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25(OH)D Concentrations Negatively Correlated With HbA1c in Type 2 Diabetes Mellitus Patients: A Cross Sectional Study in Mampang, South Jakarta

ABSTRACT

INTRODUCTION: Insulin resistance plays a central role in type 2 diabetes mellitus (T2DM). Another possible cause of T2DM is deficiency of vitamin D, although the underlying mechanism is not yet clearly understood. Most frequently used laboratory parameters for monitoring T2DM are fasting blood glucose (FBG) and HbA1c. Determining any association of vitamin D with HbA1c and FBG in T2DM.

METHODS: A cross-sectional study involving 100 T2DM patients with following characteristics: 18-year and older persons of both genders, not having kidney and liver disease, not on insulin therapy, not pregnant or lactating, and not consuming vitamin D in the last 3 months. Spearman's correlation coefficient was used to test any association of vitamin D with HbA1c and FBG at $p<0.05$.

RESULTS: Subjects comprised 74 females and 26 males with median age of 56 years. Median HbA1c, 25(OH)D, and fasting blood glucose were 8.05%, 11.2 ng/mL, and 127 mg/dL, respectively. The Spearman correlation coefficient for vitamin D and HbA1c was $r= -0.217$ ($p=0.03$), and for vitamin D and FBG it was $r = -0.153$ ($p=0.128$).

CONCLUSION: There was a significant negative correlation of vitamin D with HbA1c, but not with FBG.

Keywords: type 2 DM, 25(OH)D, blood glucose, HbA1c

INTRODUCTION

The metabolic disorder diabetes mellitus (DM) features high blood glucose levels due to defective insulin response. Insulin resistance in target tissues, particularly in the skeletal muscles and adipose tissue, is responsible for the occurrence of abnormalities of carbohydrate, fat and protein metabolism, that may result in diabetes. There are several types of DM. The 2022 American Diabetes Association guidelines categorize DM into type 1 diabetes due to autoimmune damage to the beta cells; type 2 diabetes due to insulin resistance; diabetes due to other causes such as monogenic diabetic syndrome, DM-causing drugs, exocrine pancreatic disease, and gestational diabetes [1,2].

Type 2 diabetes mellitus (T2DM) comprises the highest number of patients, both globally and in Indonesia. The global prevalence of T2DM at age 20-79 years according to the International Diabetes Federation (IDF) 2013 report was 8.3% (382 million persons) with 14 million more men than women, generally aged 40-59 years. The global number of cases is projected to increase to 592 million in the year 2035. The increased incidence of T2DM at younger ages may presumably be caused by changes in life style and unhealthy food. Obesity is also responsible for the occurrence of T2DM [1].

Insulin resistance plays a central role in T2DM. Oxidative stress, proinflammatory activation, adipokines and abnormal metabolism of glucose and lipid can lead to insulin resistance [3]. Another possible cause of T2DM is deficiency of vitamin D. Although the underlying mechanism is not yet clearly understood, low 25-hydroxyvitamin D (25(OH)D) level is connected with functional disorders of pancreatic islet Langerhans cells and insulin resistance causing changes in glucose homeostasis such as to result in the occurrence of T2DM [4].

Basically, vitamin D may increase the secretion of insulin, skeletal muscle glucose and lipid metabolism, and reduce systemic inflammation. However, several studies found no influence of vitamin D supplementation on blood glucose in patients with T2DM while other studies found inconsistencies in the connection of vitamin D with blood glucose [5].

Although low vitamin D level is correlated with higher glycosylated hemoglobin (HbA1c) and blood glucose levels in patients with T2DM, the relationship is as yet unclear as well as inconsistent, such that there is still a need for further research on the connection of vitamin D with blood glucose in T2DM patients. The purpose of our study was to explore the connection of vitamin D with HbA1c and with blood glucose in T2DM patients.

METHODS

This cross-sectional study recruited 100 patients with T2DM at Mampang District *Puskesmas*, South Jakarta, from June to July 2022, by means of consecutive sampling. The size of the required research sample was obtained using the formula for the correlation sample size, with $Z\alpha=1.96$; $Z\beta=1.28$; and $r=-0.387$ [6].

The inclusion criteria were as follows: men and women older than 18 years, not on insulin therapy, not having kidney and liver disease, not being pregnant or lactating, and not consuming vitamin D in the last 3 months. All subjects were provided with information about this study and all gave written agreement by signing the informed consent form.

The subjects were to self-report on a questionnaire on sources of 25(OH)D and the subjects' T2DM condition.

After a 10-hour fast by the respondents, venous blood was drawn from each respondent and divided into aliquots of 3, 2 and 5 mL that were respectively placed into one EDTA anticoagulant tube for measuring HbA1c, one sodium fluoride (NaF) anticoagulant tube for measuring fasting blood glucose (FBG), and one tube without anticoagulant for 25(OH)D determination. The HbA1c level was measured by High Performance Liquid Chromatography (HPLC), FBG by the hexokinase method, and of 25(OH)D by Direct Competitive Chemiluminescent Microparticle Immunoassay (CMIA). Determination of HbA1c and FBG was performed directly on the day of blood specimen collection, whereas 25(OH)D was determined simultaneously after all specimens had been collected. The blood specimens were frozen at minus 20^0C prior to being examined.

For data analysis, the Statistical Package for Social Sciences (SPSS) program version 23 was used. The Kolmogorov-Smirnov test was used to determine whether or not the data were normally distributed. Because the data distribution was non-normal, the correlation of vitamin D with HbA1c and FBG was analyzed by the Spearman correlation test at $p < 0.05$. Ethics approval was issued by the Ethics Committee of the Faculty of Medicine, Universitas Trisakti, under No. 037/KER/FK/V/2022.

RESULTS

The characteristics of the study subjects, comprised of 26 males and 74 females with median age of 56 years (35 -79 years) may be seen in Table 1. The educational level of 35% of the subjects was high school graduate, while the most frequent occupation was housewife at 68%. The most frequent 25(OH)D status was deficiency in 86% of subjects. Seventy-seven percent of the subjects had never consumed vitamin D-containing foods, 84% of the subjects had never sunbathed, and 69% wore long-sleeved clothing daily for 24 hours.

We found that the male and female subjects differed significantly in vitamin D levels, but not in HbA1c and FBG levels (Table 2).

This study also found a significant but negative correlation of vitamin D concentration with HbA1c at $r= -0.217$ ($p= 0.03$). However, no correlation was found of vitamin D with FBG, at $r= -0.153$ ($p=0.128$).

DISCUSSION

The patients with T2DM in this study were mostly females. The greater number of females who had diabetes is similar to that in the study of Mihardja, in that diabetes affects women more than men, because of their higher body mass index. This is in contrast with Japan and China, where T2DM prevalence is higher in males, which may be caused by the differing diets and behaviors of males [7]. The study by Azlin et al. shows that there are more females with T2DM than males. According to the 2002 study of Brunner and Suddart as cited by Azlin, there are more females with T2DM as compared to males, because there is proportionately more body fat in former than in the latter. Body fat deposit is one of the factors that may reduce insulin sensitivity in muscles and liver [8]. In the 2018 Basic Health Survey of Indonesia (Riskesdas), the DM prevalence in females was higher than in males, at a ratio of 1.78% to 1.21%. The Riskesdas also found that in the last 5 years the prevalence of T2DM in females showed a slight increase if compared to males [9].

The participants in our research had a median age of 56 years. In third world countries, most of the patients with T2DM are between 45 and 64 years old, whereas in first world countries the patients with T2DM are mostly older than 64 years [10]. The prevalence of diabetes also shows an increase with increasing age of the patients and peaks at age 55-64 years. In Riskesdas 2018 there were also indications of an increase in age and thus a higher risk for diabetes [9]. Sharan and team [11] reported that recent onset T2DM accounted for 79.1% in the age range of 40-60 years. Older respondents are commonly more at risk of hyperglycemia, due to decreased function of the pancreas [7].

The median 25(OH)D level in this study was 11.2 ng/mL, corresponding to vitamin D deficiency in 86% of patients. Vitamin D can be categorized as deficiency if 25(OH)D concentration < 20 ng/mL, insufficiency if 25(OH)D concentration = 21-29 ng/mL, sufficiency if 25(OH)D concentration ≥ 30 ng/mL and intoxication if 25(OH)D level ≥ 150 ng/mL [12]. The proportion of deficiency and insufficiency for vitamin D in Indonesia is relatively great, being 68.8% in Pusparini's study in South Jakarta [13] on women and men in the age range of 55-65 years. In North Sumatra, however, Keumala [14] found that 95% of adult women had vitamin D deficiency and insufficiency. Arjana's team [15] in Yogyakarta found that in adult males aged 19-25 years, the proportion of deficiency of vitamin D was 43.3%a, whereas the proportion of insufficiency of vitamin D was 51.7%.

The causative factors of vitamin D deficiency are varied, among others low exposure to sunlight, less vitamin D-containing foods in the diet, insufficient outdoor activity, a life style that avoids sunlight, sunscreen usage, covered clothing or traditional clothing covering the whole body, and dark-colored skin. Skin color is an important factor in natural vitamin D production. Darker skins need more time for the formation of vitamin D than do fair skins. Sun screens may absorb ultraviolet light (UVB-UVA) such as to inhibit the penetration of UVB into the skin [16,17]. Reduced physical activity is also a factor in vitamin D deficiency and T2DM, because physical activity may improve the vitamin D status [18].

Our findings of reduced median vitamin D level and high vitamin D deficiency prevalence was probably caused by the inadequate vitamin D intake from sunlight and the diet. This study found that the proportion of study subjects who had never been exposed to sunlight for 15-30 minutes was 84%, because the majority of study subjects were housewives (68%) such that their activity was indoors. Our study also found that the median vitamin D intake was extremely low, at 1.76 µg/day. The average vitamin D intake recommended by the Institute of Medicine (IOM) for females and males aged 18-70 years is 15 µg/day [19]. Vitamin D-containing foods are among others egg yolk, salmon, mackerel, tuna, meat, mushrooms, cod liver oil, and fortified foods [14,19]. According to the study of Masood and Iqbal, as cited by Keumala et al. [14] the inability to buy vitamin D-containing foods results in the occurrence of vitamin D deficiency. Salmon and cod liver oil are highly expensive vitamin D-containing foods on the market. It should be noted that around 69% of our subjects wore covered or long-sleeved clothing for a period of 12-24 hours, such as to prevent sunlight from penetrating into the body through the surface of the skin. According to Holick, adequate vitamin D can be obtained by twice weekly sunlight exposure of the arms and legs for 5-30 minutes between 10 AM and 3 PM (as determined by weather and skin color) [12]. Similar results were found between our study and Keumala's, in that deficiency of vitamin D correlates with indoor occupations and activities, less vitamin D-containing foods in the diet, and sunbathing of ≤ 1 hour daily [14].

Mahmodnia's team [20] reported a strong positive correlation of vitamin D and age. The age variable is a dominant factor that causes vitamin D deficiency, which is correlated with low outdoor activities resulting in low exposure to sunlight. In addition, there are also changes in the skin, namely a reduction in the content of 7-dehydroxycholesterol, which is the precursor of vitamin D3 [21]. The study of Jha et al. [22] also showed low vitamin D concentrations with increasing age. The reduced synthesis of vitamin D with increasing age, in addition to being due to reduced 7-dehydroxycholesterol concentrations, is also caused by reduced oral absorption of vitamin D.

In our study, an important difference was found in the vitamin D levels of men and women, This may have been the result of differences in physical activity and life style between males and females, where the activities of the males were presumably carried out to a greater extent out of doors, in comparison to those of the females, such that the males were more frequently exposed to sunlight. Life style, in this case the style of clothing also affects vitamin D concentrations. The majority of the female respondents in this study wore covered clothing (Muslim clothing with head scarves) causing a reduction in the exposure of the body to sunlight, such that vitamin D synthesis is reduced. Another factor that may have caused the contrasting vitamin D levels in men and women is the larger amount of body fat in women than in men. The fact that vitamin D is soluble in fats while body fat absorbs and stores vitamin D, may reduce circulatory vitamin D concentrations in females [23,24].

As stated above, our study found that vitamin D had a strong negative relationship with HbA1c, but not with FBG. This agrees with Mehta's [25] report of a significant negative correlation (r = - 0.1205) of HbA1c with vitamin D, where decreased vitamin D level is connected with increased HbA1c level. Our study also agrees with Jha's study [22] that reported an inverse relationship between vitamin D and HbA1c. However, our study results differ from those of Suguna's group [26] from Bengaluru, showing no strong relationship of HbA1c with vitamin D concentrations in T2DM patients (r= - 0.109 at p=0.05).

Research findings of Raumulfaro [24] demonstrated no association of vitamin D with HbA1c (r = 0.214 at a p value of 0.153) on the one hand and vitamin D and blood glucose (r =0.195 at p=0.193) on the other. The non-significant correlation of FBG with vitamin D may have been among other causes due to body fat percentage. A person with a high body fat percentage may have reduced vitamin D concentrations, as a large amount of body fat retains vitamin D, thereby diminishing circulatory vitamin D, leading to low serum levels. Consumption of glucocorticoids and blood-pressure-lowering medications may also influence vitamin D concentrations. The use of glucocorticoids may interfere with osteoblastic and osteoclastic functions, such that it can reduce vitamin D concentrations. Furthermore, sport increases the level of this vitamin, as reported in the study of Fernandez [24,27-29]. The study conducted by Sakung [30] found that physical activity heightens tissue sensitivity to insulin and absorption of glucose, thereby reducing blood glucose concentrations.

One study showed that vitamin D is negatively correlated with HbA1c, but not with FBG, thereby showing that adequate vitamin D in T2DM patients improves HbA1c but not FBG. This may have been due to differences in the level of glycemic control of HbA1c and FBG, where HbA1c represents the mean glucose level over the last 60 to 90 days., whereas serum glucose concentration is determined after an overnight fast. The FBG level is determined by basal insulin secretion, whereas HbA1c is determined by basal and post-prandial insulin secretion. The significant connection of vitamin D with HbA1c was presumably due to the effect of vitamin D on post-prandial but not on basal insulin release. Post-prandial vitamin D increase is connected with calcium absorption leading to increased intracellular calcium, which mediates post-prandial release of insulin, thereby improving HbA1c concentrations without an effect on FBG concentrations [31-33].

In this study no significant differences were found in HbA1c and blood glucose parameters between males and females. Vitamin D was also not strongly correlated with HbA1c or with blood glucose, after adjustment for gender subgroups (data not shown), possibly because of the disproportionately larger number of females.

According to Kautzky-Willer et al. [34] the gender differences for HbA1c cannot be related to the make-up of the body, because there are various factors affecting HbA1c level, among others genetic, age-related, ethnic, and environmental factors.

Fasting blood glucose is influenced mainly by the release of glucose in the liver [34,35]. Measurement of random glucose concentration, such as postprandial glucose, may improve glycemic control in females, particularly when there are differences between HbA1c and fasting glucose [34,36].

The American Diabetes Association recommends HbA1c instead of FBG for the diagnosis of diabetes mellitus. HbA1c constitutes an invaluable monitor of blood glucose control because it represents the glucose level accumulated over the last 60 to 90 days. HbA1c is also highly correlated with the risk of diabetic sequelae over a prolonged period of time [32].

Vitamin D is important for glucose metabolism, by stimulating insulin release from pancreatic beta cells and by increasing intracellular calcium and insulin sensitivity in peripheral muscles and adipocytes [18]. Vitamin D also stimulates insulin receptor expression and peroxisome proliferator-activated receptor delta (PPAR-δ) activation that is involved in the metabolism of fatty acids and the transport of glucose in the muscles, where these compounds will be converted into ATP as source of energy. Deficiency of vitamin D may affect calcium concentrations in cells that are sensitive to insulin, thus leading to peripheral insulin resistance that reduces glucose transport to the muscles. In addition, deficiency of vitamin D indirectly influences the resistance to insulin via the renin-angiotensin-aldosterone system, where angiotensin blocks vascular and skeletal muscle insulin, thus interfering with glucose absorption and reducing energy needs [37].

Maintaining the normal vitamin D concentration may help regulate glucose homeostasis, while in individuals with high HbA1c, screening for vitamin D insufficiency and deficiency may be performed [25].

The weak points of the present study were its cross-sectional study design which prevented delving into the relationship of vitamin D versus HbA1c or glucose, and the fact that it did not use healthy individuals as controls; and thirdly, this study performed only single blood glucose determinations that did not reflect glycemic variability.

CONCLUSION

This study found a strong negative relationship of vitamin D with HbA1c, but not with FBG. Additional research is required using healthy controls and performing serial blood glucose determinations.

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Table 1. Characteristics of Study Subjects

Characteristic	N (%)
Gender:	
Female	74 (74)
Male	26 (26)
Education	
No schooling	5 (5)
Elementary school	15 (15)
Junior high school	29 (29)
Senior high school	35 (35)
Academy	16 (16)
Employment history	
Housewife	68 (68)
Employed	25 (25)
Unemployed	7 (7)
Vitamin D status	
Deficient	86 (86)
Insufficient	12 (12)
Sufficient	2 (2)
Sunlight exposure (15-30 minutes)	
Yes	16 (16)
No	84 (84)
Wearing of long-sleeved clothing (12-24 hours)	
Yes	69 (69)
No	31 (31)
	Median (IQR)
Age (years)	56 (35-79)
Duration of DM (months)	12 (1-120)
25(OH)D concentration (ng/mL)	11.2 (2.4-31.1)
HbA1c (%)	8.05 (5.1-17.4)
Blood glucose (mg/dL)	127 (70-383)
Dietary vitamin D intake (µg/day)	1.76 (0.0 -12.44)
	Mean (SD)
Height (m)	1.54 (0.07)
Weight (kg)	62.04 (11.06)
BMI (kg/m^2)	26.09 (4.52)

Continuous data are presented as median (IQR) and mean (SD); categorical data as number (%)

Table 2. Concentrations of Vitamin D, HbA1c and Blood Glucose in Female and Male Subjects

Parameter	Concentration	P value*
25(OH)D concentration (ng/mL)		
Females	10.2 (2.4-24.7)	0.001
Males	14.7 (4.7-31.1)	
HbA1c (%)		
Females	8.3 (5.1-17.4)	0.389
Males	7.8 (5.4-12.5)	
Blood glucose (mg/dL)		
Females	125.5 (70-383)	0.804
Males	130.5 (83-248)	

*Mann-Whitney test, p significant if <0.05; Continuous data presented as median (IQR); HbA1c: hemoglobin A1c

2. Bukti konfirmasi review dan hasil review pertama
(4 APRIL 2023)

RMJ Rwanda Medical Journal

New article checklist

Go through the new articles and make the below formatting changes to all new articles

Ensure there is a title but not author names in the manuscript	√
Ensure style is: No Spacing	√
Single column	√
Single Spacing Font	√
Font: Calibri light – **size 10 font for the body of the text**	√
Titles in capitals and bold	√
Text should be justified	√
Format tables - Calibri light, **Font size 9** - No vertical lines. Horizonal lines in table can be removed (see example given below) - No table should be larger then a single A4 page - Should not be large then a single page and won't be able to fit on page of publication	√
Check keywords are MeSH (click here) and put semi-colons (;) between each each key word	√
Reference list should be MDPI style– Ideally authors should submit with Zotero references active as this will save work later	√
Has the author used a "checklist" to improve the quality? If not, suggest an appropriate checklist (click here)	√

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CORRECT REFERENCING

…. according to unicef and the World Health Organisation [1,2].

