K, Gungor ND, Basar MM, Cengiz F, Ersahin SS, Cil K (2022) Relationship between serum vitamin D levels semen parameters and sperm DNA damage in men with unexplained infertility. *Eur. Rev. Med. Pharmacol. Sci.* **26**: 499–505.

Gunter T, Yule D, Gunter K, Elisee R, Salter J (2004) Calcium and mitochondria. *FEBS Lett* **567**: 96–102.

Haq SH, AlAfaleq NO, Johari R Al (2019) Vitamin D Treatment Reverses the Induced Oxidative Stress Damage to DNA. *Pakistan J. Biol.* **22**: 8–14.

Hooijmans CR, Rovers MM, de Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW (2014) SYRCLE's risk of bias tool for animal studies. *BMC Med. Res. Methodol.* **14**: 43. https://doi.org/10.1186/1471-2288-14-43

Jensen M, Bjerrum P, Jessen T, Nielsen J, Joensen U, Olesen I, Petersen J, Juul A, Dissing S, Jorgensen N (2011) Vitamin D is positively associated with sperm motility and increases intracellular calcium in human spermatozoa. *Hum Reprod* **26**: 1307–1317.

Jurutka P, Whitfield G, Hsieh J, Thompson P, Haussler C, Haussler M (2001) Molecular Nature of the Vitamin D Receptorand its Role in Regulation of Gene Expression. *Rev Endocr Metab Disord* **2**: 203–216.

Kállay E, Bareis P, Bajna E, Kriwanek S, Bonner E, Toyokuni S, Cross HS (2002) Vitamin D receptor activity and prevention of colonic hyperproliferation and oxidative stress. *Food Chem. Toxicol.* **40**: 1191–1196. https://doi.org/10.1016/s0278-6915(02)00030-3

Khrisnan A, Trump D, Johnson C (2012) The Role of Vitamin D in Cancer Prevention and Treatment. *Rheum Dis Clin North Am* **38**: 161–78. https://doi.org/10.1016/j.rdc.2012.03.014.The

Krishnan A V., Feldman D (2010) Molecular pathways mediating the anti-inflammatory effects of calcitriol: Implications for prostate cancer chemoprevention and treatment. *Endocr. Relat. Cancer* **17**: 19–38. https://doi.org/10.1677/ERC-09-0139

Ladeira C, Pádua M, Veiga L, Viegas S, Carolino E, Gomes MC, Brito M (2015) Influence of serum levels of vitamins A, D, and E as well as vitamin D receptor polymorphisms on micronucleus frequencies and other biomarkers of genotoxicity in workers exposed to formaldehyde. *Lifestyle Genomics* **8**: 205–214.

Lan N, Luo G, Yang X, Cheng Y, Zhang Y, Wang X, Wang X, Xie T, Li G, Liu Z (2014) 25-

Hydroxyvitamin D3-deficiency enhances oxidative stress and corticosteroid resistance in severe asthma exacerbation. *PLoS One* **9**: e111599.

Liana P, Liberty IA, Murti K, Hafy Z, Salim EM, Zulkarnain M, Umar TP (2022) A systematic review on neutrophil extracellular traps and its prognostication role in COVID-19 patients. *Immunol. Res.* **70**: 449–460. https://doi.org/10.1007/s12026-022-09293-w

Liu H, Feng X, Wu S, Zong T, Li B, Zhang, Zengli (2019) Vitamin D Resists Cyclophosphamide-Induced Genomic and DNA Damage in CHL Cells In Vitro and in Mice In Vivo. *Nutr. Cancer* **71**: 1030–1039.

Machado C da S, Venancio VP, Aissa AF, Hernandes LC, de Mello MB, Del Lama JEC, Marzocchi-Machado CM, Bianchi MLP, Antunes LMG (2016) Vitamin D3 deficiency increases DNA damage and the oxidative burst of neutrophils in a hypertensive rat model. *Mutat. Res. Toxicol. Environ. Mutagen.* **798**: 19–26.

Machado CDS, Aissa AF, Ribeiro DL, Antune L, Greggi M (2019) Vitamin D supplementation alters the expression of genes associated with hypertension and did not induce DNA damage in rats. *J. Toxicol. Environ. Health* **82**: 299–313.

Maestro B, Molero S, Bajo S, Dávila N, Calle C (2002) Transcriptional activation of the human insulin receptor gene by 1, 25-dihydroxyvitamin D3. *Cell Biochem. Funct.* **20**: 227–232.

Meerza D, Naseem I, Ahmed J (2012) Effect of 1, 25$(OH)_2$ vitamin D_3 on glucose homeostasis and DNA damage in type 2 diabetic mice. *J. Diabetes Complications* **26**: 363–368. https://doi.org/10.1016/j.jdiacomp.2012.05.013

Mehri N, Haddadi R, Ganji M, Shahidi S, Soleimani Asl S, Taheri Azandariani M, Ranjbar A (2020) Effects of vitamin D in an animal model of Alzheimer's disease: Behavioral assessment with biochemical investigation of Hippocampus and serum. *Metab. Brain*

Dis. **35**: 263–274.

