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Correlation serum level of 25(OH)D and bone density in type 2 diabetes mellitus patients

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ABSTRACT

Bone density has positive relationship with levels of vitamin D, that reduce the risk of fracture. Some studies shows that vitamin D deficiency is not only related to skeletal disease, but also to non-skeletal disease such as T2DM. Vitamin D plays an important role in the regulation of blood glucose through the effect of pancreatic beta cell secretion and insulin sensitivity. To investigate correlation between serum level of 25(OH)D and bone density in type 2 diabetes mellitus patients. Observational study with cross sectional design at Internal Medicine Polyclinic Dr. Mohammad Hoesin Palembang Hospital from November 2014 to April 2015, and the result analyzed with statistical correlation. The subjects were taken by consecutive sampling in patients with T2DM who met inclusion criteria. Of 380 people who sought medication, 42 subjects met the inclusion criteria. There were 22 patients with low bone density (19 osteopenia and 3 osteoporosis), and 20 patients with normal bone density. Mean serum level of 25(OH)D in group with normal BMD of lumbar and femur was 19.48 ± 6.75 ng/ml, and in group with osteopenia and osteoporosis was 21.54 ± 6.14 ng/ml and 17.17 ± 5.66 ng/ml respectively. There was no significant correlation of 25(OH)D serum level with lumbar and femur bone density ($p < 0.05$). There is no correlation between levels of 25(OH)D serum and bone density in T2DM patients at Internal Medicine Polyclinic of Mohammad Hoesin Hospital.

Keywords: Type 2 diabetes mellitus, 25(OH)D, bone density

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INTRODUCTION

Type 2 Diabetes mellitus (T2DM) is still a serious threat to global health. In 2003, the World Health Organization (WHO) estimates that people suffering from DM in 2025 will increase to 333 million in the world, and from 8.4 million in 2000 to about 21.3 million years 2030 in Indonesia.^{1,2} Patients with DM can experience a variety of complications [1,2]. Also known that DM can affect the bone remodeling process which lead to bone disorders.² T2DM and osteoporosis is increasing in number along with increasing elderly population.

Osteoporosis is a global health problem, due to a decrease in bone microarchitecture, that will lead to the increase risk of fracture[3]. In T2DM, according to de Liefde et al. individuals with T2DM had a 69% increased risk of fracture compared with no diabetes despite having normal bone density [5]. Research showed a decrease in bone density in the group with T2DM compare to control [6,7]. According to the Gul-e-Raana et al. it is caused by decreasing bone mineralization, particularly calcium[8]. Schwartz AV et al. found that DM in elderly women will accelerate process of bone density loss compared to women without diabetes [9]. In obese patient with T2DM, there is an increasing load to the bone and insulin resistance resulting in hyperinsulinemia that will trigger bone formation. The influences of hyperglycemia on microvasculature, osteoclasts

and osteoblasts, as well as the formation of advanced glycation end products will lead to the increased of bone resorption and inhibition of bone formation [4]. Bone density has positive relationship with levels of vitamin D, that reduce the risk of fracture. Some studies shows that vitamin D deficiency is not only related to skeletal disease, but also [6] non-skeletal disease such as T2DM. Vitamin D plays an important role in the regulation of blood glucose through the effect of pancreatic beta cell secretion and insulin sensitivity. Some study showed a significant relationship between a good levels of vitamin D status and reduced risk of T2DM [11-13]. Low vitamin D in patients with T2DM, will cause decreased bone density, but it remains unclear and need further research. The literature shows that bone density is normal in patient with T2DM, but studies found that the risk of fracture is increased [5]. Located in the equatorial, do not decrease the risk of vitamin D deficiency among Indonesian people, nevertheless the research of vitamin D are also scarce, particularly bone density and T2DM in Palembang. Therefore, we conducted research to determine the correlation between levels of vitamin D [25(OH)D] and bone density in T2DM at Mohammad Hoesin Hospital (RSMH) Palembang.