COMMON MISTAKES

…. according to unicef and the World Health Organisation[1,2].

WRONG: no space between final word and reference

…. according to unicef and the World Health Organisation. [1,2]

WRONG: full stop before the reference

…. according to unicef and the World Health Organisation[1] [2],

WRONG: References not "merged" in Mendeley.

…. according to unicef and the World Health Organisation [2] [1],

WRONG: References in wrong order.

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Table format

	Muhima (n=112, 62.6%)	CHUK (n=66, 36.9%)	Both (n=178)
Male Gender	55/111 (49.5%)	37/66 (56.1%)	92/177 (52%)
Mean gestation	35.4 weeks (SD: 5.4)	31 weeks (SD: 3.8)	33.4 weeks (SD: 5.3)
Number of patients recruited by TRY-CPAP group	0/112 (0%)	13/66 (19.7%)	13/178 (7.3%)
- Group 1	18/112 (14.8%)	15/66 (22.7%)	33/178 (18.5%)
- Group 2	17/112 (13.9%)	11/66 (16.7%)	28/178 (15.7%)
- Group 3	55/112 (49.1%)	5/66 (7.6%)	60/178 (33.7%)
- Group 4	22/112 (19.6%)	22/66 (33.3%)	44/178 (24.75)
Gestational groups			
- Term (>37 weeks)	42/79 (53.1%)	6/62 (9.7%)	48/141 (34%)
- 32-37 weeks	14/79 (17.7%)	20/62 (32.3%)	34/141 (24.1%)
- 28-32 weeks	14/79 (17.7%)	24/62 (38.7%)	38/141 (27%)
- <28 weeks	9/79 (11.4%)	12/62 (19.4%)	21/141 (14.9%)
Mean birth weight (Kg)	2.4 (SD: 1.01)	1.4 (SD: 0.82)	2.0kg (SD:1.1)
Antenatal visits	45/54 (83.3%)	59/62 (95.2%)	104/116 (89.6)
Mode of delivery:			
- Vaginal	73/97 (75.2%)	35/66 (53%)	108/163 (66.25%)
- Instrumental	1 (1%)	0 (0%)	1/163 (0.06%)
- Caesarian	16 (16.5%)	16 (24.2%)	32/163 (19.6%)
Respiratory distress at birth			
- None	46/86 (53.5%)	24/66 (36.4%)	70/152 (46%)
- Mild	25/86 (29.1%)	22/66 (33.3%)	47/152 (30.9%)
- Severe	15/86 (17.4%)	20/66 (30.3%)	35/152 (23%)

figures represent where data available in chart-review, denominators reflect where data available

25(OH)D Concentrations Negatively Correlated With HbA1c in Type 2 Diabetes Mellitus Patients: A Cross Sectional Study in Mampang, South Jakarta

ABSTRACT

INTRODUCTION: Insulin resistance plays a central role in type 2 diabetes mellitus (T2DM). Another possible cause of T2DM is deficiency of vitamin D, although the underlying mechanism is not yet clearly understood. Most frequently used laboratory parameters for monitoring T2DM are fasting blood glucose (FBG) and HbA1c. Determining any association of vitamin D with HbA1c and FBG in T2DM.

METHODS: A cross-sectional study involving 100 T2DM patients with following characteristics: 18-year and older persons of both genders, not having kidney and liver disease, not on insulin therapy, not pregnant or lactating, and not consuming vitamin D in the last 3 months. Spearman's correlation coefficient was used to test any association of vitamin D with HbA1c and FBG at $p<0.05$.

RESULTS: Subjects comprised 74 females and 26 males with median age of 56 years. Median HbA1c, 25(OH)D, and fasting blood glucose were 8.05%, 11.2 ng/mL, and 127 mg/dL, respectively. The Spearman correlation coefficient for vitamin D and HbA1c was r= -0.217 (p=0.03), and for vitamin D and FBG it was r = -0.153 (p=0.128).

CONCLUSION: There was a significant negative correlation of vitamin D with HbA1c, but not with FBG.

Keywords: type 2 DM, 25(OH)D, blood glucose, HbA1c

Commented [P1]: Use MeSH keywords

~~Short running title: T2DM, 25(OH)D~~

INTRODUCTION

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The metabolic disorder diabetes mellitus (DM) features high blood glucose levels due to defective insulin response. Insulin resistance in target tissues, particularly in the skeletal muscles and adipose tissue, is responsible for the occurrence of abnormalities of carbohydrate, fat and protein metabolism, that may result in diabetes. There are several types of DM. The 2022 American Diabetes Association guidelines categorize DM into type 1 diabetes due to autoimmune damage to the beta cells; type 2 diabetes due to insulin resistance; diabetes due to other causes such as monogenic diabetic syndrome, DM-causing drugs, exocrine pancreatic disease, and gestational diabetes [1,2].

Type 2 diabetes mellitus (T2DM) comprises the highest number of patients, both globally and in Indonesia. The global prevalence of T2DM at age 20-79 years according to the International Diabetes Federation (IDF) 2013 report was 8.3% (382 million persons) with 14 million more men than women, generally aged 40-59 years. The global number of cases is projected to increase to 592 million in the year 2035. The increased incidence of T2DM at younger ages may presumably be caused by changes in life style and unhealthy food. Obesity is also responsible for the occurrence of T2DM [1].

Insulin resistance plays a central role in T2DM. Oxidative stress, proinflammatory activation, adipokines and abnormal metabolism of glucose and lipid can lead to insulin resistance [3]. Another possible cause of T2DM is deficiency of vitamin D. Although the underlying mechanism is not yet clearly understood, low 25-hydroxyvitamin D (25(OH)D) level is connected with functional disorders of pancreatic islet Langerhans cells and insulin resistance causing changes in glucose homeostasis such as to result in the occurrence of T2DM [4]. Basically, vitamin D may increase the secretion of insulin, skeletal muscle glucose and lipid metabolism, and reduce systemic inflammation. However, several studies found no influence of vitamin D supplementation on blood glucose in patients with T2DM while other studies found inconsistencies in the connection of vitamin D with blood glucose [5].

Although low vitamin D level is correlated with higher glycosylated hemoglobin (HbA1c) and blood glucose levels in patients with T2DM, the relationship is as yet unclear as well as inconsistent, such that there is still a need for further research on the connection of vitamin D with blood glucose in T2DM patients. The purpose of our study was to explore the connection of vitamin D with HbA1c and with blood glucose in T2DM patients.

METHODS

This cross-sectional study recruited 100 patients with T2DM at Mampang District *Puskesmas*, South Jakarta, from June to July 2022, by means of consecutive sampling. The size of the required research sample was obtained using the formula for the correlation sample size, with $Z\alpha=1.96$; $Z\beta=1.28$; and $r=-0.387$ [6].

The inclusion criteria were as follows: men and women older than 18 years, not on insulin therapy, not having kidney and liver disease, not being pregnant or lactating, and not consuming vitamin D in the last 3 months. All subjects were provided with information about this study and all gave written agreement by signing the informed consent form.

The subjects were to self-report on a questionnaire on sources of 25(OH)D and the subjects' T2DM condition.

After a 10-hour fast by the respondents, venous blood was drawn from each respondent and divided into aliquots of 3, 2 and 5 mL that were respectively placed into one EDTA anticoagulant tube for measuring HbA1c, one sodium fluoride (NaF) anticoagulant tube for measuring fasting blood glucose (FBG), and one tube without anticoagulant for 25(OH)D determination. The HbA1c level was measured by High Performance Liquid Chromatography (HPLC), FBG by the hexokinase method, and of 25(OH)D by Direct Competitive Chemiluminescent Microparticle Immunoassay (CMIA). Determination of HbA1c and FBG was performed directly on the day of blood specimen collection, whereas 25(OH)D was determined simultaneously after all specimens had been collected. The blood specimens were frozen at minus 20^0C prior to being examined.

For data analysis, the Statistical Package for Social Sciences (SPSS) program version 23 was used. The Kolmogorov-Smirnov test was used to determine whether or not the data were normally distributed. Because the data distribution was non-normal, the correlation of vitamin D with HbA1c and FBG was analyzed by the Spearman correlation test at $p < 0.05$. Ethics approval was issued by the Ethics Committee of the Faculty of Medicine, Universitas Trisakti, under No. 037/KER/FK/V/2022.

RESULTS

The characteristics of the study subjects, comprised of 26 males and 74 females with median age of 56 years (35 -79 years) may be seen in Table 1. The educational level of 35% of the subjects was high school graduate, while the most frequent occupation was housewife at 68%. The most frequent 25(OH)D status was deficiency in 86% of subjects. Seventy-seven percent of the subjects had never consumed vitamin D-containing foods, 84% of the subjects had never sunbathed, and 69% wore long-sleeved clothing daily for 24 hours.

We found that the male and female subjects differed significantly in vitamin D levels, but not in HbA1c and FBG levels (Table 2).

This study also found a significant but negative correlation of vitamin D concentration with HbA1c at $r=-0.217$ ($p=0.03$). However, no correlation was found of vitamin D with FBG, at $r=-0.153$ ($p=0.128$).

DISCUSSION

The patients with T2DM in this study were mostly females. The greater number of females who had diabetes is similar to that in the study of Mihardja, in that diabetes affects women more than men, because of their higher body mass index. This is in contrast with Japan and China, where T2DM prevalence is higher in males, which may be caused by the differing diets and behaviors of males [7]. The study by Azlin et al. shows that there are more females with T2DM than males. According to the 2002 study of Brunner and Suddart as cited by Azlin, there are more females with T2DM as compared to males, because there is proportionately more body fat in former than in the latter. Body fat deposit is one of the factors that may reduce insulin sensitivity in muscles and liver [8]. In the 2018 Basic Health Survey of Indonesia (Riskesdas), the DM prevalence in females was higher than in males, at a ratio of 1.78% to 1.21%. The Riskesdas also found that in the last 5 years the prevalence of T2DM in females showed a slight increase if compared to males [9].

The participants in our research had a median age of 56 years. In third world countries, most of the patients with T2DM are between 45 and 64 years old, whereas in first world countries the patients with T2DM are mostly older than 64 years [10]. The prevalence of diabetes also shows an increase with increasing age of

the patients and peaks at age 55-64 years. In Riskesdas 2018 there were also indications of an increase in age and thus a higher risk for diabetes [9]. Sharan and team [11] reported that recent onset T2DM accounted for 79.1% in the age range of 40-60 years. Older respondents are commonly more at risk of hyperglycemia, due to decreased function of the pancreas [7].
The median 25(OH)D level in this study was 11.2 ng/mL, corresponding to vitamin D deficiency in 86% of patients. Vitamin D can be categorized as deficiency if 25(OH)D concentration < 20 ng/mL, insufficiency if 25(OH)D concentration = 21-29 ng/mL, sufficiency if 25(OH)D concentration ≥ 30 ng/mL and intoxication if 25(OH)D level ≥ 150 ng/mL [12]. The proportion of deficiency and insufficiency for vitamin D in Indonesia is relatively great, being 68.8% in Pusparini's study in South Jakarta [13] on women and men in the age range of 55-65 years. In North Sumatra, however, Keumala [14] found that 95% of adult women had vitamin D deficiency and insufficiency. Arjana's team [15] in Yogyakarta found that in adult males aged 19-25 years, the proportion of deficiency of vitamin D was 43.3%a, whereas the proportion of insufficiency of vitamin D was 51.7%.
The causative factors of vitamin D deficiency are varied, among others low exposure to sunlight, less vitamin D-containing foods in the diet, insufficient outdoor activity, a life style that avoids sunlight, sunscreen usage, covered clothing or traditional clothing covering the whole body, and dark-colored skin. Skin color is an important factor in natural vitamin D production. Darker skins need more time for the formation of vitamin D than do fair skins. Sun screens may absorb ultraviolet light (UVB-UVA) such as to inhibit the penetration of UVB into the skin [16,17]. Reduced physical activity is also a factor in vitamin D deficiency and T2DM, because physical activity may improve the vitamin D status [18].
Our findings of reduced median vitamin D level and high vitamin D deficiency prevalence was probably caused by the inadequate vitamin D intake from sunlight and the diet. This study found that the proportion of study subjects who had never been exposed to sunlight for 15-30 minutes was 84%, because the majority of study subjects were housewives (68%) such that their activity was indoors. Our study also found that the median vitamin D intake was extremely low, at 1.76 µg/day. The average vitamin D intake recommended by the Institute of Medicine (IOM) for females and males aged 18-70 years is 15 µg/day [19]. Vitamin D-containing foods are among others egg yolk, salmon, mackerel, tuna, meat, mushrooms, cod liver oil, and fortified foods [14,19]. According to the study of Masood and Iqbal, as cited by Keumala et al. [14] the inability to buy vitamin D-containing foods results in the occurrence of vitamin D deficiency. Salmon and cod liver oil are highly expensive vitamin D-containing foods on the market. It should be noted that around 69% of our subjects wore covered or long-sleeved clothing for a period of 12-24 hours, such as to prevent sunlight from penetrating into the body through the surface of the skin. According to Holick, adequate vitamin D can be obtained by twice weekly sunlight exposure of the arms and legs for 5-30 minutes between 10 AM and 3 PM (as determined by weather and skin color) [12]. Similar results were found between our study and Keumala's, in that deficiency of vitamin D correlates with indoor occupations and activities, less vitamin D-containing foods in the diet, and sunbathing of ≤ 1 hour daily [14].
Mahmodnia's team [20] reported a strong positive correlation of vitamin D and age. The age variable is a dominant factor that causes vitamin D deficiency, which is correlated with low outdoor activities resulting in low exposure to sunlight. In addition, there are also changes in the skin, namely a reduction in the content of 7-dehydroxycholesterol, which is the precursor of vitamin D3 [21]. The study of Jha et al. [22] also showed low vitamin D concentrations with increasing age. The reduced synthesis of vitamin D with increasing age, in addition to being due to reduced 7-dehydroxycholesterol concentrations, is also caused by reduced oral absorption of vitamin D.
In our study, an important difference was found in the vitamin D levels of men and women, This may have been the result of differences in physical activity and life style between males and females, where the activities of the males were presumably carried out to a greater extent out of doors, in comparison to those of the females, such that the males were more frequently exposed to sunlight. Life style, in this case the style of clothing also affects vitamin D concentrations. The majority of the female respondents in this study wore covered clothing (Muslim clothing with head scarves) causing a reduction in the exposure of the body to sunlight, such that vitamin D synthesis is reduced. Another factor that may have caused the contrasting vitamin D levels in men and women is the larger amount of body fat in women than in men. The fact that vitamin D is soluble in fats while body fat absorbs and stores vitamin D, may reduce circulatory vitamin D concentrations in females [23,24].
As stated above, our study found that vitamin D had a strong negative relationship with HbA1c, but not with FBG. This agrees with Mehta's [25] report of a significant negative correlation (r = - 0.1205) of HbA1c

with vitamin D, where decreased vitamin D level is connected with increased HbA1c level. Our study also agrees with Jha's study [22] that reported an inverse relationship between vitamin D and HbA1c. However, our study results differ from those of Suguna's group [26] from Bengaluru, showing no strong relationship of HbA1c with vitamin D concentrations in T2DM patients (r= - 0.109 at p=0.05).

Research findings of Raumulfaro [24] ~~demonstrated no~~demonstrated no association of vitamin D ~~with HbA~~with HbA1c (r = 0.214 at a p value of 0.153) on the one hand and vitamin D and blood glucose (r =0.195 at p=0.193) on the other. The non-significant correlation of FBG with vitamin D may have been among other causes due to body fat percentage. A person with a high body fat percentage may have reduced vitamin D concentrations, as a large amount of body fat retains vitamin D, thereby diminishing circulatory vitamin D, leading to low serum levels. Consumption of glucocorticoids and blood-pressure-lowering medications may also influence vitamin D concentrations. The use of glucocorticoids may interfere with osteoblastic and osteoclastic functions, such that it can reduce vitamin D concentrations. Furthermore, sport increases the level of this vitamin, as reported in the study of Fernandez [24,27-29]. The study conducted by Sakung [30] found that physical activity heightens tissue sensitivity to insulin and absorption of glucose, thereby reducing blood glucose concentrations.

One study showed that vitamin D is negatively correlated with HbA1c, but not with FBG, thereby showing that adequate vitamin D in T2DM patients improves HbA1c but not FBG. This may have been due to differences in the level of glycemic control of HbA1c and FBG, where HbA1c represents the mean glucose level over the last 60 to 90 days., whereas serum glucose concentration is determined after an overnight fast. The FBG level is determined by basal insulin secretion, whereas HbA1c is determined by basal and post-prandial insulin secretion. The significant connection of vitamin D with HbA1c was presumably due to the effect of vitamin D on post-prandial but not on basal insulin release. Post-prandial vitamin D increase is connected with calcium absorption leading to increased intracellular calcium, which mediates post-prandial release of insulin, thereby improving HbA1c concentrations without an effect on FBG concentrations [31-33].

In this study no significant differences were found in HbA1c and blood glucose parameters between males and females. Vitamin D was also not strongly correlated with HbA1c or with blood glucose, after adjustment for gender subgroups (data not shown), possibly because of the disproportionately larger number of females.

According to Kautzky-Willer et al. [34] the gender differences for HbA1c cannot be related to the make-up of the body, because there are various factors affecting HbA1c level, among others genetic, age-related, ethnic, and environmental factors.

Fasting blood glucose is influenced mainly by the release of glucose in the liver [34,35]. Measurement of random glucose concentration, such as postprandial glucose, may improve glycemic control in females, particularly when there are differences between HbA1c and fasting glucose [34,36].

The American Diabetes Association recommends HbA1c instead of FBG for the diagnosis of diabetes mellitus. HbA1c constitutes an invaluable monitor of blood glucose control because it represents the glucose level accumulated over the last 60 to 90 days. HbA1c is also highly correlated with the risk of diabetic sequelae over a prolonged period of time [32].