Merino O, Sánchez R, Gregorio B, Sampaio F, Risopatrón J (2018) Effects of Diet-Induced Obesity and Deficient in Vitamin D on Spermatozoa Function and DNA Integrity in Sprague-Dawley Rats. *Biomed Res Int*

Migliaccio S, Di Nisio A, Magno S, Romano F, Barrea L, Colao AM, Muscogiuri G, Savastano S (2022) Vitamin D deficiency: a potential risk factor for cancer in obesity? *Int. J. Obes. (Lond).* **46**: 707–717. https://doi.org/10.1038/s41366-021-01045-4

Mohammed M, Aboulhoda B, Mahmoud R (2019) Vitamin D attenuates gentamicin-induced acute renal damage via prevention of oxidative stress and DNA damage. *Hum Exp Toxicol* **38**: 321–335.

Myszka M, Klinger M (2014) [The immunomodulatory role of Vitamin D]. *Postepy Hig. Med. Dosw. (Online)* **68**: 865–878. https://doi.org/10.5604/17322693.1110168

Nair-Shalliker V, Armstrong BK, Fenech M (2012a) Does vitamin D protect against DNA damage? *Mutat. Res. - Fundam. Mol. Mech. Mutagen.* **733**: 50–57. https://doi.org/10.1016/j.mrfmmm.2012.02.005

Nair-Shalliker V, Fenech M, Forder PM, Clements MS, Armstrong BK (2012b) Sunlight and vitamin D affect DNA damage, cell division and cell death in human lymphocytes: a cross-sectional study in South Australia. *Mutagenesis* **27**: 609–614.

Najeeb HA, Othman R, Salih SF, Mohammed AA, Ismaeel Q Al (2020) Vitamin D level and endogenous DNA damage in patients with cancers in Duhok city, KRG-Iraq. *Ann Med Surg* **10**: 462–467.

Ng CY, Amini F, Ahmad Bustami N, Tan ESS, Tan PY, Mitra SR (2021) Association of DNA damage with vitamin D and hair heavy metals of obese women. *Mol. Cell. Toxicol.* **17**: 429–438.

O'Callaghan-Gordo C, Kogevinas M, Fthenou E, Pedersen M, Espinosa A, Chalkiadaki G,

Daraki V, Dermitzaki E, Decordier I, Georgiou V, Merlo DF, Roumeliotaki T, Vande Loock K, Kleinjans J, Kirsch-Volders M, Chatzi L (2017) Vitamin D insufficient levels during pregnancy and micronuclei frequency in peripheral blood T lymphocytes mothers and newborns (Rhea cohort, Crete). *Clin. Nutr.* **36**: 1029–1035. https://doi.org/10.1016/j.clnu.2016.06.016

Osmancevic A, Sandström K, Gillstedt M, Landin-Wilhelmsen K, Larkö O, Wennberg Larkö A-M, F. Holick M, Krogstad A-L (2015) Vitamin D production after UVB exposure – A comparison of exposed skin regions. *J. Photochem. Photobiol. B Biol.* **143**: 38–43. https://doi.org/10.1016/j.jphotobiol.2014.12.026

Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A (2016) Rayyan—a web and mobile app for systematic reviews. *Syst. Rev.* **5**: 210. https://doi.org/10.1186/s13643-016-0384-4

Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE (2021) The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* **372**: n71. https://doi.org/10.1136/bmj.n71

Peng X, Vaishnav A, Murillo G, Alimirah F, Torres KEO, Mehta RG (2010) Protection against cellular stress by 25-hydroxyvitamin D3 in breast epithelial cells. *J. Cell. Biochem.* **110**: 1324–1333. https://doi.org/10.1002/jcb.22646

Petersen B, Wulf HC, Triguero-Mas M, Philipsen PA, Thieden E, Olsen P, Heydenreich J, Dadvand P, Basagaña X, Liljendahl TS, Harrison GI, Segerbäck D, Schmalwieser AW, Young AR, Nieuwenhuijsen MJ (2014) Sun and ski holidays improve vitamin D status, but are associated with high levels of DNA damage. *J. Invest. Dermatol.* **134**: 2806–2813. https://doi.org/10.1038/jid.2014.223

Potter TM, Neun BW, Stern. ST (2011) Assay to detect lipid peroxidation upon exposure to nanoparticles. *Mol. Biol* **697**: 181–89.

Qiao W, Yu S, Sun H, Chen L, Wang R, Wu X, Goltzman D, Miao D (2020) 1,25-Dihydroxyvitamin D insufficiency accelerates age-related bone loss by increasing oxidative stress and cell senescence. *Am. J. Transl. Res.* **12**: 507–518.

Siebert C, Santos T Dos, Bertó C, Parisi M, Coelho R, Manfredini V, Barbé-Tuana F, Wyse A (2018) Vitamin D Supplementation Reverses DNA Damage and Telomeres Shortening Caused by Ovariectomy in Hippocampus of Wistar Rats. *Neurotox Res* **34**: 538–546.