MATERIALS AND METHODS

This study was an observational study of correlation with cross sectional design. The population was patients with type 2 diabetes at the Polyclinic of Internal Medicine RSMH Palembang from November 2014 to June 2015. Using nonprobability consecutive sampling and determine sample size with correlation formula, we obtained 42 patients who met inclusion criteria; T2DM patients, aged ≥ 30 years, gave informed consent. The exclusion criteria are patients receiving long-term corticosteroid therapy (equivalent dose of prednisone $>7.5\text{mg/day}$) in the last 3 months, receiving insulin therapy or pioglitazone (class of PPAR- γ), women in menopause or estrogen therapy, suffering from chronic kidney disease, chronic obstructive pulmonary disease, chronic liver disease, hyperthyroidism disease and levothyroxine treatment over 3 months, a malignancy of the bone disease, rheumatoid arthritis, and vitamin D supplements in the last 3 months[14]. This study was approved by Unit for

Bioethics and Humanities Faculty of Medicine, University of Sriwijaya - RSMH.

Bone Density

Bone density measurements using a technique examination with DXA Bone Mineral Densitometry Stratos® brands, analyzed by Rheumatology Consultant, normal interpretation of the results when the T Score >-1 , osteopenia when the T score between -1 and -2.5 sd osteoporosis when the T score <-2.5 , or using Z Score if aged <50 years. Examination carried out at the lumbar spine and femoral neck[14].

25(OH)D

25(OH)D serum is inactive vitamin D serum, collected at 09.00 to 12.00 pm and examination of serum levels of vitamin D were based on the examination technique CLIA (chemiluminescent immunoassay), with a normal interpretation $\geq 30\text{ng/ml}$, insufficiency if the level range from 10 to 30 ng/ml, deficiency if the range <10 ng/ml [15,16].

Statistical analysis

The data was processed by using SPSS version 22 to analyze the relations [2] between the variables of categorical data using chi square test or Fisher's exact test, and analyze the correlation levels of 25(OH)D serum with bone density using Spearman and partial correlation.

RESULTS

Characteristics of Bone Density

The mean age was 51.07 ± 7.93 years, consist of women 46.06 ± 3.32 years and men 50.76 ± 8.41 years. Education level consists of uneducated one person (2.4%), elementary school 3 persons (7.1%), junior high school 5 persons (11.9%), senior high school 16 persons (38.1%), Diploma 9 persons (21.4%), graduate 4 persons (9.5%), and post graduate 4 persons (9.5%). Most of subjects were employee, consists of housewives 9 (21.4%), traders 1 (2.4%), civil servants 20 (47.6%), retired 7 (16.7%), motorcycle taxi 2 (4.8%), and unemployee 3 (7.1%). Mean BMI was 26.06 ± 25.86 , comprised of 9 (21.4%) normal, 10 (23.8%) overweight, 15 (35.7%) obese 1 and 8 (19%) obese 2. The femur bone density average was 0.896 (0.680 to 1.169) g/cm^2 , and lumbar spine was 0.906 ± 0.123 g/cm^2 . Characteristics of subjects based on bone density in table 1.

Table 1. Distribution of subject characteristic based on bone density

Characteristic	Category	Bone Density		Total n(%)	p
		Low n(%)	Normal n(%)		
Sex ^a	Male	17(40,5)	9(21,4)	26(61,9)	0,031*
	Female	5(11,9)	11(26,2)	16(38,1)	
Age ^a	≤45 y.o	6(14,3)	5(11,9)	11(26,2)	1
	>45 y.o	16(38,1)	15(35,7)	31(73,8)	
Level of education ^b	Low	6(14,3)	3(7,1)	9(21,4)	0,460
	Middle-High	16(38,1)	17(40,5)	33(78,6)	
Occupation ^b	Employee	15(35,7)	17(40,5)	32(76,2)	0,284
	Unemployee	7(16,7)	3(7,1)	10(23,8)	
Smoking ^b	Yes	8(19)	0(0)	8(19)	0,004*
	No	14(33,4)	20(47,6)	34(81)	
BMI ^b	Normal	7(16,7)	2(4,8)	9(21,4)	0,135
	High	15(35,7)	18(42,8)	33(78,6)	
Duration DM ^a	≤ 1 y.o	7(16,7)	9(21,4)	16(38,1)	0,38
	> 1 y.o	15(35,7)	11(26,2)	26(61,9)	
Drugs of DM ^b	No	4(9,5)	5(11,9)	9(21,4)	0,714
	Yes	18(42,9)	15(35,7)	33(78,6)	
Therapy period of DM ^a	≤ 1 y.o	12(28,6)	12(28,6)	24(57,1)	0,721
	> 1 y.o	10(23,8)	8(19)	18(42,9)	
Activity ^b	Easy	14(33,3)	2(4,8)	16(38,1)	0,000*
	Medium	8(19)	18(42,9)	26(61,9)	
Exposure of sun ^a	<30 minute	11(26,2)	7(16,6)	18(42,9)	0,327
	≥30 minute	11(26,2)	13(31)	24(57,1)	
Indoor Activity ^a	Indoor	17(40,5)	10(23,8)	27(64,3)	0,065
	Outdoor	5(11,9)	10(23,8)	15(35,7)	
Sun Protection ^b	No	4(9,5)	10(23,8)	14(33,4)	0,029*
	Yes	18(42,9)	10(23,8)	28(66,6)	