Vitamin D is important for glucose metabolism, by stimulating insulin release from pancreatic beta cells and by increasing intracellular calcium and insulin sensitivity in peripheral muscles and adipocytes [18]. Vitamin D also stimulates insulin receptor expression and peroxisome proliferator-activated receptor delta (PPAR-δ) activation that is involved in the metabolism of fatty acids and the transport of glucose in the muscles, where these compounds will be converted into ATP as source of energy. Deficiency of vitamin D may affect calcium concentrations in cells that are sensitive to insulin, thus leading to peripheral insulin resistance that reduces glucose transport to the muscles. In addition, deficiency of vitamin D indirectly influences the resistance to insulin via the renin-angiotensin-aldosterone system, where angiotensin blocks vascular and skeletal muscle insulin, thus interfering ~~with glucose~~with glucose ~~absorption and~~absorption ~~reducing~~and reducing energy needs [37].

Maintaining the normal vitamin D concentration may help ~~regulate glucose~~regulate glucose homeostasis, while in individuals with high HbA1c, screening for vitamin D ~~insufficiency and~~insufficiency ~~deficiency~~and deficiency may be performed [25].

The weak points of the present study were its cross-sectional study design which prevented delving into the relationship of vitamin D versus HbA1c or glucose, and the fact that it did not use healthy individuals as controls; and thirdly, this study performed only single blood glucose determinations that did not reflect glycemic variability.

CONCLUSION

This study found a strong negative relationship of vitamin D with HbA1c, but not with FBG. Additional research is required using healthy controls and performing serial blood glucose determinations.

ACKNOWLEDGMENT

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Table 1. Characteristics of Study Subjects

Characteristic	N (%)
Gender:	
Female	74 (74)
Male	26 (26)
Education	
No schooling	5 (5)
Elementary school	15 (15)
Junior high school	29 (29)
Senior high school	35 (35)
Academy	16 (16)
Employment history	
Housewife	68 (68)
Employed	25 (25)
Unemployed	7 (7)
Vitamin D status	
Deficient	86 (86)
Insufficient	12 (12)
Sufficient	2 (2)
Sunlight exposure (15-30 minutes)	
Yes	16 (16)
No	84 (84)
Wearing of long-sleeved clothing (12-24 hours)	
Yes	69 (69)
No	31 (31)
	Median (IQR)
Age (years)	56 (35-79)
Duration of DM (months)	12 (1-120)
25(OH)D concentration (ng/mL)	11.2 (2.4-31.1)
HbA1c (%)	8.05 (5.1-17.4)
Blood glucose (mg/dL)	127 (70-383)
Dietary vitamin D intake (µg/day)	1.76 (0.0 -12.44)
	Mean (SD)
Height (m)	1.54 (0.07)
Weight (kg)	62.04 (11.06)
BMI (kg/m^2)	26.09 (4.52)

Continuous data are presented as median (IQR) and mean (SD); categorical data as number (%)

Table 2. Concentrations of Vitamin D, HbA1c and Blood Glucose in Female and Male Subjects

Parameter	Concentration	P value*
25(OH)D concentration (ng/mL)		
Females	10.2 (2.4-24.7)	0.001
Males	14.7 (4.7-31.1)	
HbA1c (%)		
Females	8.3 (5.1-17.4)	0.389
Males	7.8 (5.4-12.5)	
Blood glucose (mg/dL)		
Females	125.5 (70-383)	0.804
Males	130.5 (83-248)	

*Mann-Whitney test, p significant if <0.05; Continuous data presented as median (IQR); HbA1c: hemoglobin A1c

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Thank you so much for input on the manuscript. I have corrected the manuscript according to the comments given. I've used the checklist to improve my manuscript writing and I've used the zotero reference manager for writing references. Hopefully this manuscript can be accepted at Rwanda Medical Journal.

Thank you
Best regards,
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25(OH)D CONCENTRATIONS NEGATIVELY CORRELATED WITH HbA1c IN TYPE 2 DIABETES MELLITUS PATIENTS: A CROSS SECTIONAL STUDY IN MAMPANG, SOUTH JAKARTA

ABSTRACT

INTRODUCTION: Insulin resistance plays a central role in type 2 diabetes mellitus (T2DM). Another possible cause of T2DM is deficiency of vitamin D, although the underlying mechanism is not yet clearly understood. Most frequently used laboratory parameters for monitoring T2DM are fasting blood glucose (FBG) and HbA1c. Determining any association of vitamin D with HbA1c and FBG in T2DM.
METHODS: A cross-sectional study involving 100 T2DM patients with following characteristics: 18-year and older persons of both genders, not having kidney and liver disease, not on insulin therapy, not pregnant or lactating, and not consuming vitamin D in the last 3 months. Spearman's correlation coefficient was used to test any association of vitamin D with HbA1c and FBG at $p<0.05$.
RESULTS: Subjects comprised 74 females and 26 males with median age of 56 years. Median HbA1c, 25(OH)D, and fasting blood glucose were 8.05%, 11.2 ng/mL, and 127 mg/dL, respectively. The Spearman correlation coefficient for vitamin D and HbA1c was r= -0.217 (p=0.03), and for vitamin D and FBG it was r = -0.153 (p=0.128).
CONCLUSION: There was a significant negative correlation of vitamin D with HbA1c, but not with FBG.

Keywords: type 2 DM; 25(OH)D; blood glucose; HbA1c

INTRODUCTION

The metabolic disorder diabetes mellitus (DM) features high blood glucose levels due to defective insulin response. Insulin resistance in target tissues, particularly in the skeletal muscles and adipose tissue, is responsible for the occurrence of abnormalities of carbohydrate, fat and protein metabolism, that may result in diabetes. There are several types of DM. The 2022 American Diabetes Association guidelines categorize DM into type 1 diabetes due to autoimmune damage to the beta cells; type 2 diabetes due to insulin resistance; diabetes due to other causes such as monogenic diabetic syndrome, DM-causing drugs, exocrine pancreatic disease, and gestational diabetes [1,2].

Type 2 diabetes mellitus (T2DM) comprises the highest number of patients, both globally and in Indonesia. The global prevalence of T2DM at age 20-79 years according to the International Diabetes Federation (IDF) 2013 report was 8.3% (382 million persons) with 14 million more men than women, generally aged 40-59 years. The global number of cases is projected to increase to 592 million in the year 2035. The increased incidence of T2DM at younger ages may presumably be caused by changes in life style and unhealthy food. Obesity is also responsible for the occurrence of T2DM [1].

Insulin resistance plays a central role in T2DM. Oxidative stress, proinflammatory activation, adipokines and abnormal metabolism of glucose and lipid can lead to insulin resistance [3]. Another possible cause of T2DM is deficiency of vitamin D. Although the underlying mechanism is not yet clearly understood, low 25-hydroxyvitamin D (25(OH)D) level is connected with functional disorders of pancreatic islet Langerhans cells and insulin resistance causing changes in glucose homeostasis such as to result in the occurrence of T2DM [4].

Basically, vitamin D may increase the secretion of insulin, skeletal muscle glucose and lipid metabolism, and reduce systemic inflammation. However, several studies found no influence of vitamin D supplementation on blood glucose in patients with T2DM while other studies found inconsistencies in the connection of vitamin D with blood glucose [5].

Although low vitamin D level is correlated with higher glycosylated hemoglobin (HbA1c) and blood glucose levels in patients with T2DM, the relationship is as yet unclear as well as inconsistent, such that there is still a need for further research on the connection of vitamin D with blood glucose in T2DM patients. The purpose of our study was to explore the connection of vitamin D with HbA1c and with blood glucose in T2DM patients.

METHODS

This cross-sectional study recruited 100 patients with T2DM at Mampang District *Puskesmas*, South Jakarta, from June to July 2022, by means of consecutive sampling. The size of the required research sample was obtained using the formula for the correlation sample size, with $Z\alpha$=1.96; $Z\beta$=1.28; and r=- 0.387 [6].

The inclusion criteria were as follows: men and women older than 18 years, not on insulin therapy, not having kidney and liver disease, not being pregnant or lactating, and not consuming vitamin D in the last 3 months. All subjects were provided with information about this study and all gave written agreement by signing the informed consent form.

The subjects were to self-report on a questionnaire on sources of 25(OH)D and the subjects' T2DM condition.

After a 10-hour fast by the respondents, venous blood was drawn from each respondent and divided into aliquots of 3, 2 and 5 mL that were respectively placed into one EDTA anticoagulant tube for measuring HbA1c, one sodium fluoride (NaF) anticoagulant tube for measuring fasting blood glucose (FBG), and one tube without anticoagulant for 25(OH)D determination. The HbA1c level was measured by High Performance Liquid Chromatography (HPLC), FBG by the hexokinase method, and of 25(OH)D by Direct Competitive Chemiluminescent Microparticle Immunoassay (CMIA). Determination of HbA1c and FBG was performed directly on the day of blood specimen collection, whereas 25(OH)D was determined simultaneously after all specimens had been collected. The blood specimens were frozen at minus 20^0C prior to being examined.

For data analysis, the Statistical Package for Social Sciences (SPSS) program version 23 was used. The Kolmogorov-Smirnov test was used to determine whether or not the data were normally distributed. Because the data distribution was non-normal, the correlation of vitamin D with HbA1c and FBG was analyzed by the Spearman correlation test at $p < 0.05$. Ethics approval was issued by the Ethics Committee of the Faculty of Medicine, Universitas Trisakti, under No. 037/KER/FK/V/2022.

RESULTS

The characteristics of the study subjects, comprised of 26 males and 74 females with median age of 56 years (35 -79 years) may be seen in Table 1. The educational level of 35% of the subjects was high school graduate, while the most frequent occupation was housewife at 68%. The most frequent 25(OH)D status was deficiency in 86% of subjects. Seventy-seven percent of the subjects had never consumed vitamin D-containing foods, 84% of the subjects had never sunbathed, and 69% wore long-sleeved clothing daily for 24 hours.

We found that the male and female subjects differed significantly in vitamin D levels, but not in HbA1c and FBG levels (Table 2).

This study also found a significant but negative correlation of vitamin D concentration with HbA1c at r= -0.217 (p= 0.03). However, no correlation was found of vitamin D with FBG, at r= - 0.153 (p=0.128).

DISCUSSION

The patients with T2DM in this study were mostly females. The greater number of females who had diabetes is similar to that in the study of Mihardja, in that diabetes affects women more than men, because of their higher body mass index. This is in contrast with Japan and China, where T2DM prevalence is higher in males, which may be caused by the differing diets and behaviors of males [7]. The study by Azlin et al. shows that there are more females with T2DM than males. According to the 2002 study of Brunner and Suddart as cited by Azlin, there are more females with T2DM as compared to males, because there is proportionately more body fat in former than in the latter. Body fat deposit is one of the factors that may reduce insulin sensitivity in muscles and liver [8]. In the 2018 Basic Health Survey of Indonesia (Riskesdas), the DM prevalence in females was higher than in males, at a ratio of 1.78% to 1.21%. The Riskesdas also found that in the last 5 years the prevalence of T2DM in females showed a slight increase if compared to males [9].

The participants in our research had a median age of 56 years. In third world countries, most of the patients with T2DM are between 45 and 64 years old, whereas in first world countries the patients with T2DM are mostly older than 64 years [10]. The prevalence of diabetes also shows an increase with increasing age of the patients and peaks at age 55-64 years. In Riskesdas 2018 there were also indications of an increase in age and thus a higher risk for diabetes [9]. Sharan and team [11] reported that recent onset T2DM

accounted for 79.1% in the age range of 40-60 years. Older respondents are commonly more at risk of hyperglycemia, due to decreased function of the pancreas [7].
The median 25(OH)D level in this study was 11.2 ng/mL, corresponding to vitamin D deficiency in 86% of patients. Vitamin D can be categorized as deficiency if 25(OH)D concentration < 20 ng/mL, insufficiency if 25(OH)D concentration = 21-29 ng/mL, sufficiency if 25(OH)D concentration ≥ 30 ng/mL and intoxication if 25(OH)D level ≥ 150 ng/mL [12]. The proportion of deficiency and insufficiency for vitamin D in Indonesia is relatively great, being 68.8% in Pusparini's study in South Jakarta [13] on women and men in the age range of 55-65 years. In North Sumatra, however, Keumala [14] found that 95% of adult women had vitamin D deficiency and insufficiency. Arjana's team [15] in Yogyakarta found that in adult males aged 19-25 years, the proportion of deficiency of vitamin D was 43.3%a, whereas the proportion of insufficiency of vitamin D was 51.7%.
The causative factors of vitamin D deficiency are varied, among others low exposure to sunlight, less vitamin D-containing foods in the diet, insufficient outdoor activity, a life style that avoids sunlight, sunscreen usage, covered clothing or traditional clothing covering the whole body, and dark-colored skin. Skin color is an important factor in natural vitamin D production. Darker skins need more time for the formation of vitamin D than do fair skins. Sun screens may absorb ultraviolet light (UVB-UVA) such as to inhibit the penetration of UVB into the skin [16,17]. Reduced physical activity is also a factor in vitamin D deficiency and T2DM, because physical activity may improve the vitamin D status [18].
Our findings of reduced median vitamin D level and high vitamin D deficiency prevalence was probably caused by the inadequate vitamin D intake from sunlight and the diet. This study found that the proportion of study subjects who had never been exposed to sunlight for 15-30 minutes was 84%, because the majority of study subjects were housewives (68%) such that their activity was indoors. Our study also found that the median vitamin D intake was extremely low, at 1.76 µg/day. The average vitamin D intake recommended by the Institute of Medicine (IOM) for females and males aged 18-70 years is 15 µg/day [19]. Vitamin D-containing foods are among others egg yolk, salmon, mackerel, tuna, meat, mushrooms, cod liver oil, and fortified foods [14,19]. According to the study of Masood and Iqbal, as cited by Keumala et al. [14] the inability to buy vitamin D-containing foods results in the occurrence of vitamin D deficiency. Salmon and cod liver oil are highly expensive vitamin D-containing foods on the market. It should be noted that around 69% of our subjects wore covered or long-sleeved clothing for a period of 12-24 hours, such as to prevent sunlight from penetrating into the body through the surface of the skin. According to Holick, adequate vitamin D can be obtained by twice weekly sunlight exposure of the arms and legs for 5-30 minutes between 10 AM and 3 PM (as determined by weather and skin color) [12]. Similar results were found between our study and Keumala's, in that deficiency of vitamin D correlates with indoor occupations and activities, less vitamin D-containing foods in the diet, and sunbathing of ≤ 1 hour daily [14].
Mahmodnia's team [20] reported a strong positive correlation of vitamin D and age. The age variable is a dominant factor that causes vitamin D deficiency, which is correlated with low outdoor activities resulting in low exposure to sunlight. In addition, there are also changes in the skin, namely a reduction in the content of 7-dehydroxycholesterol, which is the precursor of vitamin D3 [21]. The study of Jha et al. [22] also showed low vitamin D concentrations with increasing age. The reduced synthesis of vitamin D with increasing age, in addition to being due to reduced 7-dehydroxycholesterol concentrations, is also caused by reduced oral absorption of vitamin D.
In our study, an important difference was found in the vitamin D levels of men and women, This may have been the result of differences in physical activity and life style between males and females, where the activities of the males were presumably carried out to a greater extent out of doors, in comparison to those of the females, such that the males were more frequently exposed to sunlight. Life style, in this case the style of clothing also affects vitamin D concentrations. The majority of the female respondents in this study wore covered clothing (Muslim clothing with head scarves) causing a reduction in the exposure of the body to sunlight, such that vitamin D synthesis is reduced. Another factor that may have caused the contrasting vitamin D levels in men and women is the larger amount of body fat in women than in men. The fact that vitamin D is soluble in fats while body fat absorbs and stores vitamin D, may reduce circulatory vitamin D concentrations in females [23,24].
As stated above, our study found that vitamin D had a strong negative relationship with HbA1c, but not with FBG. This agrees with Mehta's [25] report of a significant negative correlation (r = - 0.1205) of HbA1c with vitamin D, where decreased vitamin D level is connected with increased HbA1c level. Our study also agrees with Jha's study [22] that reported an inverse relationship between vitamin D and HbA1c. However,

our study results differ from those of Suguna's group [26] from Bengaluru, showing no strong relationship of HbA1c with vitamin D concentrations in T2DM patients (r= - 0.109 at p=0.05).

Research findings of Raumulfaro [24] revealed no association between vitamin D and HbA1c (r = 0.214 at a p value of 0.153) on the one hand and vitamin D and blood glucose (r =0.195 at p=0.193) on the other. The non-significant correlation of FBG with vitamin D may have been among other causes due to body fat percentage. A person with a high body fat percentage may have reduced vitamin D concentrations, as a large amount of body fat retains vitamin D, thereby diminishing circulatory vitamin D, leading to low serum levels. Consumption of glucocorticoids and blood-pressure-lowering medications may also influence vitamin D concentrations. The use of glucocorticoids may interfere with osteoblastic and osteoclastic functions, such that it can reduce vitamin D concentrations. Furthermore, sport increases the level of this vitamin, as reported in the study of Fernandez [24,27–29]. The study conducted by Sakung [30] found that physical activity heightens tissue sensitivity to insulin and absorption of glucose, thereby reducing blood glucose concentrations.

One study showed that vitamin D is negatively correlated with HbA1c, but not with FBG, thereby showing that adequate vitamin D in T2DM patients improves HbA1c but not FBG. This may have been due to differences in the level of glycemic control of HbA1c and FBG, where HbA1c represents the mean glucose level over the last 60 to 90 days., whereas serum glucose concentration is determined after an overnight fast. The FBG level is determined by basal insulin secretion, whereas HbA1c is determined by basal and post-prandial insulin secretion. The significant connection of vitamin D with HbA1c was presumably due to the effect of vitamin D on post-prandial but not on basal insulin release. Post-prandial vitamin D increase is connected with calcium absorption leading to increased intracellular calcium, which mediates post-prandial release of insulin, thereby improving HbA1c concentrations without an effect on FBG concentrations [31–33].

In this study no significant differences were found in HbA1c and blood glucose parameters between males and females. Vitamin D was also not strongly correlated with HbA1c or with blood glucose, after adjustment for gender subgroups (data not shown), possibly because of the disproportionately larger number of females.

According to Kautzky-Willer et al. [34] the gender differences for HbA1c cannot be related to the make-up of the body, because there are various factors affecting HbA1c level, among others genetic, age-related, ethnic, and environmental factors.