Smith SM, Zwart SR, Block G, Rice BL, Davis-Street JE (2005) The nutritional status of astronauts is altered after long-term space flight aboard the International Space Station. *J. Nutr.* **135**: 437–443. https://doi.org/10.1093/jn/135.3.437

Sterne JAC, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, Henry D, Altman DG, Ansari MT, Boutron I, Carpenter JR, Chan A-W, Churchill R, Deeks JJ, Hróbjartsson A, Kirkham J, Jüni P, Loke YK, Pigott TD, Ramsay CR et al. (2016) ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* **355**: i4919. https://doi.org/10.1136/bmj.i4919

Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, Cates CJ, Cheng H-Y, Corbett MS, Eldridge SM, Emberson JR, Hernán MA, Hopewell S, Hróbjartsson A, Junqueira DR, Jüni P, Kirkham JJ, Lasserson T, Li T, McAleenan A et al. (2019) RoB 2: a revised tool for assessing risk of bias in randomised trials. *BMJ* **366**: l4898. https://doi.org/10.1136/bmj.l4898

Talaei A, Mohamadi M, Adgi Z (2013) The effect of vitamin D on insulin resistance in patients with type 2 diabetes. *Diabetol. Metab. Syndr.* **5**: 8. https://doi.org/10.1186/1758-5996-5-8

Tilstra JS, Robinson AR, Wang J, Gregg SQ, Clauson CL, Reay DP, Nasto LA, St Croix CM, Usas A, Vo N, Huard J, Clemens PR, Stolz DB, Guttridge DC, Watkins SC, Garinis GA, Wang Y, Niedernhofer LJ, Robbins PD (2012) NF-κB inhibition delays DNA damage-

induced senescence and aging in mice. *J. Clin. Invest.* **122**: 2601–2612. https://doi.org/10.1172/JCI45785

Umar TP, Samudra MG, Nashor KMN, Agustini D, Syakurah RA (2022) Health professional student's volunteering activities during the COVID-19 pandemic: A systematic literature review. *Front. Med.* **9**: 797153. https://doi.org/10.3389/fmed.2022.797153

Umar TP, Siburian R (2022) Routine Laboratory Testing Role For Covid-19 Identification: A Systematic Review. *Med. Sci. J. Adv. Res.* **3**: 99–106.

Usman M, Woloshynowych M, Britto JC, Bilkevic I, Glassar B, Chapman S, Ford-Adams ME, Desai A, Bain M, Tewfik I, Volpi E V. (2021) Obesity, oxidative DNA damage and vitamin D as predictors of genomic instability in children and adolescents. *Int. J. Obes.* **45**: 2095–2107. https://doi.org/10.1038/s41366-021-00879-2

Vuolo L, Di Somma C, Faggiano A, Colao A (2012) Vitamin D and Cancer. *Front. Endocrinol. (Lausanne).* **3**: 1–13. https://doi.org/10.3389/fendo.2012.00058

Wacker M, Holiack MF (2013) Vitamin D-effects on skeletal and extraskeletal health and the need for supplementation. *Nutrients* **5**: 111–148. https://doi.org/10.3390/nu5010111

Wang EW, Collins AR, Pang MYC, Siu PPM, Lai CKY, Woo J, Benzie IFF (2016) Vitamin D and oxidation-induced DNA damage: is there a connection? *Mutagenesis* **31**: 655–659. https://doi.org/10.1093/mutage/gew033

Wenclewska S, Szymczak-Pajor I, Drzewoski J, Bunk M, A Sliwińska (2019) Vitamin D Supplementation Reduces Both Oxidative DNA Damage and Insulin Resistance in the Elderly with Metabolic Disorders. *Int J Mol Sci* **20**: 2891.

Yavuzer H, Yavuzer S, Cengiz M, Erman H, Doventas A, Balci H, Erdincler DS, Uzun H (2016) Biomarkers of lipid peroxidation related to hypertension in aging. *Hypertens. Res* **39**: 342–48.

Tables

Table 1. Data extraction for animal studies

Author (year)	Country	Experimental animal	Disease model and inducer	Parameter	Method	Major findings
(Alfawaz et al., 2014)	Saudi Arabia	Western Albino rats (±21 days, n=28)	Neurotoxicity by PPA	• Tail length • Tail DNA (%) • Tail Moment (Unit)	Comet DNA assay	• There is a potential impact of vitamin D in protecting and treating PPA neurotoxicity, while ameliorating the DNA-damaging effects of PPA. • Prevention impact of vitamin D against PPA-induced DNA damage is more profound than its treatment effect
(Chen et al., 2018)	China	1α(OH)ase$^{-/-}$and wild-type mice (120 pairs divided into four groups)	Vitamin D-deficient model	• γH2AX • 8-OHdG	Immunohistochemistry	• There is an increase of DNA damage in 1,25(OH)2D3-deficient mice as measured by γH2AX and 8-OHdG on tumor cells according to immunohistochemistry
(Elhusseini et al., 2018)	United States	Female C57BL/6 mice, (4–6 weeks old), control (n = 10), vitamin D-deficient diet group (n = 10)	Vitamin D-deficient model	• γH2AX • DNA repair (RAD50 and RAD51)	Immunohistochemistry	• Vitamin D-deficient mice showed increased γ-H2AX in myometrial tissue compared to healthy controls (P<0.05) • Vitamin D deficiency caused a significant reduction in the expression of DNA repair genes such as RAD50 (P<0.005) and RAD51 (P<0.005) compared to healthy controls.
(Haq et al., 2019)	Saudi Arabia	Wistar Albino rats (1 week, n = 4)	Hydrogen peroxide (H_2O_2)	• Chromosomal aberration (DNA fragmentation) • 8-OHdG	• Gel electrophoresis • ELISA	• Vitamin D, which was given for 24 hours prior to the induced oxidative stress by H_2O_2 significantly ($p<0.001$) reversing the deleterious and damaging effect of H_2O_2 alone as presented by DNA fragmentation percentage • Significantly lower value of 8-OHdG following the administration of vitamin D+ H_2O_2 than H_2O_2 alone ($p<0.05$)
(Liu et al., 2019)	China	Male mice (7–8 weeks)	CP	• DNA damage score, total DNA in the tail (%), tail length, tail moment • Micronuclei formation	• Alkaline comet assay • Buccal Micronuclei Cytome assay	• Vitamin D3 suppressed CP-induced micronucleus formation in mice Buccal cells, with an alleviation range of 36.73–44.46% ($p<0.05$) • Vitamin D3 injection for a dose of 5,000 IU significantly reduced CP-induced DNA damage, with a 46.6% decrease in tail DNA percentage ($p<0.05$), a 24.2% decrease in tail length ($p<0.05$), and 37 ,3% decrease in olive tail moment