Note : ^a Chi Square Test, ^b Fisher Test

Table 2. Characteristic subjects based on level of 25(OH)D

Characteristic	Category	Level of 25(OH)D Serum		Total n(%)	p
		Sufficiency n(%)	Insufficiency n(%)		
Sex ^a	Female	0(0)	16(38,1)	16(38,1)	0,138
	Male	5(11,9)	21(50)	26(61,9)	
Age ^a	≤45 y.o	0(0)	11(26,2)	11(26,2)	0,315
	>45 y.o	5(11,9)	26(61,9)	31(73,8)	
Occupation ^a	Unemployee	4(9,5)	6(14,3)	10(23,8)	0,008*
	Employee	1(2,4)	31(73,8)	32(76,2)	
Smoking ^a	Yes	0(0)	8(19)	8(19)	0,564
	No	5(11,9)	29(69,1)	34(81)	
BMI ^a	Normal	1(2,4)	8(19)	9(21,4)	1
	High	4(9,5)	29(69,1)	33(78,6)	
Sun Exposure ^a	<30 minute	0(0)	18(42,9)	18(42,9)	0,06
	≥30 minute	5(11,9)	19(45,2)	24(57,1)	
Room Activity ^a	Outdoor	3(7,1)	12(28,6)	15(35,7)	0,329
	Indoor	2(4,8)	25(59,5)	27(64,3)	
Sun Protection ^a	No	3(7,1)	11(26,2)	14(33,3)	0,313
	Yes	2(4,8)	26(61,9)	28(66,7)	

Note : ^a Fisher Test

Table 3. Correlation between level of 25(OH)D serum and bone density

BMD	Level of 25(OH)D Serum (ng/ml)			r	p
	Normal Mean±SD	Osteopenia Mean±SD	Osteoporosis Mean±SD		
Lumbal	19,62±7,05	21,60±5,62	17,17±5,66	0,107	0,501
Femur	20,30±6,37	19,82±7,40	-	0	1
Lumbal+Femur	19,48±6,75	21,54±6,14	17,17±5,66	0,114	0,473

3

Characteristics levels of 25(OH)D serum

The mean levels of 25(OH)D serum was 20.25 ± 6.4 ng/ml. There were 5 subjects with sufficiency and 37 subjects insufficiency. The mean levels of both groups were 32.02 ± 2.6 ng/ml and 18.65 ± 4.91 ng/ml respectively. The characteristics of subjects according to the levels of 25(OH)D serum in Table 2.

1

Correlation Levels of 25(OH)D serum With Bone Density

The Spearman correlation analysis showed that there was no significant correlation between the levels of 25(OH)D serum with bone density in patients with T2DM (Table 3). Similarly, after controlling (significance analyzed bivariate) the variables sex, smoking, activity category, occupation and the use of sun protection, analyzed with partial correlation.