Fasting blood glucose is influenced mainly by the release of glucose in the liver [34,35]. Measurement of random glucose concentration, such as postprandial glucose, may improve glycemic control in females, particularly when there are differences between HbA1c and fasting glucose [34,36].

The American Diabetes Association recommends HbA1c instead of FBG for the diagnosis of diabetes mellitus. HbA1c constitutes an invaluable monitor of blood glucose control because it represents the glucose level accumulated over the last 60 to 90 days. HbA1c is also highly correlated with the risk of diabetic sequelae over a prolonged period of time [32].

Vitamin D is important for glucose metabolism, by stimulating insulin release from pancreatic beta cells and by increasing intracellular calcium and insulin sensitivity in peripheral muscles and adipocytes [18]. Vitamin D also stimulates insulin receptor expression and peroxisome proliferator-activated receptor delta (PPAR-δ) activation that is involved in the metabolism of fatty acids and the transport of glucose in the muscles, where these compounds will be converted into ATP as source of energy. Deficiency of vitamin D may affect calcium concentrations in cells that are sensitive to insulin, thus leading to peripheral insulin resistance that reduces glucose transport to the muscles. In addition, deficiency of vitamin D indirectly influences the resistance to insulin via the renin-angiotensin-aldosterone system, where angiotensin blocks vascular and skeletal muscle insulin, thus interfering energy needs [37].

Maintaining the normal vitamin D concentration may help glucose homeostasis, while in individuals with high HbA1c, screening for vitamin D may be performed [25].

The weak points of the present study were its cross-sectional study design which prevented delving into the relationship of vitamin D versus HbA1c or glucose, and the fact that it did not use healthy individuals as controls; and thirdly, this study performed only single blood glucose determinations that did not reflect glycemic variability.

CONCLUSION
This study found a strong negative relationship of vitamin D with HbA1c, but not with FBG. Additional research is required using healthy controls and performing serial blood glucose determinations.

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Table 1. Characteristics of Study Subjects

Characteristic	N (%)
Gender:	
Female	74 (74)
Male	26 (26)
Education	
No schooling	5 (5)
Elementary school	15 (15)
Junior high school	29 (29)
Senior high school	35 (35)
Academy	16 (16)
Employment history	
Housewife	68 (68)
Employed	25 (25)
Unemployed	7 (7)
Vitamin D status	
Deficient	86 (86)
Insufficient	12 (12)
Sufficient	2 (2)
Sunlight exposure (15-30 minutes)	
Yes	16 (16)
No	84 (84)
Wearing of long-sleeved clothing (12-24 hours)	
Yes	69 (69)
No	31 (31)
	Median (IQR)
Age (years)	56 (35-79)
Duration of DM (months)	12 (1-120)
25(OH)D concentration (ng/mL)	11.2 (2.4-31.1)
HbA1c (%)	8.05 (5.1-17.4)
Blood glucose (mg/dL)	127 (70-383)
Dietary vitamin D intake (µg/day)	1.76 (0.0 -12.44)
	Mean (SD)
Height (m)	1.54 (0.07)
Weight (kg)	62.04 (11.06)
BMI (kg/m^2)	26.09 (4.52)

Continuous data are presented as median (IQR) and mean (SD); categorical data as number (%)

Table 2. Concentrations of Vitamin D, HbA1c and Blood Glucose in Female and Male Subjects

Parameter	Concentration	P value*
25(OH)D concentration (ng/mL)		
Females	10.2 (2.4-24.7)	0.001
Males	14.7 (4.7-31.1)	
HbA1c (%)		
Females	8.3 (5.1-17.4)	0.389
Males	7.8 (5.4-12.5)	
Blood glucose (mg/dL)		
Females	125.5 (70-383)	0.804
Males	130.5 (83-248)	

*Mann-Whitney test, p significant if <0.05; Continuous data presented as median (IQR); HbA1c: hemoglobin A1c

4. Bukti konfirmasi review dan hasil review kedua (30 Juni 2023)

UNIVERSITAS TRISAKTI

Alvina fk <dr.alvina@trisakti.ac.id>

SUGGESTED MINOR CHANGES BEFORE ACCEPTANCE

3 messages

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Here are some minor changes needed before acceptance for publication
Please respond to the added comments.

Thank you for considering the RMJ.

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Thank you for the input to my manuscripts. I have corrected the manuscripts. Hopefully my manuscripts can be accepted.
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25(OH)D -HYDROXY VITAMIN D CCONCENTRATIONS NEGATIVELY CORRELATED WITH HbA1c IN TYPE 2 DIABETES MELLITUS PATIENTS: A CROSS SECTIONAL STUDYSECTIONAL STUDY IN MAMPANG, SOUTH JAKARTA

ABSTRACT

INTRODUCTION: Insulin resistance plays a central role in type 2 diabetes mellitus (T2DM). Another possible cause of T2DM is deficiency of 25-hydroxy vitamin D [25(OH)D]of vitamin D, although the underlying mechanism is not yet clearly understood. Most frequently used laboratory parameters for monitoring T2DM are fasting blood glucose (FBG) and HbA1c. Determining any association of vitamin D with HbA1c and FBG in T2DM.

METHODS: A cross-sectional study involving 100 T2DM patients with following characteristics: 18-year and older persons of both genders, not having kidney and liver disease, not on insulin therapy, not pregnant or lactating, and not consuming vitamin D in the last 3 months. Spearman's correlation coefficient was used to test any association of vitamin Dof -25(OH)D with HbA1c and FBG at p<0.05.

RESULTS: Subjects comprised 74 females and 26 males with median age of 56 years. Median HbA1c, 25(OH)D, and fasting blood glucose were 8.05%, 11.2 ng/mL, and 127 mg/dL, respectively. The Spearman correlation coefficient for vitamin D and HbA1c was r= -0.217 (p=0.03), and for vitaminfor vitamin D and FBG it was rwas r = -0.153 (p=0.128).

CONCLUSION: There was a significant negative correlation of vitamin D with HbA1c, but not with FBG.

Keywords: type 2 DM; 25(OH)D; blood glucose; HbA1c

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Commented [P2]: Use at least 5 MeSH key words

INTRODUCTION

The metabolic disorder diabetes mellitus (DM) features high blood glucose levels due to defective insulin response. Insulin resistance in target tissues, particularly in the skeletal muscles and adipose tissue, is responsible for the occurrence of abnormalities of carbohydrate, fat and protein metabolism, that may result in diabetes. There are several types of DM. The 2022 American Diabetes Association guidelines categorize DM into type 1 diabetes due to autoimmune damage to the beta cells; type 2 diabetes due to insulin resistance; diabetes due to other causes such as monogenic diabetic syndrome, DM-causing drugs, exocrine pancreatic disease, and gestational diabetes [1,2].

Type 2 diabetes mellitus (T2DM) comprises the highest number of patients, both globally and in Indonesia. The global prevalence of T2DM at age 20-79 years according to the International Diabetes Federation (IDF) 2013 report was 8.3% (382 million persons) with 14 million more men than women, generally aged 40-59 years. The global number of cases is projected to increase to 592 million in the year 2035. The increased incidence of T2DM at younger ages may presumably be caused by changes in life style and unhealthy food. Obesity is also responsible for the occurrence of T2DM [1].

Insulin resistance plays a central role in T2DM. Oxidative stress, proinflammatory activation, adipokines and abnormal metabolism of glucose and lipid can lead to insulin resistance [3]. Another possible cause of T2DM is deficiency of vitamin D. Although the underlying mechanism is not yet clearly understood, low 25-hydroxyvitamin D [(25(OH)D]) level is connected with functional disorders of pancreatic islet Langerhans cells and insulin resistance causing changes in glucose homeostasis such as to result in the occurrence of T2DM [4]. Basically, vitamin D may increase the secretion of insulin, skeletal muscle glucose and lipid metabolism, and reduce systemic inflammation. However, several studies found no influence of vitamin D supplementation on blood glucose in patients with T2DM while other studies found inconsistencies in the connection of vitamin D with blood glucose [5].

Although low vitamin D level is correlated with higher glycosylated hemoglobin (HbA1c) and blood glucose levels in patients with T2DM, the relationship is as yet unclear as well as inconsistent, such that there is still a need for further research on the connection of vitamin D with blood glucose in T2DM patients. The purpose of our study was to explore the connection of vitamin D with HbA1c and with blood glucose in T2DM patients.

METHODS

This cross-sectional study recruited 100 patients with T2DM at Mampang District *Puskesmas*, South Jakarta, from June to July 2022, by means of consecutive sampling. The size of the required research sample was obtained using the formula for the correlation sample size, with $Z\alpha=1.96$; $Z\beta=1.28$; and $r=-0.387$ [6].
The inclusion criteria were as follows: men and women older than 18 years, not on insulin therapy, not having kidney and liver disease, not being pregnant or lactating, and not consuming vitamin D in the last 3 months. All subjects were provided with information about this study and all gave written agreement by signing the informed consent form.
The subjects were to self-report on a questionnaire on sources of 25(OH)D and the subjects' T2DM condition.
After a 10-hour fast by the respondents, venous blood was drawn from each respondent and divided into aliquots of 3, 2 and 5 mL that were respectively placed into one EDTA anticoagulant tube for measuring HbA1c, one sodium fluoride (NaF) anticoagulant tube for measuring fasting blood glucose (FBG), and one tube without anticoagulant for 25(OH)D determination. The HbA1c level was measured by High Performance Liquid Chromatography (HPLC), FBG by the hexokinase method, and of 25(OH)D by Direct Competitive Chemiluminescent Microparticle Immunoassay (CMIA). Determination of HbA1c and FBG was performed directly on the day of blood specimen collection, whereas 25(OH)D was determined simultaneously after all specimens had been collected. The blood specimens were frozen at minus 20^0C prior to being examined.

For data analysis, the Statistical Package for Social Sciences (SPSS) program version 23 was used. The Kolmogorov-Smirnov test was used to determine whether or not the data were normally distributed. Because the data distribution was non-normal, the correlation of vitamin D with HbA1c and FBG was analyzed by the Spearman correlation test at $p < 0.05$.
Ethics approval was issued by the Ethics Committee of the Faculty of Medicine, Universitas Trisakti, under No. 037/KER/FK/V/2022.

RESULTS

The characteristics of the study subjects, comprised of 26 males and 74 females with median age of 56 years (35 -79 years) may be seen in Table 1. The educational level of 35% of the subjects was high school graduate, while the most frequent occupation was housewife (at 68%). The most frequent 25(OH)D status was deficiency in 86% of subjects. Seventy-seven percent of the subjects had never consumed vitamin D-containing foods, 84% of the subjects had never sunbathed, and 69% wore long-sleeved clothing daily for 24 hours.
We found that the male and female subjects differed significantly in vitamin D levels, but not in HbA1c and FBG levels (Table 2).
This study also found a significant but negative correlation of vitamin D concentration with HbA1c at $r=-0.217$ ($p=0.03$). However, no correlation was found of vitamin D with FBG, at $r=-0.153$ ($p=0.128$).

DISCUSSION

The patients with T2DM in this study were mostly females. The greater number of females who had diabetes is similar to that in the study of Mihardja, in that diabetes affects women more than men, because of their higher body mass index. This is in contrast with Japan and China, where T2DM prevalence is higher in males, which may be caused by the differing diets and behaviors of males [7]. The study by Azlin et al. shows that there are more females with T2DM than males. According to the 2002 study of Brunner and Suddart as cited by Azlin, there are more females with T2DM as compared to males, because there is proportionately more body fat in former than in the latter. Body fat deposit is one of the factors that may reduce insulin sensitivity in muscles and liver [8]. In the 2018 Basic Health Survey of ~~Indonesia (~~Indonesia (Riskesdas), the DM prevalence in females was higher than in males, at a ratio of 1.78% to 1.21%. The Riskesdas also found that in the last 5 years the prevalence of T2DM in females showed a slight increase if compared to males [9].
The participants in our research had a median age of 56 years. In third world countries, most of the patients with T2DM are between 45 and 64 years old, whereas in first world countries the patients with T2DM are mostly older than 64 years [10]. The prevalence of diabetes also shows an increase with increasing age of the ~~patients and~~patients and peaks at age 55-64 years. In Riskesdas 2018 there were also indications of

Commented [P3]: Rephrase this to make it clear and add some more details

Commented [P4]: Add all tables directly in this article an increase in age and thus a higher risk for diabetes [9]. Sharan and team [11] reported that recent onset T2DM accounted for 79.1% in the age range of 40-60 years. Older respondents are commonly more at risk of hyperglycemia, due to decreased function of the pancreas [7].

The median 25(OH)D level in this study was 11.2 ng/mL, corresponding to vitamin D deficiency in 86% of patients. Vitamin D can be categorized as deficiency if 25(OH)D concentration < 20 ng/mL, insufficiency if 25(OH)D concentration = 21-29 ng/mL, sufficiency if 25(OH)D concentration ≥ 30 ng/mL and intoxication if 25(OH)D level ≥ 150 ng/mL [12]. The proportion of deficiency and insufficiency for vitamin D in Indonesia is relatively great, being 68.8% in Pusparini's study in South Jakarta [13] on women and men in the age range of 55-65 years. In North Sumatra, however, Keumala [14] found that 95% of adult women had vitamin D deficiency and insufficiency. Arjana's team [15] in Yogyakarta found that in adult males aged 19-25 years, the proportion of deficiency of vitamin D was 43.3%a, whereas the proportion of insufficiency of vitamin D was 51.7%.

The causative factors of vitamin D deficiency are varied, among others low exposure to sunlight, less vitamin D-containing foods in the diet, insufficient outdoor activity, a life style that avoids sunlight, sunscreen usage, covered clothing or traditional clothing covering the whole body, and dark-colored skin. Skin color is an important factor in natural vitamin D production. Darker skins need more time for the formation of vitamin D than do fair skins. Sun screens may absorb ultraviolet light (UVB-UVA) such as to inhibit the penetration of UVB into the skin [16,17]. Reduced physical activity is also a factor in vitamin D deficiency and T2DM, because physical activity may improve the vitamin D status [18].

Our findings of reduced median vitamin D level and high vitamin D deficiency prevalence was probably caused by the inadequate vitamin D intake from sunlight and the diet. This study found that the proportion of study subjects who had never been exposed to sunlight for 15-30 minutes was 84%, because the majority of study subjects were housewives (68%) such that their activity was indoors. Our study also found that the median vitamin D intake was extremely low, at 1.76 µg/day. The average vitamin D intake recommended by the Institute of Medicine (IOM) for females and males aged 18-70 years is 15 µg/day [19]. Vitamin D-containing foods are among others egg yolk, salmon, mackerel, tuna, meat, mushrooms, cod liver oil, and fortified foods [14,19]. According to the study of Masood and Iqbal, as cited by Keumala et al. [14] the inability to buy vitamin D-containing foods results in the occurrence of vitamin D deficiency. Salmon and cod liver oil are highly expensive vitamin D-containing foods on the market. It should be noted that around 69% of our subjects wore covered or long-sleeved clothing for a period of 12-24 hours, such as to prevent sunlight from penetrating into the body through the surface of the skin. According to Holick, adequate vitamin D can be obtained by twice weekly sunlight exposure of the arms and legs for 5-30 minutes between 10 AM and 3 PM (as determined by weather and skin color) [12]. Similar results were found between our study and Keumala's, in that deficiency of vitamin D correlates with indoor occupations and activities, less vitamin D-containing foods in the diet, and sunbathing of ≤ 1 hour daily [14].

Mahmodnia's team [20] reported a strong positive correlation of vitamin D and age. The age variable is a dominant factor that causes vitamin D deficiency, which is correlated with low outdoor activities resulting in low exposure to sunlight. In addition, there are also changes in the skin, namely a reduction in the content of 7-dehydroxycholesterol, which is the precursor of vitamin D3 [21]. The study of Jha et al. [22] also showed low vitamin D concentrations with increasing age. The reduced synthesis of vitamin D with increasing age, in addition to being due to reduced 7-dehydroxycholesterol concentrations, is also caused by reduced oral absorption of vitamin D.

In our study, an important difference was found in the vitamin D levels of men and women, This may have been the result of differences in physical activity and life style between males and females, where the activities of the males were presumably carried out to a greater extent out of doors, in comparison to those of the females, such that the males were more frequently exposed to sunlight. Life style, in this case the style of clothing also affects vitamin D concentrations. The majority of the female respondents in this study wore covered clothing (Muslim clothing with head scarves) causing a reduction in the exposure of the body to sunlight, such that vitamin D synthesis is reduced. Another factor that may have caused the contrasting vitamin D levels in men and women is the larger amount of body fat in women than in men. The fact that vitamin D is soluble in fats while body fat absorbs and stores vitamin D, may reduce circulatory vitamin D concentrations in females [23,24].