						(P<.05). However, it is not significant at the 1,000 or 10,000 IU dose
(Machado et al., 2016)	Brazil	Male Spontaneously hypertensive rats (SHR) and WKY (20 weeks) divided into six groups)	Hypertension model	• DNA damage score, total DNA in the tail (%), tail length, tail moment • Micronuclei formation	• Alkaline comet assay • Micronucleus test	• Vitamin D3 deficient diet was able to increase the percentage of DNA damage in both SHR (49%) and WKY rats (54%) • SHR rats with a vitamin D3 deficient diet showed a significant increase in the incidence of micronuclei formation in bone marrow and peripheral blood ($p<0.05$)
(Machado et al., 2019)	Brazil	Male Spontaneously hypertensive rats (SHR) and WKY (20 weeks) divided into six groups)	Hypertension model	• Total DNA in the tail (%)	Alkaline comet assay	• There was no significant difference in the percentage of DNA in comet tails ($p>0.05$) in the vitamin D deficiency group when compared to the control group. Vitamin D3 supplementation or deficiency did not significantly affect cardiac genotoxicity.
(Meerza et al., 2012)	India	Female albino mice	Diabetes model by alloxan (200 mg/kgBW)	• DNA tail length	Comet assay	• Vitamin D-supplemented group showed a significant decrease in liver (21.80 ± 2.40 μm) and pancreatic (19.25 ± 1.90 μm) DNA tail length in diabetic mice
(Mehri et al., 2020)	Iran	Wistar rats (200–240g, n=48)	Alzheimer's Disease by 5 μl of Aβ-containing solution	• 8-OHdG	ELISA	• The level of DNA damage in Vitamin D and Aβ + Vitamin D groups in hippocampus and Vitamin D group of serum samples was significantly lower than that of Aβ group ($p < 0.0001$)
(Merino et al., 2018)	Chile	Male Sprague-Dawley rats (4 months, n=20 divided into four groups)	High-fat diet and vitamin-D deficient-diet	• DNA Fragmentation	Flow cytometry	• Vitamin D supplementation results in lower DNA fragmentation, either at the control or experimental group ($p<0.05$) • The interaction between the vitamin D deficiency and diet-induced obesity was significant in DNA fragmentation ($p = 0.0359$)
(Mohammed et al., 2019)	Egypt	Male albino rats (8-10 weeks, n=24 divided into four groups)	Acute renal damage by gentamycin 100 mg/kgBW	• 8-OHdG	Immunohistochemistry	• Vitamin D administration significantly reduce 8-OHdG immunohistochemical expression when compared to control group ($p< 0.01$)

(Qiao et al., 2020)	China	Cyp27b1$^{+/-}$ and wild type mice (9 months)	Aging model	• γ-H2AX	Western blot	• 1,25(OH)2D insufficiency increases γ-H2AX expression significantly ($p<0.001$) compared with the wild type mice
(Siebert et al., 2018)	Brazil	Wistar Albino rats (90 days or 180 days, divided into four groups)	OVX	• DNA damage index • Telomere length	• Alkaline comet assay • RT-PCR	• OVX significantly increases DNA damage ($p < 0.001$) when compared to control. Vitamin D alone decreased the DNA damage index ($p < 0.05$ and $p < 0.001$), but when associated with OVX (OVX + VIT D), partially reversed DNA damage induced by OVX ($p < 0.001$). • Vitamin D did not change telomere length ($p > 0.05$), and when associated with OVX (OVX + VIT D), Vitamin D supplementation was able to reverse the observed telomere shortening ($p < 0.005$).

Abbreviation: γ-H2AX = γ phosphorylated form of the histone H2AX, 1,25(OH)2D3 = 1,25-dihydroxyvitamin D3, 1α(OH)ase = 1alpha-Hydroxylase, 8-OHdG = 8-Hydroxyguanosine, Aβ = Amyloid beta, C57BL/6 = C57 black 6, CP = Cyclophosphamide, Cyp27b1 = Cytochrome p450 27B1, DNA = Deoxyribonucleic acid, ELISA = Enzyme-linked immunosorbent assay, H_2O_2 = Hydrogen peroxide, IU = International unit, mg/kgBW = milligrams/kilograms body weight, OVX = Ovariectomy, PPA = Propionic acid, RAD50 = DNA repair protein RAD50, RAD51 = DNA repair protein RAD51, RT-PCR = Reverse transcription polymerase chain reaction, WKY = Wistar Kyoto rat