DISCUSSION

Dutta et al. and Yaturu et al. found low bone density in patients with diabetes mellitus type 2 [6,7]. However, at the opposite, de Liefde et al., Cutrim et al., and Hadzibegovic et al. showed normal bone density. In this study, we found more T2DM patients with low bone density [5,16,17]. We found more subjects with insufficiency compare to sufficiency based on levels of 25(OH)D. This result is similar to Chagas et al. and Candido et al., they conclude that vitamin D has a role in diabetes [18,19]. The decline in vitamin D will interfere the function of pancreatic β cells in insulin secretion and decrease insulin sensitivity due to systemic inflammation [20].

The number of male in this study is more than female with ratio of 1.62:1. This can be explained because we use certain exclusion criteria for female such as menopause and use of hormonal contraceptive. More male are found to have low bone density with ratio of 3.4 times higher than female and showed a significant relationship. This happened because the number of male are more than female as subject, also because we have 7 females aged <45 y.o (70%) and 23 males aged >45 y.o (71.9%). By contrast, we found more male than female in the 25(OH)D serum insufficiency group, while in 25(OH)D serum level sufficiency there were only male found. The results showed no significant relationships between the sexes and the serum levels of 25(OH)D.

Subjects with moderate activity have better bone density than mild activity, and the results showed a significant correlation. Physical activity can improve bone density by improving muscle mass and stimulating contraction. Rotikan stated that sufficient activity can improve bone strength and density, the statement is supported by Muir JM et al. who reported that regular activity in elderly women can prevent loss of bone density [21,22].

All subjects who are active smoker have low bone density, and there is a significant relationship between smoking and bone density. Setyohadi and Supervia et al. stated that smoking is a risk factor for osteoporosis. Smoking decreases intestinal calcium absorption, provoke early menopause, disrupt estrogen metabolism, and give toxic effect to bone vascularization [23,24].

We found more subjects with low bone density when they use sun protection. The results show a significant relationship. Sun exposure to UV-B is required in the activation of 7-dehydrocholesterol in the skin to be converted into pre-vitamin D₃, which then turns into vitamin D₃ in the process of non-enzymatic dependent on exposure to UV-B rays of the sun, which is necessary for calcium intestinal absorption in the process of mineral balance [14].

There are several studies that reported an association between vitamin D levels and bone density [14]. AACE and IOF claimed that the levels of 25(OH)D serum needed to prevent secondary hyperparathyroidism which will have an effect on bone is 30 ng/ml. Levels of $1,25(\text{OH})_2\text{D}$ will increase calcium absorption in the small intestine and at osteoblast receptors, causing an increase in RANKL expression and induce preosteoclasts into osteoclasts which are important in the resorption of calcium and phosphorus from the bone.¹⁴ In addition, in T2DM, there are several mechanisms of disorders associated with decreased vitamin D, such as impaired insulin secretion and insulin insensitivity [20].

This study showed no significant correlation between 25(OH)D and bone density (lumbal, femoral or both) in patients with T2DM. This is similar to Winckler et al. who conducted cohort in 415 patients with T2DM, as well as Kalamanathan et al. in 194 patients and Bakalov et al. in 100 patients with also reported the similar study result [25-27]. It might happen because of

25(OH)D measured was an inactive form vitamin D.

11 et al. found significant correlation between 25(OH)D serum and bone density of the femur and lumbar, which was conducted only in 82 male patients T2DM, whom 49 patient (60%) found osteoporosis [28], whilst we found only 7.2% who have osteoporosis. A retrospective study conducted by Jingjia et al. with a larger sample size (1050 patients with T2DM) show 1 a significant positive correlation between the levels of 25(OH)D serum and bone density of the femur and pelvis [29]. Both of these studies have a number of samples greater than this study, but in Jingjia study, there were 377 female subjects with menopausal women might be included. Both studies did not describe the factors that may affect the levels of 25(OH)D serum and bone density in study subjects such as exposure to sunlight, activity, and smoking habits.

CONCLUSION

There is no correlation between the levels of 25(OH)D serum and bone density in patients with T2DM at the Polyclinic of Internal Medicine RSMH Palembang. Further research is required to use the active form of vitamin D [1,25(OH)₂D], PTH, bone remodeling markers (CTX, NTX, BSAP, TRAb), histopathologic of bone, as well as gene polymorphism in patients with T2DM.

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Conflict of Interest

The authors have no conflict of interest to disclose.

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