As stated above, our study found that vitamin D had a strong negative relationship with HbA1c, but not with FBG. This agrees with Mehta's [25] report of a significant negative correlation (r = - 0.1205) of HbA1c with vitamin D, where decreased vitamin D level is connected with increased HbA1c level. Our study also

agrees with Jha's study [22] that reported an inverse relationship between vitamin D and HbA1c. However, our study results differ from those of Suguna's group [26] from Bengaluru, showing no strong relationship of HbA1c with vitamin D concentrations in T2DM patients (r= - 0.109 at p=0.05).
Research findings of Raumulfaro [24] revealed no association between vitamin D and HbA1c (r = 0.214 at a p value of 0.153) on the one hand and vitamin D and blood glucose (r =0.195 at p=0.193) on the other. The non-significant correlation of FBG with vitamin D may have been among other causes due to body fat percentage. A person with a high body fat percentage may have reduced vitamin D concentrations, as a large amount of body fat retains vitamin D, thereby diminishing circulatory vitamin D, leading to low serum levels. Consumption of glucocorticoids and blood-pressure-lowering medications may also influence vitamin D concentrations. The use of glucocorticoids may interfere with osteoblastic and osteoclastic functions, such that it can reduce vitamin D concentrations. Furthermore, sport increases the level of this vitamin, as reported in the study of Fernandez [24,27–29]. The study conducted by Sakung [30] found that physical activity heightens tissue sensitivity to insulin and absorption of glucose, thereby reducing blood glucose concentrations.
One study showed that vitamin D is negatively correlated with HbA1c, but not with FBG, thereby showing that adequate vitamin D in T2DM patients improves HbA1c but not FBG. This may have been due to differences in the level of glycemic control of HbA1c and FBG, where HbA1c represents ~~the mean~~the mean glucose level over the last 60 to 90 days., whereas serum glucose concentration is determined after an overnight fast. The FBG level is determined by basal insulin secretion, whereas HbA1c is determined by basal and post-prandial insulin secretion. The significant connection of vitamin D with HbA1c was presumably due to the effect of vitamin D on post-prandial but not on basal insulin release. Post-prandial vitamin D increase is connected with calcium absorption leading to increased intracellular calcium, which mediates post-prandial release of insulin, thereby improving HbA1c concentrations without an effect on FBG concentrations [31–33].
In this study no significant differences were found in HbA1c and blood glucose parameters between males and females. Vitamin D was also not strongly correlated with HbA1c or with blood glucose, after adjustment for gender subgroups (data not shown), possibly because of the disproportionately larger number of females.
According to Kautzky-Willer et al. [34] the gender differences for HbA1c cannot be related to the make-up of the body, because there are various factors affecting HbA1c level, among others genetic, age-related, ethnic, and environmental factors.
Fasting blood glucose is influenced mainly by the release of glucose in the liver [34,35]. Measurement of random glucose concentration, such as postprandial glucose, may improve glycemic control in females, particularly when there are differences between HbA1c and fasting glucose [34,36].
The American Diabetes Association recommends HbA1c instead of FBG for the diagnosis of diabetes mellitus. HbA1c constitutes an invaluable monitor of blood glucose control because it represents the glucose level accumulated over the last 60 to 90 days. HbA1c is also highly correlated with the risk of diabetic sequelae over a prolonged period of time [32].
Vitamin D is important for glucose metabolism, by stimulating insulin release from pancreatic beta cells and by increasing intracellular calcium and insulin sensitivity in peripheral muscles and adipocytes [18]. Vitamin D also stimulates insulin receptor expression and peroxisome proliferator-activated receptor delta (PPAR-δ) activation that is involved in the metabolism of fatty acids and the transport of glucose in the muscles, where these compounds will be converted into ATP as source of energy. Deficiency of vitamin D may affect calcium concentrations in cells that are sensitive to insulin, thus leading to peripheral insulin resistance that reduces glucose transport to the muscles. In addition, deficiency of vitamin D indirectly influences the resistance to insulin via the renin-angiotensin-aldosterone system, where angiotensin blocks vascular and skeletal muscle insulin, thus interfering energy needs [37].
Maintaining the normal vitamin D concentration may help glucose homeostasis, while in individuals with high HbA1c, screening for vitamin D may be performed [25].
The weak points of the present study were its cross-sectional study design which prevented delving into the relationship of vitamin D versus HbA1c or glucose, and the fact that it did not use healthy individuals as controls; and thirdly, this study performed only single blood glucose determinations that did not reflect glycemic variability.

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CONCLUSION

This study found a strong negative relationship of vitamin D with HbA1c, but not with FBG. Additional research is required using healthy controls and performing serial blood glucose determinations.

ACKNOWLEDGMENT

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Table 1. Characteristics of Study Subjects

Characteristic	N (%)
Gender:	
Female	74 (74)
Male	26 (26)
Education	
No schooling	5 (5)
Elementary school	15 (15)
Junior high school	29 (29)
Senior high school	35 (35)
Academy	16 (16)
Employment history	
Housewife	68 (68)
Employed	25 (25)
Unemployed	7 (7)
Vitamin D status	
Deficient	86 (86)
Insufficient	12 (12)
Sufficient	2 (2)
Sunlight exposure (15-30 minutes)	
Yes	16 (16)
No	84 (84)
Wearing of long-sleeved clothing (12-24 hours)	
Yes	69 (69)
No	31 (31)
	Median (IQR)
Age (years)	56 (35-79)
Duration of DM (months)	12 (1-120)
25(OH)D concentration (ng/mL)	11.2 (2.4-31.1)
HbA1c (%)	8.05 (5.1-17.4)
Blood glucose (mg/dL)	127 (70-383)
Dietary vitamin D intake (µg/day)	1.76 (0.0 -12.44)
	Mean (SD)
Height (m)	1.54 (0.07)
Weight (kg)	62.04 (11.06)
BMI (kg/m^2)	26.09 (4.52)

Continuous data are presented as median (IQR) and mean (SD); categorical data as number (%)

Table 2. Concentrations of Vitamin D, HbA1c and Blood Glucose in Female and Male Subjects

Parameter	Concentration	P value*
25(OH)D concentration (ng/mL)		
Females	10.2 (2.4-24.7)	0.001
Males	14.7 (4.7-31.1)	
HbA1c (%)		
Females	8.3 (5.1-17.4)	0.389
Males	7.8 (5.4-12.5)	
Blood glucose (mg/dL)		
Females	125.5 (70-383)	0.804
Males	130.5 (83-248)	

*Mann-Whitney test, p significant if <0.05; Continuous data presented as median (IQR); HbA1c: hemoglobin A1c

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UNIVERSITAS TRISAKTI

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25 HYDROXY VITAMIN D CONCENTRATIONS NEGATIVELY CORRELATED WITH HbA1c IN TYPE 2 DIABETES MELLITUS PATIENTS: A CROSS-SECTIONAL STUDY IN MAMPANG, SOUTH JAKARTA

ABSTRACT

INTRODUCTION: Insulin resistance plays a central role in type 2 diabetes mellitus (T2DM). Another possible cause of T2DM is deficiency of 25-hydroxy vitamin D [25(OH)D], although the underlying mechanism is not yet clearly understood. Most frequently used laboratory parameters for monitoring T2DM are fasting blood glucose (FBG) and HbA1c. Determining any association of vitamin D with HbA1c and FBG in T2DM.

METHODS: A cross-sectional study involving 100 T2DM patients with following characteristics: 18-year and older persons of both genders, not having kidney and liver disease, not on insulin therapy, not pregnant or lactating, and not consuming vitamin D in the last 3 months. Spearman's correlation coefficient was used to test any association of 25(OH)D with HbA1c and FBG at $p<0.05$.

RESULTS: Subjects comprised 74 females and 26 males with median age of 56 years. Median HbA1c, 25(OH)D, and fasting blood glucose were 8.05%, 11.2 ng/mL, and 127 mg/dL, respectively. The Spearman correlation coefficient for vitamin D and HbA1c was r= -0.217 (p=0.03), and for vitamin D and FBG it was r = -0.153 (p=0.128).

CONCLUSION: There was a significant negative correlation of vitamin D with HbA1c, but not with FBG. Administration of vitamin D should be considered for additional treatment of T2DM.

Keywords: type 2 DM; 25(OH)D; blood glucose; HbA1c; FBG

INTRODUCTION

The metabolic disorder diabetes mellitus (DM) features high blood glucose levels due to defective insulin response. Insulin resistance in target tissues, particularly in the skeletal muscles and adipose tissue, is responsible for the occurrence of abnormalities of carbohydrate, fat and protein metabolism, that may result in diabetes. There are several types of DM. The 2022 American Diabetes Association guidelines categorize DM into type 1 diabetes due to autoimmune damage to the beta cells; type 2 diabetes due to insulin resistance; diabetes due to other causes such as monogenic diabetic syndrome, DM-causing drugs, exocrine pancreatic disease, and gestational diabetes [1,2].

Type 2 diabetes mellitus (T2DM) comprises the highest number of patients, both globally and in Indonesia. The global prevalence of T2DM at age 20-79 years according to the International Diabetes Federation (IDF) 2013 report was 8.3% (382 million persons) with 14 million more men than women, generally aged 40-59 years. The global number of cases is projected to increase to 592 million in the year 2035. The increased incidence of T2DM at younger ages may presumably be caused by changes in life style and unhealthy food. Obesity is also responsible for the occurrence of T2DM [1].

Insulin resistance plays a central role in T2DM. Oxidative stress, proinflammatory activation, adipokines and abnormal metabolism of glucose and lipid can lead to insulin resistance [3]. Another possible cause of T2DM is deficiency of vitamin D. Although the underlying mechanism is not yet clearly understood, low 25-hydroxyvitamin D [25(OH)D] level is connected with functional disorders of pancreatic islet Langerhans cells and insulin resistance causing changes in glucose homeostasis such as to result in the occurrence of T2DM [4]. Basically, vitamin D may increase the secretion of insulin, skeletal muscle glucose and lipid metabolism, and reduce systemic inflammation. However, several studies found no influence of vitamin D supplementation on blood glucose in patients with T2DM while other studies found inconsistencies in the connection of vitamin D with blood glucose [5].

Although low vitamin D level is correlated with higher glycosylated hemoglobin (HbA1c) and blood glucose levels in patients with T2DM, the relationship is as yet unclear as well as inconsistent, such that there is still a need for further research on the connection of vitamin D with blood glucose in T2DM patients. The purpose of our study was to explore the connection of vitamin D with HbA1c and with blood glucose in T2DM patients.

METHODS

This cross-sectional study recruited 100 patients with T2DM at Mampang District *Puskesmas*, South Jakarta, from June to July 2022, by means of consecutive sampling. The size of the required research sample was obtained using the formula for the correlation sample size, with $Z\alpha=1.96$; $Z\beta=1.28$; and $r=-0.387$ [6].

The inclusion criteria were as follows: men and women older than 18 years, not on insulin therapy, not having kidney and liver disease, not being pregnant or lactating, and not consuming vitamin D in the last 3 months. All subjects were provided with information about this study and all gave written agreement by signing the informed consent form.

A self-reported questionnaire was used comprising items on subjects' employment, education, sunbathing habit, use of covered clothing, and consumption of vitamin D rich foods, and T2DM condition.

After a 10-hour fast by the respondents, venous blood was drawn from each respondent and divided into aliquots of 3, 2 and 5 mL that were respectively placed into one EDTA anticoagulant tube for measuring HbA1c, one sodium fluoride (NaF) anticoagulant tube for measuring fasting blood glucose (FBG), and one tube without anticoagulant for 25(OH)D determination. The HbA1c level was measured by High Performance Liquid Chromatography (HPLC), FBG by the hexokinase method, and of 25(OH)D by Direct Competitive Chemiluminescent Microparticle Immunoassay (CMIA). Determination of HbA1c and FBG was performed directly on the day of blood specimen collection, whereas 25(OH)D was determined simultaneously after all specimens had been collected. The blood specimens were frozen at minus 20^0C prior to being examined.

For data analysis, the Statistical Package for Social Sciences (SPSS) program version 23 was used. The Kolmogorov-Smirnov test was used to determine whether or not the data were normally distributed. Because the data distribution was non-normal, the correlation of vitamin D with HbA1c and FBG was analyzed by the Spearman correlation test at $p < 0.05$.

Ethics approval was issued by the Ethics Committee of the Faculty of Medicine, Universitas Trisakti, under No. 037/KER/FK/V/2022.

RESULTS

The characteristics of the study subjects, comprised of 26 males and 74 females with median age of 56 years (35 -79 years) may be seen in Table 1. The educational level of 35% of the subjects was high school graduate, while the most frequent occupation was housewife (68%). The most frequent 25(OH)D status was deficiency in 86% of subjects. Seventy-seven percent of the subjects had never consumed vitamin D-containing foods, 84% of the subjects had never sunbathed, and 69% wore long-sleeved clothing daily for 24 hours.

Table 1. Characteristics of Study Subjects

Characteristic	N (%)
Gender:	
Female	74 (74)
Male	26 (26)
Education	
No schooling	5 (5)
Elementary school	15 (15)
Junior high school	29 (29)
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Employment history	
Housewife	68 (68)
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Insufficient	12 (12)
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Sunlight exposure (15-30 minutes)	
Yes	16 (16)
No	84 (84)
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Yes	69 (69)
No	31 (31)
	Median (IQR)
Age (years)	56 (35-79)
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	Mean (SD)
Height (m)	1.54 (0.07)
Weight (kg)	62.04 (11.06)
BMI (kg/m^2)	26.09 (4.52)

Continuous data are presented as median (IQR) and mean (SD); categorical data as number (%)

We found that the male and female subjects differed significantly in vitamin D levels, but not in HbA1c and FBG levels (Table 2).

This study also found a significant but negative correlation of vitamin D concentration with HbA1c at r= - 0.217 (p= 0.03). However, no correlation was found of vitamin D with FBG, at r= - 0.153 (p=0.128).

Table 2. Concentrations of Vitamin D, HbA1c and Blood Glucose in Female and Male Subjects

Parameter	Concentration	P value*
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*Mann-Whitney test, p significant if <0.05; Continuous data presented as median (IQR); HbA1c: hemoglobin A1c

DISCUSSION

The patients with T2DM in this study were mostly females. The greater number of females who had diabetes is similar to that in the study of Mihardja, in that diabetes affects women more than men, because of their higher body mass index. This is in contrast with Japan and China, where T2DM prevalence is higher in males, which may be caused by the differing diets and behaviors of males [7]. The study by Azlin et al. shows that there are more females with T2DM than males. According to the 2002 study of Brunner and Suddart as cited by Azlin, there are more females with T2DM as compared to males, because there is proportionately more body fat in former than in the latter. Body fat deposit is one of the factors that may reduce insulin sensitivity in muscles and liver [8]. In the 2018 Basic Health Survey of Indonesia, the DM prevalence in females was higher than in males, at a ratio of 1.78% to 1.21%. The Basic Health Survey of Indonesia also found that in the last 5 years the prevalence of T2DM in females showed a slight increase if compared to males [9].

The participants in our research had a median age of 56 years. In third world countries, most of the patients with T2DM are between 45 and 64 years old, whereas in first world countries the patients with T2DM are mostly older than 64 years [10]. The prevalence of diabetes also shows an increase with increasing age of the patients and peaks at age 55-64 years. In Basic Health Survey of Indonesia 2018 there were also indications of an increase in age and thus a higher risk for diabetes [9]. Sharan and team [11] reported that recent onset T2DM accounted for 79.1% in the age range of 40-60 years. Older respondents are commonly more at risk of hyperglycemia, due to decreased function of the pancreas [7].

The median 25(OH)D level in this study was 11.2 ng/mL, corresponding to vitamin D deficiency in 86% of patients. Vitamin D can be categorized as deficiency if 25(OH)D concentration < 20 ng/mL, insufficiency if 25(OH)D concentration = 21-29 ng/mL, sufficiency if 25(OH)D concentration ≥ 30 ng/mL and intoxication if 25(OH)D level ≥ 150 ng/mL [12]. The proportion of deficiency and insufficiency for vitamin D in Indonesia is relatively great, being 68.8% in Pusparini's study in South Jakarta [13] on women and men in the age range of 55-65 years. In North Sumatra, however, Keumala [14] found that 95% of adult women had vitamin D deficiency and insufficiency. Arjana's team [15] in Yogyakarta found that in adult males aged 19-25 years, the proportion of deficiency of vitamin D was 43.3%a, whereas the proportion of insufficiency of vitamin D was 51.7%.

The causative factors of vitamin D deficiency are varied, among others low exposure to sunlight, less vitamin D-containing foods in the diet, insufficient outdoor activity, a life style that avoids sunlight, sunscreen usage, covered clothing or traditional clothing covering the whole body, and dark-colored skin. Skin color is an important factor in natural vitamin D production. Darker skins need more time for the formation of vitamin D than do fair skins. Sun screens may absorb ultraviolet light (UVB-UVA) such as to inhibit the penetration of UVB into the skin [16,17]. Reduced physical activity is also a factor in vitamin D deficiency and T2DM, because physical activity may improve the vitamin D status [18].

Our findings of reduced median vitamin D level and high vitamin D deficiency prevalence was probably caused by the inadequate vitamin D intake from sunlight and the diet. This study found that the proportion of study subjects who had never been exposed to sunlight for 15-30 minutes was 84%, because the majority of study subjects were housewives (68%) such that their activity was indoors. Our study also found that the median vitamin D intake was extremely low, at 1.76 µg/day. The average vitamin D intake recommended by the Institute of Medicine (IOM) for females and males aged 18-70 years is 15 µg/day [19]. Vitamin D-containing foods are among others egg yolk, salmon, mackerel, tuna, meat, mushrooms, cod liver oil, and fortified foods [14,19]. According to the study of Masood and Iqbal, as cited by Keumala et al. [14] the inability to buy vitamin D-containing foods results in the occurrence of vitamin D deficiency. Salmon and cod liver oil are highly expensive vitamin D-containing foods on the market. It should be noted that around 69% of our subjects wore covered or long-sleeved clothing for a period of 12-24 hours, such as to prevent sunlight from penetrating into the body through the surface of the skin. According to Holick, adequate vitamin D can be obtained by twice weekly sunlight exposure of the arms and legs for 5-30 minutes between 10 AM and 3 PM (as determined by weather and skin color) [12]. Similar results were found between our study and Keumala's, in that deficiency of vitamin D correlates with indoor occupations and activities, less vitamin D-containing foods in the diet, and sunbathing of ≤ 1 hour daily [14].

Mahmodnia's team [20] reported a strong positive correlation of vitamin D and age. The age variable is a dominant factor that causes vitamin D deficiency, which is correlated with low outdoor activities resulting in low exposure to sunlight. In addition, there are also changes in the skin, namely a reduction in the content of 7-dehydroxycholesterol, which is the precursor of vitamin D3 [21]. The study of Jha et al. [22] also showed low vitamin D concentrations with increasing age. The reduced synthesis of vitamin D with increasing age, in addition to being due to reduced 7-dehydroxycholesterol concentrations, is also caused by reduced oral absorption of vitamin D.

In our study, an important difference was found in the vitamin D levels of men and women, This may have been the result of differences in physical activity and life style between males and females, where the activities of the males were presumably carried out to a greater extent out of doors, in comparison to those of the females, such that the males were more frequently exposed to sunlight. Life style, in this case the style of clothing also affects vitamin D concentrations. The majority of the female respondents in this study wore covered clothing (Muslim clothing with head scarves) causing a reduction in the exposure of the body to sunlight, such that vitamin D synthesis is reduced. Another factor that may have caused the contrasting vitamin D levels in men and women is the larger amount of body fat in women than in men. The fact that vitamin D is soluble in fats while body fat absorbs and stores vitamin D, may reduce circulatory vitamin D concentrations in females [23,24].

As stated above, our study found that vitamin D had a strong negative relationship with HbA1c, but not with FBG. This agrees with Mehta's [25] report of a significant negative correlation (r = - 0.1205) of HbA1c with vitamin D, where decreased vitamin D level is connected with increased HbA1c level. Our study also agrees with Jha's study [22] that reported an inverse relationship between vitamin D and HbA1c. However, our study results differ from those of Suguna's group [26] from Bengaluru, showing no strong relationship of HbA1c with vitamin D concentrations in T2DM patients (r= - 0.109 at p=0.05).