Table 2. Data extraction for human studies

Author (year)	Location	Design	Age	% Male	Population	Method	Outcome
(Fagundes et al., 2019)	Brazil	Prospective cohort	62.11 ± 9.64[a,d]	37	75 patients with type 2 diabetes melitus who were given supplementation of vitamin D3 4000 IU/day for 8 weeks	• Comet assay • Buccal micronuclei cytome assay	• Decreased DNA damage index (comet assay) ($p\leq0.05$) and micronuclei formation ($p\leq0.05$) following supplementation with vitamin D3 and wash-out period • There is a negative correlation between DNA damage index and vitamin D levels ($r=0.2569$; $p<0.0001$) but not in micronuclei.
(Gungor et al., 2022)	Turkey	Non-RCT	34.67± 4.01[a,c]	100	58 men with unexplained infertility (+50 controls)	• Aniline blue staining (sperm DNA damage)	• There was a negative and significant correlation between vitamin D levels and sperm DNA damage ($r = -0.605$, $p<0.001$)
(Ladeira et al., 2015)	Portugal	Cross-sectional	39.64 ± 11.5[a,c]	65.45	55 workers occupationally exposed to Formaldehyde (+80 controls)	• CBMN assay (buccal and lymphocyte)	• Vitamin D has no association with frequency of micronuclei (lymphocytes or buccal cells) in workers exposed to formaldehyde
(Lan et al., 2014)	China	Cross-sectional	50 ± 10[a,c]	50	16 patients with severe asthma and vitamin D <30ng/ml (+16 controls	• Comet assay (DNA damage score)	• Total DNA damage score for subject with Vitamin D deficiency was significantly increased compared to the scores in Vitamin D sufficiency ($p = 0.002$).

					and 16 patients with vitamin D >30ng/ml)		
(Nair-Shalliker et al., 2012b)	Australia	Cross-sectional	46.0 (27.1-61.4)[b,d]	NA	207 participants	• CBMN assay	• There is no association between log serum 25(OH)D concentration and log-transformed frequency of any CBMN-cyt assay biomarker (p=0.3)
(Najeeb et al., 2020)	Iraq	Cross-sectional	61.87 ± 12.77[a,c]	48.89	45 cancer patients (+ 35 controls)	• Comet assay	• Correlation between Tail DNA% and plasma vitamin D is significant in cancer patients (r^2=0.3707; p<0.0001) and control (r^2=0.2824; p<0.001) with higher damage at lower vitamin D level
(Ng et al., 2021)	Malaysia	Cross-sectional	29.96 ± 0.63[a,c]	0	134 participants (47 obese, 87 non-obese)	• Alkaline comet assay	• Multivariate analysis revealed that individuals with serum 25(OH)D level of ≥ 31 nmol/L had a significantly lower tail moment (1.06 ± 0.22 nmol/L vs. 2.37 ± 0.60 nmol/L; p = 0.029) and tail olive moment (2.36 ± 0.24 nmol/L versus 3.41 ± 0.46 nmol/L; p = 0.031) compared to those with lower serum 25(OH)D level, in the obese group
(O'Callaghan-Gordo et al., 2017)	Spain	Cross-sectional	NA	NA	344 participants (173 mothers and 171 newborns)	• CBMN assay	• In cord blood, 25(OH)D insufficient values (<50 nmol/L) were associated with increased lymphocyte micronuclei frequency (adjusted IRR = 1.32 (1.00, 1.72)), but not in maternal blood
(Petersen et al., 2014)	Denmark	Cross-sectional	39.74 ± 7.22[a,d]	46.48	71 participants	• Urinary cyclobutane thymine (T–T) dimers	• Association between cyclobutane thymine dimers (T–T dimers) and vitamin D is significant (r^2 = 0.76; p<0.0001), strongly indicating that the harmful DNA effects of ultraviolet radiation are unavoidable
(Usman et al., 2021)	United Kingdom	Cross-sectional	14.60 ± 2.05[a,c]	45.28	132 adolescents (53 obese, control: 59 non-obese)	• Buccal micronucleus cytome assay (buccal epithelial cells) • ELISA (measures 8-OHdG from urine)	• Vitamin D has significant correlation with 8-OHdG (r^2=-0.245; p<0.01) and buccal micronuclei (r^2=-0.305; p<0.01)
(Wang et al., 2016)	China	Cross-sectional	20.69 ± 1.50[a,d]	36.36	121 participants (44 males, 77 females)	• Comet Assay IV Lite scoring system	• No significant correlation was observed between 25(OH) D level and DNA damage (r = −0.0824; P > 0.05).

(Wenclewska et al., 2019)	Poland	RCT	63.43 ± 1.57[a,c]	29.17	92 people with vitamin D deficiency (intervention: 48 people, control: 44 people (14 with T2DM, 30 healthy)	• Comet assay (peripheral lymphocyte)	• The percentage of DNA in the tail decreased in the intervention group when compared to the control group, either with or without T2DM ($p<0.05$) • DNA oxidative parameters (Fpg) decreased in the intervention group (113.63 ± 4.26 vs. 104.19 ± 3.06; $p<0.05$), especially in the T2DM group when compared to the control group ($p<0.01$).