Research findings of Raumulfaro [24] revealed no association between vitamin D and HbA1c (r = 0.214 at a p value of 0.153) on the one hand and vitamin D and blood glucose (r =0.195 at p=0.193) on the other. The non-significant correlation of FBG with vitamin D may have been among other causes due to body fat percentage. A person with a high body fat percentage may have reduced vitamin D concentrations, as a large amount of body fat retains vitamin D, thereby diminishing circulatory vitamin D, leading to low serum levels. Consumption of glucocorticoids and blood-pressure-lowering medications may also influence vitamin D concentrations. The use of glucocorticoids may interfere with osteoblastic and osteoclastic functions, such that it can reduce vitamin D concentrations. Furthermore, sport increases the level of this vitamin, as reported in the study of Fernandez [24,27–29]. The study conducted by Sakung [30] found that physical activity heightens tissue sensitivity to insulin and absorption of glucose, thereby reducing blood glucose concentrations.

One study showed that vitamin D is negatively correlated with HbA1c, but not with FBG, thereby showing that adequate vitamin D in T2DM patients improves HbA1c but not FBG. This may have been due to differences in the level of glycemic control of HbA1c and FBG, where HbA1c represents the mean glucose level over the last 60 to 90 days., whereas serum glucose concentration is determined after an overnight fast. The FBG level is determined by basal insulin secretion, whereas HbA1c is determined by basal and post-prandial insulin secretion. The significant connection of vitamin D with HbA1c was presumably due to the effect of vitamin D on post-prandial but not on basal insulin release. Post-prandial vitamin D increase is connected with calcium absorption leading to increased intracellular calcium, which mediates post-prandial release of insulin, thereby improving HbA1c concentrations without an effect on FBG concentrations [31–33].

In this study no significant differences were found in HbA1c and blood glucose parameters between males and females. Vitamin D was also not strongly correlated with HbA1c or with blood glucose, after adjustment for gender subgroups (data not shown), possibly because of the disproportionately larger number of females.

According to Kautzky-Willer et al. [34] the gender differences for HbA1c cannot be related to the make-up of the body, because there are various factors affecting HbA1c level, among others genetic, age-related, ethnic, and environmental factors.

Fasting blood glucose is influenced mainly by the release of glucose in the liver [34,35]. Measurement of random glucose concentration, such as postprandial glucose, may improve glycemic control in females, particularly when there are differences between HbA1c and fasting glucose [34,36].

The American Diabetes Association recommends HbA1c instead of FBG for the diagnosis of diabetes mellitus. HbA1c constitutes an invaluable monitor of blood glucose control because it represents the glucose level accumulated over the last 60 to 90 days. HbA1c is also highly correlated with the risk of diabetic sequelae over a prolonged period of time [32].

Vitamin D is important for glucose metabolism, by stimulating insulin release from pancreatic beta cells and by increasing intracellular calcium and insulin sensitivity in peripheral muscles and adipocytes [18]. Vitamin D also stimulates insulin receptor expression and peroxisome proliferator-activated receptor delta (PPAR-δ) activation that is involved in the metabolism of fatty acids and the transport of glucose in the muscles, where these compounds will be converted into ATP as source of energy. Deficiency of vitamin D may affect calcium concentrations in cells that are sensitive to insulin, thus leading to peripheral insulin resistance that reduces glucose transport to the muscles. In addition, deficiency of vitamin D indirectly influences the resistance to insulin via the renin-angiotensin-aldosterone system, where angiotensin blocks vascular and skeletal muscle insulin, thus interfering energy needs [37].

Maintaining the normal vitamin D concentration may help glucose homeostasis, while in individuals with high HbA1c, screening for vitamin D may be performed [25].

The weak points of the present study were its cross-sectional study design which prevented delving into the relationship of vitamin D versus HbA1c or glucose, and the fact that it did not use healthy individuals as controls; and thirdly, this study performed only single blood glucose determinations that did not reflect glycemic variability. Further studies need to be conducted on vitamin D supplementation in the form of a randomized double blind placebo controlled trial to determine the effects of vitamin D on HbA1c and FBG.

CONCLUSION

This study found a strong negative relationship of vitamin D with HbA1c, but not with FBG. Additional research is required using healthy controls and performing serial blood glucose determinations.

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ORIGINAL ARTICLE Open Access

25-hydroxy vitamin D concentrations negatively correlated with HbA1c in type 2 diabetes mellitus patients: a cross-sectional study in Mampang, South Jakarta

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ABSTRACT

INTRODUCTION: Insulin resistance plays a central role in type 2 diabetes mellitus (T2DM). Another possible cause of T2DM is a deficiency of 25-hydroxy vitamin D [25(OH)D], although the underlying mechanism is not yet clearly understood. The most frequently used laboratory parameters for monitoring T2DM are fasting blood glucose (FBG) and HbA1c. Determining any association of vitamin D with HbA1c and FBG in T2DM.

METHODS: A cross-sectional study involving 100 T2DM patients with the following characteristics: 18-year and older persons of both genders, not having kidney and liver disease, not on insulin therapy, not pregnant or lactating, and not consuming vitamin D in the last three months. Spearman's correlation coefficient was used to test any association of 25(OH)D with HbA1c and fasting blood glucose (FBG) ($p<0.05$).

RESULTS: Subjects comprised 74 females and 26 males with a median age of 56 years. Median HbA1c, 25(OH)D, and FBG were 8.05%, 11.2 ng/mL, and 127 mg/dL, respectively. The Spearman correlation coefficient for vitamin D and HbA1c was $r=-0.217$ ($p=0.03$), and for vitamin D and FBG, it was $r=-0.153$ ($p=0.128$).

CONCLUSION: There was a significant negative correlation of vitamin D with HbA1c but not with FBG. Administration of vitamin D should be considered for additional treatment of T2DM.

Keywords: Hepatitis B Vaccine, Immunological response, Healthcare Workers, Rwanda Military Hospital

INTRODUCTION

The metabolic disorder diabetes mellitus (DM) features high blood glucose levels due to defective insulin response. Insulin resistance in target tissues, particularly in the skeletal muscles and adipose tissue, is responsible for abnormalities in carbohydrate, fat and protein metabolism, which may result in diabetes. There are several types of DM. The 2022 American Diabetes Association guidelines categorize DM into type 1 diabetes due to autoimmune damage to the beta cells, type 2 diabetes due to insulin resistance; diabetes due to other causes such as monogenic diabetic syndrome, DM-causing drugs, exocrine pancreatic disease, and gestational diabetes [1,2].

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Type 2 diabetes mellitus (T2DM) comprises the highest number of patients globally and in Indonesia. The global prevalence of T2DM at age 20-79 years, according to the International Diabetes Federation (IDF) 2013 report, was 8.3% (382 million persons), with 14 million more men than women, generally aged 40-59 years. The global number of cases is projected to increase to 592 million in the year 2035. Changes in lifestyle and unhealthy food may presumably cause the increased incidence of T2DM at younger ages. Obesity is also responsible for the occurrence of T2DM [1].

Insulin resistance plays a central role in T2DM. Oxidative stress, proinflammatory activation, adipokines, and abnormal glucose and lipid metabolism can lead to insulin resistance [3]. Another possible cause of T2DM is a deficiency of vitamin D. Although the underlying mechanism is not yet clearly understood, low 25-hydroxyvitamin D [25(OH)D] level is connected with functional disorders of pancreatic islet Langerhans cells and insulin resistance causing changes in glucose homeostasis such as to result in the occurrence of T2DM [4].

Vitamin D may increase insulin secretion, skeletal muscle glucose, and lipid metabolism and reduce systemic inflammation. However, several studies found no influence of vitamin D supplementation on blood glucose in patients with T2DM, while other studies found inconsistencies in the connection of vitamin D with blood glucose [5].

Although low vitamin D level is correlated with higher glycosylated hemoglobin (HbA1c) and blood glucose levels in patients with T2DM, the relationship is as yet unclear as well as inconsistent, such that there is still a need for further research on the connection of vitamin D with blood glucose in T2DM patients. Our study aimed to explore the connection of vitamin D with HbA1c and blood glucose in T2DM patients.

METHODS

This cross-sectional study recruited 100 patients with T2DM at Mampang District Puskesmas, South Jakarta, from June to July 2022 through consecutive sampling. The size of the required research sample was obtained using the formula for the correlation sample size, with $Z\alpha$=1.96; $Z\beta$=1.28; and r=- 0.387 [6].

The inclusion criteria were as follows: men and women older than 18 years, not on insulin therapy, not having kidney and liver disease, not being pregnant or lactating, and not consuming vitamin D in the last three months. All subjects were provided with information about this study, and all gave written agreement by signing the informed consent form.

A self-reported questionnaire was used with items on subjects' employment, education, sunbathing habits, use of covered clothing, consumption of vitamin D-rich foods, and T2DM condition.

After a 10-hour fast by the respondents, venous blood was drawn from each respondent and divided into aliquots of 3, 2, and 5 mL that were respectively placed into one EDTA anticoagulant tube for measuring HbA1c, one sodium fluoride (NaF) anticoagulant tube for measuring fasting blood glucose (FBG), and one tube without anticoagulant for 25(OH)D determination. The HbA1c level was measured by High-Performance Liquid Chromatography (HPLC), FBG by the hexokinase method, and 25(OH)D by Direct Competitive Chemiluminescent Microparticle Immunoassay (CMIA). Determination of HbA1c and FBG was performed directly on the day of blood specimen collection, whereas 25(OH)D was determined simultaneously after all specimens had been collected. The blood specimens were frozen at minus 200C prior to being examined.

For data analysis, the Statistical Package for Social Sciences (SPSS) program version 23 was used. The Kolmogorov-Smirnov test was used to determine whether or not the data were normally distributed. Because the data distribution was non-normal, the correlation of vitamin D with HbA1c and FBG was analyzed by the Spearman correlation test at $p < 0.05$.

Ethics approval was issued by the Faculty of Medicine Ethics Committee, Universitas Trisakti, under No. 037/KER/FK/V/2022.

RESULTS

The characteristics of the study subjects, comprised of 26 males and 74 females with median age of 56 years (35 -79 years) may be seen in Table 1. The educational level of 35% of the subjects was high school graduate, while the most frequent occupation was housewife (68%). The most frequent 25(OH)D status was deficiency in 86% of subjects. Seventy-seven percent of the subjects

Table 1. Characteristics of study subjects

Characteristic	N (%)
Gender	
Female	74 (74)
Male	26 (26)
Education	
No schooling	5 (5)
Elementary school	15 (15)
Junior high school	29 (29)
Senior high school	35 (35)
Academy	16 (16)
Employment history	
Housewife	68 (68)
Employed	25 (25)
Unemployed	7 (7)
Vitamin D status	
Deficient	86 (86)
Insufficient	12 (12)
Sufficient	2 (2)
Sunlight exposure (15-30 minutes)	
Yes	16 (16)
No	84 (84)
Wearing of long-sleeved clothing (12-24 hours)	
Yes	69 (69)
No	31 (31)
	Median (IQR)
Age (years)	56 (35-79)
Duration of DM (months)	12 (1-120)
25(OH)D concentration (ng/mL)	11.2 (2.4-31.1)
HbA1c (%)	8.05 (5.1-17.4)
Blood glucose (mg/dL)	127 (70-383)
Dietary vitamin D intake (µg/day)	1.76 (0.0-12.44)
	Mean (SD)
Height (m)	1.54 (0.07)
Weight (kg)	62.04 (11.06)
BMI (kg/m^2)	26.09 (4.52)

BMI: Body Mass Index, DM: Diabetes Mellitus, IQR: Interquartile, SD: Standard deviation, HbA1c: Hemoglobin A1c

Table 2. Cconcentrations of vitamin D, HbA1c, and blood glucose in female and famle Subjects

Parameter	Concentration	P value
25(OH)D concentration (ng/mL)		
Females	10.2 (2.4-24.7)	0.001*
Males	14.7 (4.7-31.1)	
HbA1c (%)		
Females	8.3 (5.1-17.4)	0.389
Males	7.8 (5.4-12.5)	
Blood glucose (mg/dL)		
Females	125.5 (70-383)	0.804
Males	130.5 (83-248)	

**Statistically significant, HbA1c: Hemoglobin A1c*

had never consumed vitamin D-containing foods, 84% of the subjects had never sunbathed, and 69% wore long-sleeved clothing daily for 24 hours.

We found that the male and female subjects differed significantly in vitamin D levels but not in HbA1c and FBG levels (Table 2).
This study also found a significant but negative correlation of vitamin D concentration with HbA1c at r= - 0.217 (p= 0.03). However, no correlation of vitamin D with FBG was found at r= - 0.153 (p=0.128).

DISCUSSION

The patients with T2DM in this study were mostly females. The greater number of females who had diabetes is similar to that in the study of Mihardja, in that diabetes affects women more than men because of their higher body mass index. This is in contrast with Japan and China, where T2DM prevalence is higher in males, which may be caused by the differing diets and behaviors of males [7]. The study by Azlin et al. shows that there are more females with T2DM than males. According to the 2002 study of Brunner and Suddart, as cited by Azlin, there are more females with T2DM as compared to males because there is proportionately more body fat in the former than in the latter. Body fat deposit is one of the factors that may reduce insulin sensitivity in muscles and liver [8]. In the 2018 Basic Health Survey of Indonesia, the DM prevalence in females was higher than in males, at a ratio of 1.78% to 1.21%. The Basic Health Survey of Indonesia also found that in the last five years, the prevalence of T2DM in females showed a slight increase compared to males [9].
The participants in our research had a median age of 56 years. In third-world countries, most of the patients with T2DM are between 45 and 64 years old, whereas in first-world countries, the patients with T2DM are mostly older than 64 years [10]. The prevalence of diabetes also shows an increase with increasing age of the patients and peaks at age 55-64 years. In the Basic Health Survey of Indonesia 2018, there were also indications of an increase in age and, thus, a higher risk for diabetes [9]. Sharan and team [11] reported that recent onset T2DM accounted for 79.1% in the age range of 40-60 years. Older respondents are commonly more at risk of hyperglycemia due to decreased function of the pancreas [7].
The median 25(OH)D level in this study was 11.2 ng/mL, corresponding to vitamin D deficiency in 86% of patients. Vitamin D can be categorized as deficiency if 25(OH)D concentration < 20 ng/mL, insufficiency if 25(OH)D concentration = 21-29 ng/mL, sufficiency if 25(OH)D concentration ≥ 30 ng/mL and intoxication if 25(OH)D level ≥ 150 ng/mL [12]. The proportion of deficiency and insufficiency for vitamin D in Indonesia is relatively great, being 68.8% in Pusparini's study in South Jakarta [13] on women and men aged 55-65 years. In North Sumatra, however, Keumala [14] found that 95% of adult women had vitamin D deficiency and insufficiency. Arjana's team [15] in Yogyakarta found that in adult males aged 19-25 years, the proportion of deficiency of vitamin D was 43.3%,

whereas the proportion of insufficiency of vitamin D was 51.7%.

The causative factors of vitamin D deficiency are varied, among others, low exposure to sunlight, fewer vitamin D-containing foods in the diet, insufficient outdoor activity, a lifestyle that avoids sunlight, sunscreen usage, covered clothing or traditional clothing covering the whole body, and dark-colored skin. Skin color is an important factor in natural vitamin D production. Darker skins need more time to form vitamin D than fair skins. Sunscreens may absorb ultraviolet light (UVB-UVA) to inhibit UVB penetration into the skin [16,17]. Reduced physical activity is also a factor in vitamin D deficiency and T2DM because physical activity may improve the vitamin D status [18].

Our findings of reduced median vitamin D level and high vitamin D deficiency prevalence were probably caused by inadequate vitamin D intake from sunlight and the diet. This study found that the proportion of study subjects who had never been exposed to sunlight for 15-30 minutes was 84% because the majority of study subjects were housewives (68%) such that their activity was indoors. Our study also found that the median vitamin D intake was extremely low, at 1.76 µg/day. The average vitamin D intake recommended by the Institute of Medicine (IOM) for females and males aged 18-70 years is 15 µg/day [19]. Vitamin D-containing foods are among others egg yolk, salmon, mackerel, tuna, meat, mushrooms, cod liver oil, and fortified foods [14,19]. According to the study of Masood and Iqbal, as cited by Keumala et al. [14] the inability to buy vitamin D-containing foods results in the occurrence of vitamin D deficiency. Salmon and cod liver oil are highly expensive vitamin D-containing foods on the market. It should be noted that around 69% of our subjects wore covered or long-sleeved clothing for 12-24 hours, such as to prevent sunlight from penetrating the body through the skin's surface. According to Holick, adequate vitamin D can be obtained by twice weekly sunlight exposure of the arms and legs for 5-30 minutes between 10 AM and 3 PM (as determined by weather and skin color) [12]. Similar results were found between our study and Keumala's, in that deficiency of vitamin D correlates with indoor occupations and activities, less vitamin D-containing foods in the diet, and sunbathing of ≤ 1 hour daily [14].

Mahmodnia's team [20] reported a strong positive correlation of vitamin D and age. The age variable is a dominant factor that causes vitamin D deficiency, which is correlated with low outdoor activities, resulting in low exposure to sunlight. In addition, there are changes in the skin, namely a reduction in the content of 7-dehydroxycholesterol, the precursor of vitamin D3 [21]. The study of Jha et al. [22] also showed low vitamin D concentrations with increasing age. The reduced synthesis of vitamin D with increasing age, in addition to being due to reduced 7-dehydroxycholesterol concentrations, is also caused by reduced oral absorption of vitamin D.

In our study, an important difference was found in the vitamin D levels of men and women, This may have been the result of differences in physical activity and lifestyle between males and females, where the activities of the males were presumably carried out to a greater extent out of doors, in comparison to those of the females, such that the males were more frequently exposed to sunlight. Lifestyle, in this case, the style of clothing, also affects vitamin D concentrations. The majority of the female respondents in this study wore covered clothing (Muslim clothing with headscarves), causing a reduction in the exposure of the body to sunlight, such that vitamin D synthesis is reduced. Another factor that may have caused the contrasting vitamin D levels in men and women is the larger amount of body fat in women than in men. Vitamin D is soluble in fats, while body fat absorbs and stores vitamin D, which may reduce circulatory vitamin D concentrations in females [23,24].

As stated above, our study found that vitamin D had a strong negative relationship with HbA1c but not with FBG. This agrees with Mehta's report of a significant negative correlation ($r = -0.1205$) of HbA1c with vitamin D, where decreased vitamin D level is connected with increased HbA1c level [25]. Our study also agrees with Jha et al. [22], which reported an inverse relationship between vitamin D and HbA1c. However, our study results differ from those of Suguna's group [26] from Bengaluru, showing no strong relationship of HbA1c with vitamin D concentrations in T2DM patients ($r= -0.109$ at $p=0.05$).

Research findings of Raumulfaro [24] revealed no association between vitamin D and HbA1c ($r = 0.214$, $P=0.153$) on the one hand and vitamin D and blood glucose ($r =0.195$, $p=0.193$) on the other. The non-significant correlation of FBG with vitamin D may have been among other causes

due to body fat percentage. A person with a high body fat percentage may have reduced vitamin D concentrations, as a large amount of body fat retains vitamin D, thereby diminishing circulatory vitamin D, leading to low serum levels. Consumption of glucocorticoids and blood-pressure-lowering medications may also influence vitamin D concentrations. The use of glucocorticoids may interfere with osteoblastic and osteoclastic functions, such that it can reduce vitamin D concentrations. Furthermore, sport increases the level of this vitamin, as reported in the study by Fernandez [24,27–29]. The study conducted by Sakung [30] found that physical activity heightens tissue sensitivity to insulin and absorption of glucose, thereby reducing blood glucose concentrations.

One study showed that vitamin D is negatively correlated with HbA1c, but not with FBG, thereby showing that adequate vitamin D in T2DM patients improves HbA1c but not FBG. This may have been due to differences in the level of glycemic control of HbA1c and FBG, where HbA1c represents the mean glucose level over the last 60 to 90 days., whereas serum glucose concentration is determined after an overnight fast. The FBG level is determined by basal insulin secretion, whereas HbA1c is determined by basal and post-prandial insulin secretion. The significant connection of vitamin D with HbA1c was presumably due to the effect of vitamin D on post-prandial but not on basal insulin release. Post-prandial vitamin D increase is connected with calcium absorption, leading to increased intracellular calcium, which mediates post-prandial insulin release, thereby improving HbA1c concentrations without affecting FBG concentrations [31–33].

No significant differences were found in HbA1c and blood glucose parameters between males and females in this study. Vitamin D was also not strongly correlated with HbA1c or with blood glucose after adjustment for gender subgroups (data not shown), possibly because of the disproportionately larger number of females.

According to Kautzky-Willer et al. [34] the gender differences for HbA1c cannot be related to the makeup of the body, because there are various factors affecting HbA1c level, among others, genetic, age-related, ethnic, and environmental factors.

Fasting blood glucose is influenced mainly by the release of glucose in the liver [34,35]. Measurement of random glucose concentration, such as post-prandial glucose, may improve glycemic control in females, particularly when there are differences between HbA1c and fasting glucose [34,36].

The American Diabetes Association recommends HbA1c instead of FBG for the diagnosis of diabetes mellitus. HbA1c constitutes an invaluable monitor of blood glucose control because it represents the glucose level accumulated over the last 60 to 90 days. HbA1c is also highly correlated with the risk of diabetic sequelae over a prolonged period of time [32].

Vitamin D is important for glucose metabolism, stimulating insulin release from pancreatic beta cells and increasing intracellular calcium and insulin sensitivity in peripheral muscles and adipocytes [18]. Vitamin D also stimulates insulin receptor expression and peroxisome proliferator-activated receptor delta (PPAR-δ) activation involved in the metabolism of fatty acids and the transport of glucose in the muscles, where these compounds will be converted into ATP as an energy source. Vitamin D deficiency may affect calcium concentrations in cells that are sensitive to insulin, thus leading to peripheral insulin resistance that reduces glucose transport to the muscles. In addition, vitamin D deficiency indirectly influences insulin resistance via the renin-angiotensin-aldosterone system, where angiotensin blocks vascular and skeletal muscle insulin, thus interfering with energy needs [37].

Maintaining a normal vitamin D concentration may help glucose homeostasis, while in individuals with high HbA1c, screening for vitamin D may be performed [25].

The weak points of the present study were its cross-sectional study design, which prevented delving into the relationship of vitamin D versus HbA1c or glucose, and the fact that it did not use healthy individuals as controls; and thirdly, this study performed only single blood glucose determinations that did not reflect glycemic variability. Further studies need to be conducted on vitamin D supplementation in the form of a ` randomized, double-blind, placebo-controlled trial to determine the effects of vitamin D on HbA1c and FBG.

CONCLUSION

This study found a strong negative relationship of vitamin D with HbA1c, but not with FBG. Additional

research is required using healthy controls and performing serial blood glucose determinations.

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ORIGINAL ARTICLE Open Access

25-hydroxy vitamin D concentrations negatively correlated with HbA1c in type 2 diabetes mellitus patients: a cross-sectional study in Mampang, South Jakarta

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ABSTRACT

INTRODUCTION: Insulin resistance plays a central role in type 2 diabetes mellitus (T2DM). Another possible cause of T2DM is a deficiency of 25-hydroxy vitamin D [25(OH)D], although the underlying mechanism is not yet clearly understood. The most frequently used laboratory parameters for monitoring T2DM are fasting blood glucose (FBG) and HbA1c. Determining any association of vitamin D with HbA1c and FBG in T2DM.
METHODS: A cross-sectional study involving 100 T2DM patients with the following characteristics: 18-year and older persons of both genders, not having kidney and liver disease, not on insulin therapy, not pregnant or lactating, and not consuming vitamin D in the last three months. Spearman's correlation coefficient was used to test any association of 25(OH)D with HbA1c and fasting blood glucose (FBG) ($p<0.05$).
RESULTS: Subjects comprised 74 females and 26 males with a median age of 56 years. Median HbA1c, 25(OH)D, and FBG were 8.05%, 11.2 ng/mL, and 127 mg/dL, respectively. The Spearman correlation coefficient for vitamin D and HbA1c was $r=-0.217$ ($p=0.03$), and for vitamin D and FBG, it was $r=-0.153$ ($p=0.128$).
CONCLUSION: There was a significant negative correlation of vitamin D with HbA1c but not with FBG. Administration of vitamin D should be considered for additional treatment of T2DM.

Keywords: Hepatitis B Vaccine, Immunological response, Healthcare Workers, Rwanda Military Hospital

INTRODUCTION

The metabolic disorder diabetes mellitus (DM) features high blood glucose levels due to defective insulin response. Insulin resistance in target tissues, particularly in the skeletal muscles and adipose tissue, is responsible for abnormalities in carbohydrate, fat and protein metabolism, which may result in diabetes. There are several types of DM. The 2022 American Diabetes Association guidelines categorize DM into type 1 diabetes due to autoimmune damage to the beta cells, type 2 diabetes due to insulin resistance; diabetes due to other causes such as monogenic diabetic syndrome, DM-causing drugs, exocrine pancreatic disease, and gestational diabetes [1,2].

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Type 2 diabetes mellitus (T2DM) comprises the highest number of patients globally and in Indonesia. The global prevalence of T2DM at age 20-79 years, according to the International Diabetes Federation (IDF) 2013 report, was 8.3% (382 million persons), with 14 million more men than women, generally aged 40-59 years. The global number of cases is projected to increase to 592 million in the year 2035. Changes in lifestyle and unhealthy food may presumably cause the increased incidence of T2DM at younger ages. Obesity is also responsible for the occurrence of T2DM [1].

Insulin resistance plays a central role in T2DM. Oxidative stress, proinflammatory activation, adipokines, and abnormal glucose and lipid metabolism can lead to insulin resistance [3]. Another possible cause of T2DM is a deficiency of vitamin D. Although the underlying mechanism is not yet clearly understood, low 25-hydroxyvitamin D [25(OH)D] level is connected with functional disorders of pancreatic islet Langerhans cells and insulin resistance causing changes in glucose homeostasis such as to result in the occurrence of T2DM [4].

Vitamin D may increase insulin secretion, skeletal muscle glucose, and lipid metabolism and reduce systemic inflammation. However, several studies found no influence of vitamin D supplementation on blood glucose in patients with T2DM, while other studies found inconsistencies in the connection of vitamin D with blood glucose [5].

Although low vitamin D level is correlated with higher glycosylated hemoglobin (HbA1c) and blood glucose levels in patients with T2DM, the relationship is as yet unclear as well as inconsistent, such that there is still a need for further research on the connection of vitamin D with blood glucose in T2DM patients. Our study aimed to explore the connection of vitamin D with HbA1c and blood glucose in T2DM patients.

METHODS

This cross-sectional study recruited 100 patients with T2DM at Mampang District Puskesmas, South Jakarta, from June to July 2022 through consecutive sampling. The size of the required research sample was obtained using the formula for the correlation sample size, with $Z\alpha=1.96$; $Z\beta=1.28$; and $r=-0.387$ [6].

The inclusion criteria were as follows: men and women older than 18 years, not on insulin therapy, not having kidney and liver disease, not being pregnant or lactating, and not consuming vitamin D in the last three months. All subjects were provided with information about this study, and all gave written agreement by signing the informed consent form.

A self-reported questionnaire was used with items on subjects' employment, education, sunbathing habits, use of covered clothing, consumption of vitamin D-rich foods, and T2DM condition.

After a 10-hour fast by the respondents, venous blood was drawn from each respondent and divided into aliquots of 3, 2, and 5 mL that were respectively placed into one EDTA anticoagulant tube for measuring HbA1c, one sodium fluoride (NaF) anticoagulant tube for measuring fasting blood glucose (FBG), and one tube without anticoagulant for 25(OH)D determination. The HbA1c level was measured by High-Performance Liquid Chromatography (HPLC), FBG by the hexokinase method, and 25(OH)D by Direct Competitive Chemiluminescent Microparticle Immunoassay (CMIA). Determination of HbA1c and FBG was performed directly on the day of blood specimen collection, whereas 25(OH)D was determined simultaneously after all specimens had been collected. The blood specimens were frozen at minus 200C prior to being examined.

For data analysis, the Statistical Package for Social Sciences (SPSS) program version 23 was used. The Kolmogorov-Smirnov test was used to determine whether or not the data were normally distributed. Because the data distribution was non-normal, the correlation of vitamin D with HbA1c and FBG was analyzed by the Spearman correlation test at $p < 0.05$.

Ethics approval was issued by the Faculty of Medicine Ethics Committee, Universitas Trisakti, under No. 037/KER/FK/V/2022.

RESULTS

The characteristics of the study subjects, comprised of 26 males and 74 females with median age of 56 years (35 -79 years) may be seen in Table 1. The educational level of 35% of the subjects was high school graduate, while the most frequent occupation was housewife (68%). The most frequent 25(OH)D status was deficiency in 86% of subjects. Seventy-seven percent of the subjects

Table 1. Characteristics of study subjects

Characteristic	N (%)
Gender	
Female	74 (74)
Male	26 (26)
Education	
No schooling	5 (5)
Elementary school	15 (15)
Junior high school	29 (29)
Senior high school	35 (35)
Academy	16 (16)
Employment history	
Housewife	68 (68)
Employed	25 (25)
Unemployed	7 (7)
Vitamin D status	
Deficient	86 (86)
Insufficient	12 (12)
Sufficient	2 (2)
Sunlight exposure (15-30 minutes)	
Yes	16 (16)
No	84 (84)
Wearing of long-sleeved clothing (12-24 hours)	
Yes	69 (69)
No	31 (31)
	Median (IQR)
Age (years)	56 (35-79)
Duration of DM (months)	12 (1-120)
25(OH)D concentration (ng/mL)	11.2 (2.4-31.1)
HbA1c (%)	8.05 (5.1-17.4)
Blood glucose (mg/dL)	127 (70-383)
Dietary vitamin D intake (µg/day)	1.76 (0.0-12.44)
	Mean (SD)
Height (m)	1.54 (0.07)
Weight (kg)	62.04 (11.06)
BMI (kg/m^2)	26.09 (4.52)

BMI: Body Mass Index, DM: Diabetes Mellitus, IQR: Interquartile, SD: Standard deviation, HbA1c: Hemoglobin A1c

Table 2. Cconcentrations of vitamin D, HbA1c, and blood glucose in female and famle Subjects

Parameter	Concentration	P value
25(OH)D concentration (ng/mL)		
Females	10.2 (2.4-24.7)	0.001*
Males	14.7 (4.7-31.1)	
HbA1c (%)		
Females	8.3 (5.1-17.4)	0.389
Males	7.8 (5.4-12.5)	
Blood glucose (mg/dL)		
Females	125.5 (70-383)	0.804
Males	130.5 (83-248)	

**Statistically significant, HbA1c: Hemoglobin A1c*

had never consumed vitamin D-containing foods, 84% of the subjects had never sunbathed, and 69% wore long-sleeved clothing daily for 24 hours.

We found that the male and female subjects differed significantly in vitamin D levels but not in HbA1c and FBG levels (Table 2).
This study also found a significant but negative correlation of vitamin D concentration with HbA1c at r= - 0.217 (p= 0.03). However, no correlation of vitamin D with FBG was found at r= - 0.153 (p=0.128).

DISCUSSION

The patients with T2DM in this study were mostly females. The greater number of females who had diabetes is similar to that in the study of Mihardja, in that diabetes affects women more than men because of their higher body mass index. This is in contrast with Japan and China, where T2DM prevalence is higher in males, which may be caused by the differing diets and behaviors of males [7]. The study by Azlin et al. shows that there are more females with T2DM than males. According to the 2002 study of Brunner and Suddart, as cited by Azlin, there are more females with T2DM as compared to males because there is proportionately more body fat in the former than in the latter. Body fat deposit is one of the factors that may reduce insulin sensitivity in muscles and liver [8]. In the 2018 Basic Health Survey of Indonesia, the DM prevalence in females was higher than in males, at a ratio of 1.78% to 1.21%. The Basic Health Survey of Indonesia also found that in the last five years, the prevalence of T2DM in females showed a slight increase compared to males [9].
The participants in our research had a median age of 56 years. In third-world countries, most of the patients with T2DM are between 45 and 64 years old, whereas in first-world countries, the patients with T2DM are mostly older than 64 years [10]. The prevalence of diabetes also shows an increase with increasing age of the patients and peaks at age 55-64 years. In the Basic Health Survey of Indonesia 2018, there were also indications of an increase in age and, thus, a higher risk for diabetes [9]. Sharan and team [11] reported that recent onset T2DM accounted for 79.1% in the age range of 40-60 years. Older respondents are commonly more at risk of hyperglycemia due to decreased function of the pancreas [7].
The median 25(OH)D level in this study was 11.2 ng/mL, corresponding to vitamin D deficiency in 86% of patients. Vitamin D can be categorized as deficiency if 25(OH)D concentration < 20 ng/mL, insufficiency if 25(OH)D concentration = 21-29 ng/mL, sufficiency if 25(OH)D concentration ≥ 30 ng/mL and intoxication if 25(OH)D level ≥ 150 ng/mL [12]. The proportion of deficiency and insufficiency for vitamin D in Indonesia is relatively great, being 68.8% in Pusparini's study in South Jakarta [13] on women and men aged 55-65 years. In North Sumatra, however, Keumala [14] found that 95% of adult women had vitamin D deficiency and insufficiency. Arjana's team [15] in Yogyakarta found that in adult males aged 19-25 years, the proportion of deficiency of vitamin D was 43.3%,

whereas the proportion of insufficiency of vitamin D was 51.7%.

The causative factors of vitamin D deficiency are varied, among others, low exposure to sunlight, fewer vitamin D-containing foods in the diet, insufficient outdoor activity, a lifestyle that avoids sunlight, sunscreen usage, covered clothing or traditional clothing covering the whole body, and dark-colored skin. Skin color is an important factor in natural vitamin D production. Darker skins need more time to form vitamin D than fair skins. Sunscreens may absorb ultraviolet light (UVB-UVA) to inhibit UVB penetration into the skin [16,17]. Reduced physical activity is also a factor in vitamin D deficiency and T2DM because physical activity may improve the vitamin D status [18].

Our findings of reduced median vitamin D level and high vitamin D deficiency prevalence were probably caused by inadequate vitamin D intake from sunlight and the diet. This study found that the proportion of study subjects who had never been exposed to sunlight for 15-30 minutes was 84% because the majority of study subjects were housewives (68%) such that their activity was indoors. Our study also found that the median vitamin D intake was extremely low, at 1.76 µg/day. The average vitamin D intake recommended by the Institute of Medicine (IOM) for females and males aged 18-70 years is 15 µg/day [19]. Vitamin D-containing foods are among others egg yolk, salmon, mackerel, tuna, meat, mushrooms, cod liver oil, and fortified foods [14,19]. According to the study of Masood and Iqbal, as cited by Keumala et al. [14] the inability to buy vitamin D-containing foods results in the occurrence of vitamin D deficiency. Salmon and cod liver oil are highly expensive vitamin D-containing foods on the market. It should be noted that around 69% of our subjects wore covered or long-sleeved clothing for 12-24 hours, such as to prevent sunlight from penetrating the body through the skin's surface. According to Holick, adequate vitamin D can be obtained by twice weekly sunlight exposure of the arms and legs for 5-30 minutes between 10 AM and 3 PM (as determined by weather and skin color) [12]. Similar results were found between our study and Keumala's, in that deficiency of vitamin D correlates with indoor occupations and activities, less vitamin D-containing foods in the diet, and sunbathing of ≤ 1 hour daily [14].

Mahmodnia's team [20] reported a strong positive correlation of vitamin D and age. The age variable is a dominant factor that causes vitamin D deficiency, which is correlated with low outdoor activities, resulting in low exposure to sunlight. In addition, there are changes in the skin, namely a reduction in the content of 7-dehydroxycholesterol, the precursor of vitamin D3 [21]. The study of Jha et al. [22] also showed low vitamin D concentrations with increasing age. The reduced synthesis of vitamin D with increasing age, in addition to being due to reduced 7-dehydroxycholesterol concentrations, is also caused by reduced oral absorption of vitamin D.

In our study, an important difference was found in the vitamin D levels of men and women, This may have been the result of differences in physical activity and lifestyle between males and females, where the activities of the males were presumably carried out to a greater extent out of doors, in comparison to those of the females, such that the males were more frequently exposed to sunlight. Lifestyle, in this case, the style of clothing, also affects vitamin D concentrations. The majority of the female respondents in this study wore covered clothing (Muslim clothing with headscarves), causing a reduction in the exposure of the body to sunlight, such that vitamin D synthesis is reduced. Another factor that may have caused the contrasting vitamin D levels in men and women is the larger amount of body fat in women than in men. Vitamin D is soluble in fats, while body fat absorbs and stores vitamin D, which may reduce circulatory vitamin D concentrations in females [23,24].

As stated above, our study found that vitamin D had a strong negative relationship with HbA1c but not with FBG. This agrees with Mehta's report of a significant negative correlation ($r = - 0.1205$) of HbA1c with vitamin D, where decreased vitamin D level is connected with increased HbA1c level [25]. Our study also agrees with Jha et al. [22], which reported an inverse relationship between vitamin D and HbA1c. However, our study results differ from those of Suguna's group [26] from Bengaluru, showing no strong relationship of HbA1c with vitamin D concentrations in T2DM patients ($r= -0.109$ at $p=0.05$).