Abbreviation: 25(OH)D = 25 hydroxy vitamin D, 8-OHdG = 8-Hydroxyguanosine, T–T dimers = cyclobutane thymine dimers, CBMN = cytokinesis-block micronuclei, Cyp27b1 = Cytochrome p450 27B1, DNA = Deoxyribonucleic acid, ELISA = Enzyme-linked immunosorbent assay, H_2O_2 = Hydrogen peroxide, IU = International unit, IRR = Incidence rate ratio, RCT = Randomized controlled trial, T2DM = Type 2 diabetes mellitus. Data was presented as: (a) mean ± SD or (B) median (min-max), (c) exposed mean, (d) overall mean

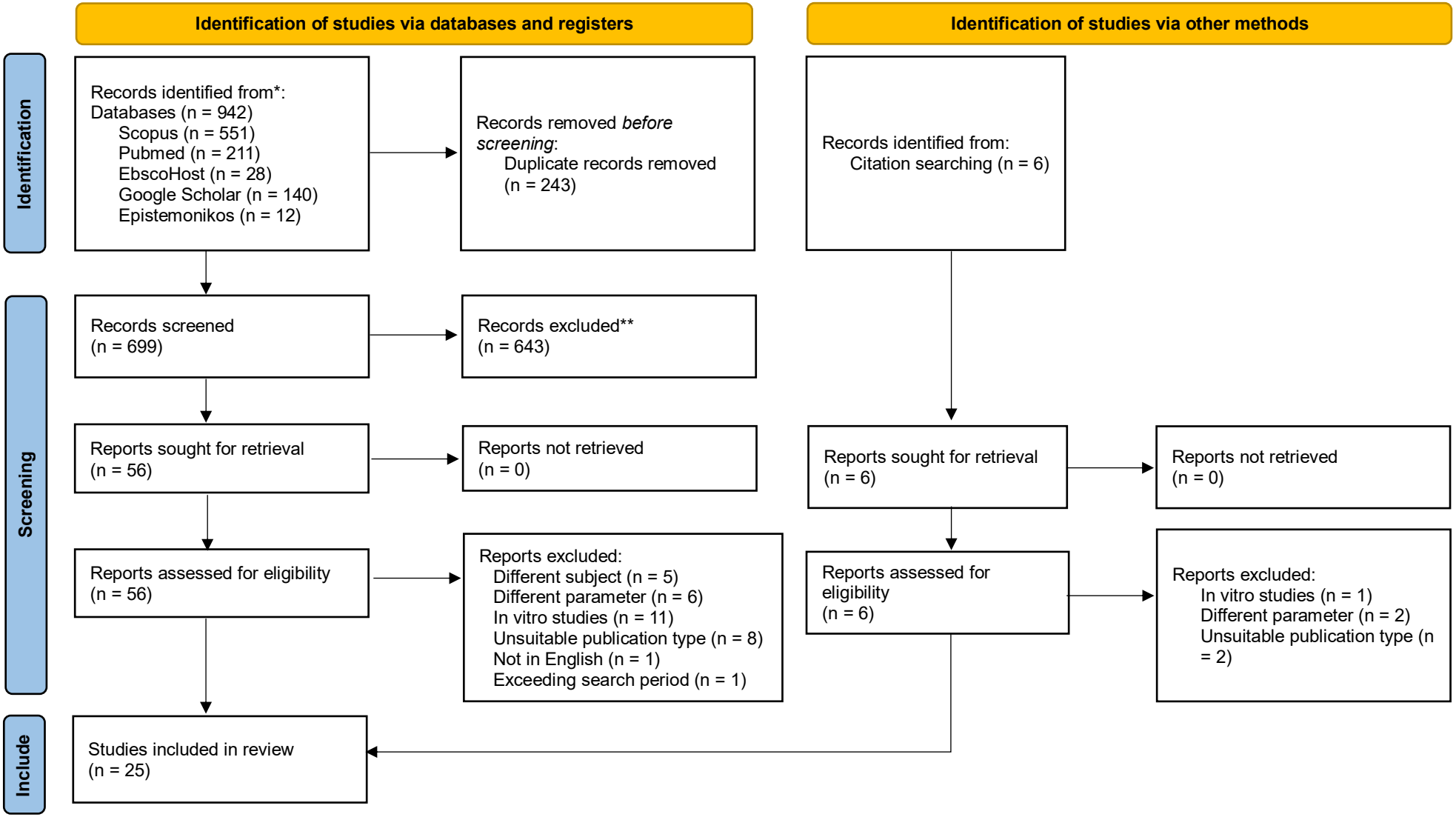

Figure 1. Study selection and selection flow

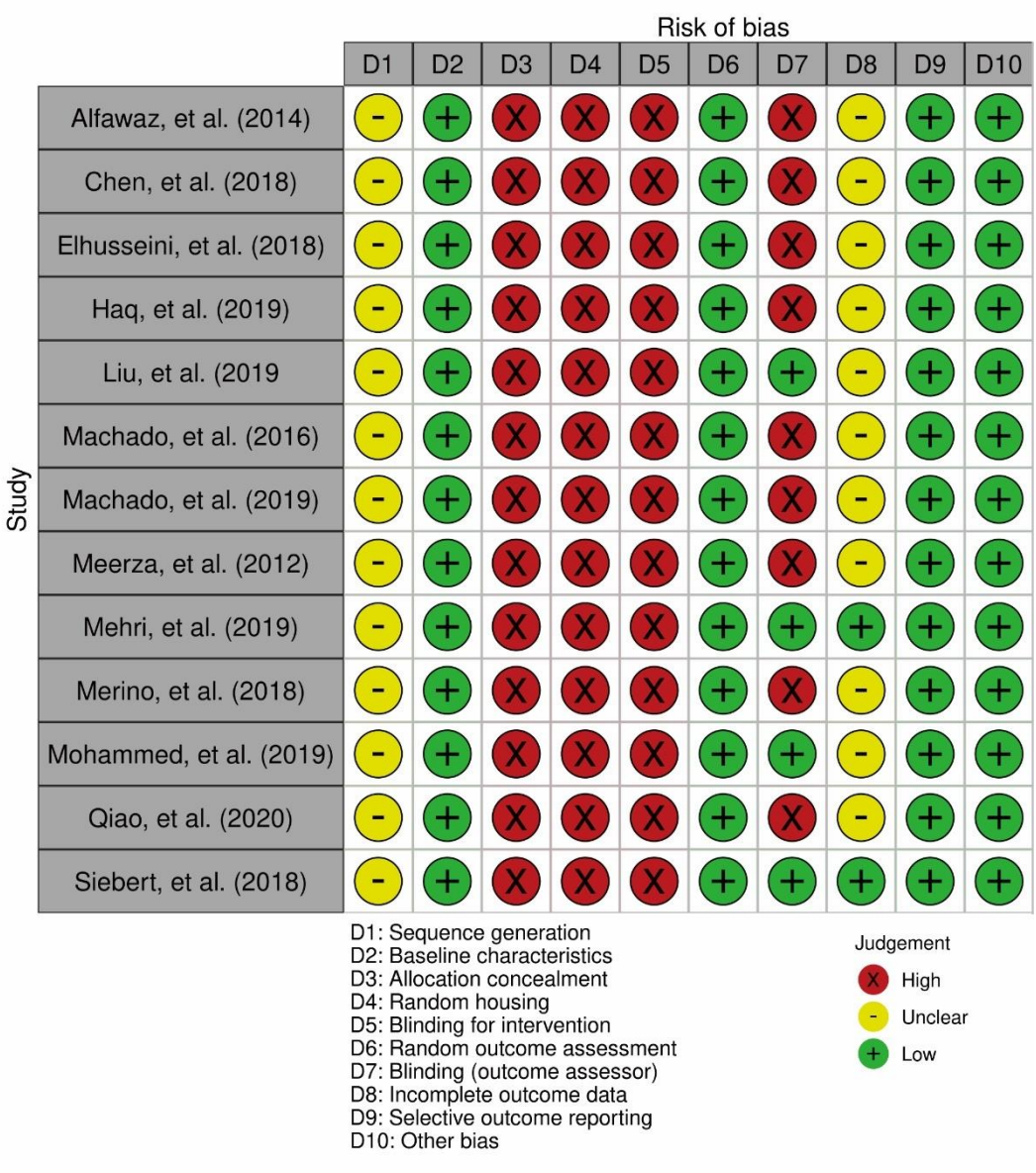

Figure 2. Risk of Bias for Animal Studies

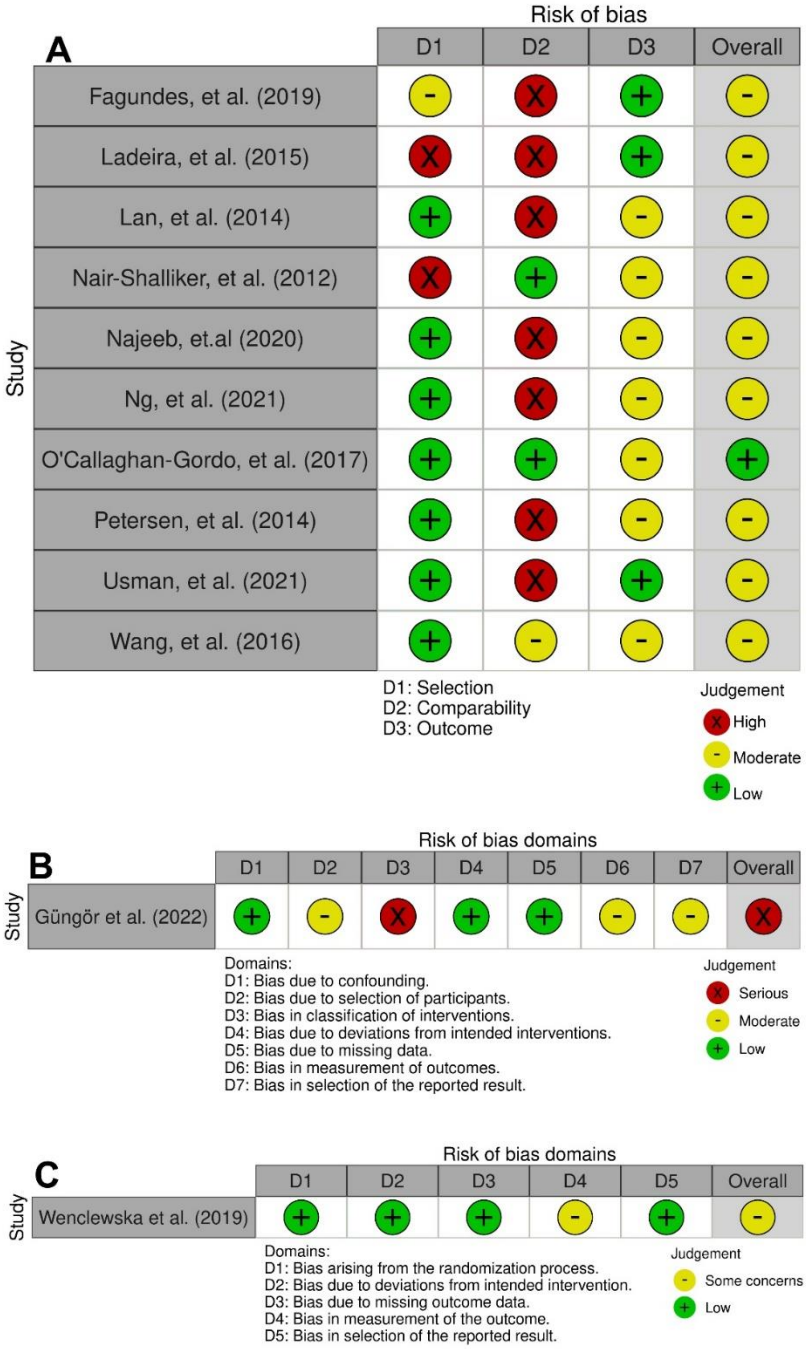

Figure 3. Risk of Bias for Human Studies. A = Newcastle-Ottawa Scale; B = Risk Of Bias In Non-randomised Studies - of Interventions (ROBINS-I); C = Version 2 of the Cochrane risk-of-bias tool for randomized trials (ROB-2)

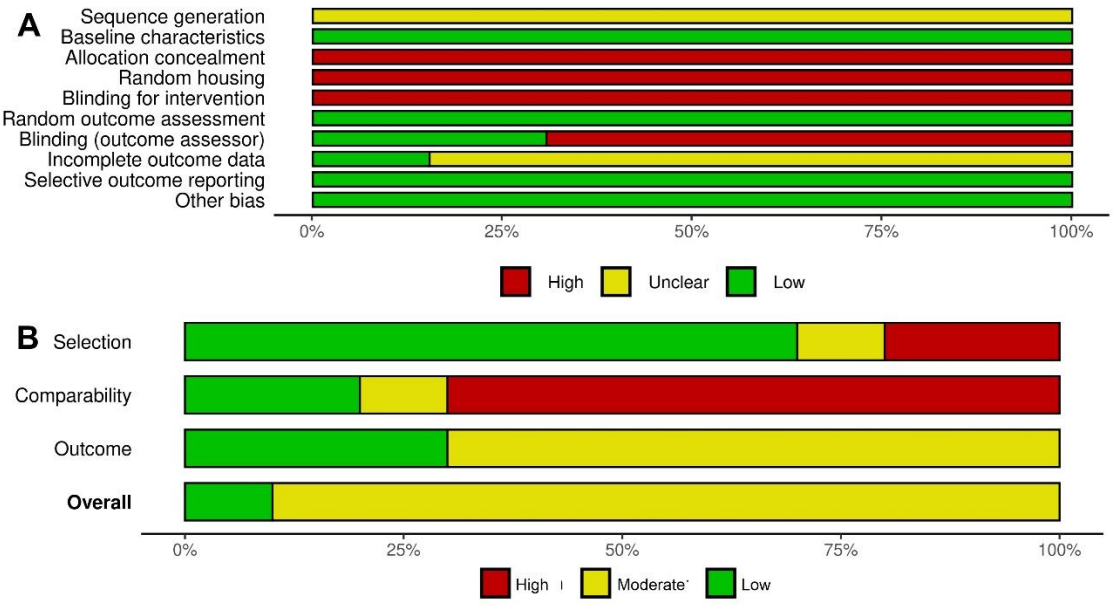

Figure 4. Risk of Bias Summary. A = Animal studies, B = Human studies (limited to observational studies since experimental research only consisted of one study)

Bukti Konfirmasi Artikel Accepted dan Artikel Lengkap (15 Mei 2023)

Krisna Murti unsri <krisna.arinafril@unsri.ac.id>

[ABP] Editor Decision reference number 6641 Accept

abp@ptbioch.edu.pl <abp@ptbioch.edu.pl> Mon, May 15, 2023 at 6:33 PM
Reply-To: Dr Mikołaj Olejniczak <m.olejniczak@abp.ptbioch.edu.pl>
To: Mayang Indah Lestari <mayangindah@fk.unsri.ac.id>, Krisna Murti <krisna.arinafril@unsri.ac.id>, Iche Andriyani Liberty <icheandriyaniliberty@fk.unsri.ac.id>, Zen Hafy <zenhafy@gmail.com>, Violantina Linardi <violantinalinardi10@gmail.com>, Muhammad Khoirudin <mkhoirudin32@gmail.com>, Tungki Pratama Umar <tungkipratama@gmail.com>

Dear Dr. Murti,

I am pleased to inform you that your article with reference number 6641 entitled: "Association of Vitamin D with Deoxyribonucleic Acid (DNA) Damage: A Systematic Review of Animal and Human Studies: Vitamin D and DNA Damage" has been accepted for publication in Acta Biochimica Polonica.

As manuscript is to be forwarded for lingual and editorial correction please upload the clean, without markup, WORD file of the accepted version of your article in Copyeditng under Copyediting Discussions.

Please klick Copyediting link and under Add Message upload the requested file.

Sincerely

Dr Mikołaj Olejniczak
m.olejniczak@abp.ptbioch.edu.pl

Associate Editor

Acta Biochimica Polonica

The following message is being delivered on behalf of Acta Biochimica Polonica.