Research findings of Raumulfaro [24] revealed no association between vitamin D and HbA1c ($r = 0.214$, $P=0.153$) on the one hand and vitamin D and blood glucose ($r =0.195$, $p=0.193$) on the other. The non-significant correlation of FBG with vitamin D may have been among other causes

due to body fat percentage. A person with a high body fat percentage may have reduced vitamin D concentrations, as a large amount of body fat retains vitamin D, thereby diminishing circulatory vitamin D, leading to low serum levels. Consumption of glucocorticoids and blood-pressure-lowering medications may also influence vitamin D concentrations. The use of glucocorticoids may interfere with osteoblastic and osteoclastic functions, such that it can reduce vitamin D concentrations. Furthermore, sport increases the level of this vitamin, as reported in the study by Fernandez [24,27–29]. The study conducted by Sakung [30] found that physical activity heightens tissue sensitivity to insulin and absorption of glucose, thereby reducing blood glucose concentrations.

One study showed that vitamin D is negatively correlated with HbA1c, but not with FBG, thereby showing that adequate vitamin D in T2DM patients improves HbA1c but not FBG. This may have been due to differences in the level of glycemic control of HbA1c and FBG, where HbA1c represents the mean glucose level over the last 60 to 90 days., whereas serum glucose concentration is determined after an overnight fast. The FBG level is determined by basal insulin secretion, whereas HbA1c is determined by basal and post-prandial insulin secretion. The significant connection of vitamin D with HbA1c was presumably due to the effect of vitamin D on post-prandial but not on basal insulin release. Post-prandial vitamin D increase is connected with calcium absorption, leading to increased intracellular calcium, which mediates post-prandial insulin release, thereby improving HbA1c concentrations without affecting FBG concentrations [31–33].

No significant differences were found in HbA1c and blood glucose parameters between males and females in this study. Vitamin D was also not strongly correlated with HbA1c or with blood glucose after adjustment for gender subgroups (data not shown), possibly because of the disproportionately larger number of females.

According to Kautzky-Willer et al. [34] the gender differences for HbA1c cannot be related to the makeup of the body, because there are various factors affecting HbA1c level, among others, genetic, age-related, ethnic, and environmental factors.

Fasting blood glucose is influenced mainly by the release of glucose in the liver [34,35]. Measurement of random glucose concentration, such as post-prandial glucose, may improve glycemic control in females, particularly when there are differences between HbA1c and fasting glucose [34,36].

The American Diabetes Association recommends HbA1c instead of FBG for the diagnosis of diabetes mellitus. HbA1c constitutes an invaluable monitor of blood glucose control because it represents the glucose level accumulated over the last 60 to 90 days. HbA1c is also highly correlated with the risk of diabetic sequelae over a prolonged period of time [32].

Vitamin D is important for glucose metabolism, stimulating insulin release from pancreatic beta cells and increasing intracellular calcium and insulin sensitivity in peripheral muscles and adipocytes [18]. Vitamin D also stimulates insulin receptor expression and peroxisome proliferator-activated receptor delta (PPAR-δ) activation involved in the metabolism of fatty acids and the transport of glucose in the muscles, where these compounds will be converted into ATP as an energy source. Vitamin D deficiency may affect calcium concentrations in cells that are sensitive to insulin, thus leading to peripheral insulin resistance that reduces glucose transport to the muscles. In addition, vitamin D deficiency indirectly influences insulin resistance via the renin-angiotensin-aldosterone system, where angiotensin blocks vascular and skeletal muscle insulin, thus interfering with energy needs [37].

Maintaining a normal vitamin D concentration may help glucose homeostasis, while in individuals with high HbA1c, screening for vitamin D may be performed [25].

The weak points of the present study were its cross-sectional study design, which prevented delving into the relationship of vitamin D versus HbA1c or glucose, and the fact that it did not use healthy individuals as controls; and thirdly, this study performed only single blood glucose determinations that did not reflect glycemic variability. Further studies need to be conducted on vitamin D supplementation in the form of a ` randomized, double-blind, placebo-controlled trial to determine the effects of vitamin D on HbA1c and FBG.

CONCLUSION

This study found a strong negative relationship of vitamin D with HbA1c, but not with FBG. Additional

research is required using healthy controls and performing serial blood glucose determinations.

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8. Bukti konfirmasi publikasi artikel dan artikel yang dipublikasi
(6 Oktober 2023)

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4 messages

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Dear Alvina,

The Rwanda Medical Journal (RMJ) Editorial Team is pleased to let you know that your article was published in the RMJ Vol. 80, Issue 3

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Dear
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Thank you for my article which was published in the RMJ Vol. 80, Issue 3. But after I saw the published article, the article had not been corrected. I have replied via email on September 4, 2023 for the corrected version.

Thank you
Best regards,
Alvina,MD
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Rwanda Medical Journal <rwandamedicaljournal@gmail.com> Fri, Oct 6, 2023 at 11:15 PM
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Dear Alvina,

Please send us your latest corrections in case we did not see them.

Thank you for considering the RMJ

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Alvina fk <dr.alvina@trisakti.ac.id> Sat, Oct 7, 2023 at 7:11 AM
To: Rwanda Medical Journal <rwandamedicaljournal@gmail.com>

Dear
Editorial Team RMJ

There are some minor errors in the PDF:
- typo authors (In PDF: P. Pusparini), the CORRECT name is Pusparini. The name is just Pusparini.
- typo keyword (In PDF, keywords: Hepatitis B Vaccine, Immunological response, Healthcare Workers, Rwanda Military Hospital), the CORRECT KEYWORDS: type 2 DM; 25(OH)D; blood glucose; HbA1c; FBG
- typo title table 2 (In PDF: Cconcentration; famle), the CORRECT are: Concentrations; male

I received an email from RMJ regarding the corrected version on September 3, 2023 and I replied to the email that the corrected version was OK on September 4, 2023.

Attached is the corrected version.

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ORIGINAL ARTICLE Open Access

25-hydroxy vitamin D concentrations negatively correlated with HbA1c in type 2 diabetes mellitus patients: a cross-sectional study in Mampang, South Jakarta

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ABSTRACT

INTRODUCTION: Insulin resistance plays a central role in type 2 diabetes mellitus (T2DM). Another possible cause of T2DM is a deficiency of 25-hydroxy vitamin D [25(OH)D], although the underlying mechanism is not yet clearly understood. The most frequently used laboratory parameters for monitoring T2DM are fasting blood glucose (FBG) and HbA1c. Determining any association of vitamin D with HbA1c and FBG in T2DM.

METHODS: A cross-sectional study involving 100 T2DM patients with the following characteristics: 18-year and older persons of both genders, not having kidney and liver disease, not on insulin therapy, not pregnant or lactating, and not consuming vitamin D in the last three months. Spearman's correlation coefficient was used to test any association of 25(OH)D with HbA1c and fasting blood glucose (FBG) ($p<0.05$).

RESULTS: Subjects comprised 74 females and 26 males with a median age of 56 years. Median HbA1c, 25(OH)D, and FBG were 8.05%, 11.2 ng/mL, and 127 mg/dL, respectively. The Spearman correlation coefficient for vitamin D and HbA1c was $r=-0.217$ ($p=0.03$), and for vitamin D and FBG, it was $r=-0.153$ ($p=0.128$).

CONCLUSION: There was a significant negative correlation of vitamin D with HbA1c but not with FBG. Administration of vitamin D should be considered for additional treatment of T2DM.

Keywords: Type 2 Diabetes Mellitus, 25-hydroxy Vitamin D, Blood Glucose, HbA1c, Fasting Blood Glucose

INTRODUCTION

The metabolic disorder diabetes mellitus (DM) features high blood glucose levels due to defective insulin response. Insulin resistance in target tissues, particularly in the skeletal muscles and adipose tissue, is responsible for abnormalities in carbohydrate, fat and protein metabolism, which may result in diabetes. There are several types of DM. The 2022 American Diabetes Association guidelines categorize DM into type 1 diabetes due to autoimmune damage to the beta cells, type 2 diabetes due to insulin resistance; diabetes due to other causes such as monogenic diabetic syndrome, DM-causing drugs, exocrine pancreatic disease, and gestational diabetes [1,2].

***Corresponding author:** Alvina, Department of Clinical Pathology, Universitas Trisakti, Jakarta, Indonesia, email: dr.alvina@trisakti.ac.id; **Potential Conflicts of Interest (CoI):** All authors: no potential conflicts of interest disclosed; **Funding:** All authors: no funding has been sought or gained for this project; **Academic Integrity.** All authors confirm that they have made substantial academic contributions to this manuscript as defined by the ICMJE; **Ethics of human subject participation:** The study was approved by the local Institutional Review Board. Informed consent was sought and gained where applicable; **Originality:** All authors: this manuscript is original has not been published elsewhere; **Review:** This manuscript was peer-reviewed by three reviewers in a double-blind review process; **Type-editor:** King (USA).

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Type 2 diabetes mellitus (T2DM) comprises the highest number of patients globally and in Indonesia. The global prevalence of T2DM at age 20-79 years, according to the International Diabetes Federation (IDF) 2013 report, was 8.3% (382 million persons), with 14 million more men than women, generally aged 40-59 years. The global number of cases is projected to increase to 592 million in the year 2035. Changes in lifestyle and unhealthy food may presumably cause the increased incidence of T2DM at younger ages. Obesity is also responsible for the occurrence of T2DM [1].

Insulin resistance plays a central role in T2DM. Oxidative stress, proinflammatory activation, adipokines, and abnormal glucose and lipid metabolism can lead to insulin resistance [3]. Another possible cause of T2DM is a deficiency of vitamin D. Although the underlying mechanism is not yet clearly understood, low 25-hydroxyvitamin D [25(OH)D] level is connected with functional disorders of pancreatic islet Langerhans cells and insulin resistance causing changes in glucose homeostasis such as to result in the occurrence of T2DM [4].

Vitamin D may increase insulin secretion, skeletal muscle glucose, and lipid metabolism and reduce systemic inflammation. However, several studies found no influence of vitamin D supplementation on blood glucose in patients with T2DM, while other studies found inconsistencies in the connection of vitamin D with blood glucose [5].

Although low vitamin D level is correlated with higher glycosylated hemoglobin (HbA1c) and blood glucose levels in patients with T2DM, the relationship is as yet unclear as well as inconsistent, such that there is still a need for further research on the connection of vitamin D with blood glucose in T2DM patients. Our study aimed to explore the connection of vitamin D with HbA1c and blood glucose in T2DM patients.

METHODS

This cross-sectional study recruited 100 patients with T2DM at Mampang District Puskesmas, South Jakarta, from June to July 2022 through consecutive sampling. The size of the required research sample was obtained using the formula for the correlation sample size, with $Z\alpha=1.96$; $Z\beta=1.28$; and $r=-0.387$ [6].

The inclusion criteria were as follows: men and women older than 18 years, not on insulin therapy, not having kidney and liver disease, not being pregnant or lactating, and not consuming vitamin D in the last three months. All subjects were provided with information about this study, and all gave written agreement by signing the informed consent form.

A self-reported questionnaire was used with items on subjects' employment, education, sunbathing habits, use of covered clothing, consumption of vitamin D-rich foods, and T2DM condition.

After a 10-hour fast by the respondents, venous blood was drawn from each respondent and divided into aliquots of 3, 2, and 5 mL that were respectively placed into one EDTA anticoagulant tube for measuring HbA1c, one sodium fluoride (NaF) anticoagulant tube for measuring fasting blood glucose (FBG), and one tube without anticoagulant for 25(OH)D determination. The HbA1c level was measured by High-Performance Liquid Chromatography (HPLC), FBG by the hexokinase method, and 25(OH)D by Direct Competitive Chemiluminescent Microparticle Immunoassay (CMIA). Determination of HbA1c and FBG was performed directly on the day of blood specimen collection, whereas 25(OH)D was determined simultaneously after all specimens had been collected. The blood specimens were frozen at minus 200C prior to being examined.

For data analysis, the Statistical Package for Social Sciences (SPSS) program version 23 was used. The Kolmogorov-Smirnov test was used to determine whether or not the data were normally distributed. Because the data distribution was non-normal, the correlation of vitamin D with HbA1c and FBG was analyzed by the Spearman correlation test at $p < 0.05$.

Ethics approval was issued by the Faculty of Medicine Ethics Committee, Universitas Trisakti, under No. 037/KER/FK/V/2022.

RESULTS

The characteristics of the study subjects, comprised of 26 males and 74 females with median age of 56 years (35 -79 years) may be seen in Table 1. The educational level of 35% of the subjects was high school graduate, while the most frequent occupation was housewife (68%). The most frequent 25(OH)D status was deficiency in 86% of subjects. Seventy-seven percent of the subjects

Table 1. Characteristics of study subjects

Characteristic	N (%)
Gender	
Female	74 (74)
Male	26 (26)
Education	
No schooling	5 (5)
Elementary school	15 (15)
Junior high school	29 (29)
Senior high school	35 (35)
Academy	16 (16)
Employment history	
Housewife	68 (68)
Employed	25 (25)
Unemployed	7 (7)
Vitamin D status	
Deficient	86 (86)
Insufficient	12 (12)
Sufficient	2 (2)
Sunlight exposure (15-30 minutes)	
Yes	16 (16)
No	84 (84)
Wearing of long-sleeved clothing (12-24 hours)	
Yes	69 (69)
No	31 (31)
	Median (IQR)
Age (years)	56 (35-79)
Duration of DM (months)	12 (1-120)
25(OH)D concentration (ng/mL)	11.2 (2.4-31.1)
HbA1c (%)	8.05 (5.1-17.4)
Blood glucose (mg/dL)	127 (70-383)
Dietary vitamin D intake (µg/day)	1.76 (0.0-12.44)
	Mean (SD)
Height (m)	1.54 (0.07)
Weight (kg)	62.04 (11.06)
BMI (kg/m^2)	26.09 (4.52)

BMI: Body Mass Index, DM: Diabetes Mellitus, IQR: Interquartile, SD: Standard deviation, HbA1c: Hemoglobin A1c

Table 2. Concentrations of vitamin D, HbA1c, and blood glucose in female and male Subjects

Parameter	Concentration	P value
25(OH)D concentration (ng/mL)		
Females	10.2 (2.4-24.7)	0.001*
Males	14.7 (4.7-31.1)	
HbA1c (%)		
Females	8.3 (5.1-17.4)	0.389
Males	7.8 (5.4-12.5)	
Blood glucose (mg/dL)		
Females	125.5 (70-383)	0.804
Males	130.5 (83-248)	

**Statistically significant, HbA1c: Hemoglobin A1c*

had never consumed vitamin D-containing foods, 84% of the subjects had never sunbathed, and 69% wore long-sleeved clothing daily for 24 hours.

We found that the male and female subjects differed significantly in vitamin D levels but not in HbA1c and FBG levels (Table 2).
This study also found a significant but negative correlation of vitamin D concentration with HbA1c at r= - 0.217 (p= 0.03). However, no correlation of vitamin D with FBG was found at r= - 0.153 (p=0.128).

DISCUSSION

The patients with T2DM in this study were mostly females. The greater number of females who had diabetes is similar to that in the study of Mihardja, in that diabetes affects women more than men because of their higher body mass index. This is in contrast with Japan and China, where T2DM prevalence is higher in males, which may be caused by the differing diets and behaviors of males [7]. The study by Azlin et al. shows that there are more females with T2DM than males. According to the 2002 study of Brunner and Suddart, as cited by Azlin, there are more females with T2DM as compared to males because there is proportionately more body fat in the former than in the latter. Body fat deposit is one of the factors that may reduce insulin sensitivity in muscles and liver [8]. In the 2018 Basic Health Survey of Indonesia, the DM prevalence in females was higher than in males, at a ratio of 1.78% to 1.21%. The Basic Health Survey of Indonesia also found that in the last five years, the prevalence of T2DM in females showed a slight increase compared to males [9].
The participants in our research had a median age of 56 years. In third-world countries, most of the patients with T2DM are between 45 and 64 years old, whereas in first-world countries, the patients with T2DM are mostly older than 64 years [10]. The prevalence of diabetes also shows an increase with increasing age of the patients and peaks at age 55-64 years. In the Basic Health Survey of Indonesia 2018, there were also indications of an increase in age and, thus, a higher risk for diabetes [9]. Sharan and team [11] reported that recent onset T2DM accounted for 79.1% in the age range of 40-60 years. Older respondents are commonly more at risk of hyperglycemia due to decreased function of the pancreas [7].
The median 25(OH)D level in this study was 11.2 ng/mL, corresponding to vitamin D deficiency in 86% of patients. Vitamin D can be categorized as deficiency if 25(OH)D concentration < 20 ng/mL, insufficiency if 25(OH)D concentration = 21-29 ng/mL, sufficiency if 25(OH)D concentration ≥ 30 ng/mL and intoxication if 25(OH)D level ≥ 150 ng/mL [12]. The proportion of deficiency and insufficiency for vitamin D in Indonesia is relatively great, being 68.8% in Pusparini's study in South Jakarta [13] on women and men aged 55-65 years. In North Sumatra, however, Keumala [14] found that 95% of adult women had vitamin D deficiency and insufficiency. Arjana's team [15] in Yogyakarta found that in adult males aged 19-25 years, the proportion of deficiency of vitamin D was 43.3%,

whereas the proportion of insufficiency of vitamin D was 51.7%.

The causative factors of vitamin D deficiency are varied, among others, low exposure to sunlight, fewer vitamin D-containing foods in the diet, insufficient outdoor activity, a lifestyle that avoids sunlight, sunscreen usage, covered clothing or traditional clothing covering the whole body, and dark-colored skin. Skin color is an important factor in natural vitamin D production. Darker skins need more time to form vitamin D than fair skins. Sunscreens may absorb ultraviolet light (UVB-UVA) to inhibit UVB penetration into the skin [16,17]. Reduced physical activity is also a factor in vitamin D deficiency and T2DM because physical activity may improve the vitamin D status [18].

Our findings of reduced median vitamin D level and high vitamin D deficiency prevalence were probably caused by inadequate vitamin D intake from sunlight and the diet. This study found that the proportion of study subjects who had never been exposed to sunlight for 15-30 minutes was 84% because the majority of study subjects were housewives (68%) such that their activity was indoors. Our study also found that the median vitamin D intake was extremely low, at 1.76 µg/day. The average vitamin D intake recommended by the Institute of Medicine (IOM) for females and males aged 18-70 years is 15 µg/day [19]. Vitamin D-containing foods are among others egg yolk, salmon, mackerel, tuna, meat, mushrooms, cod liver oil, and fortified foods [14,19]. According to the study of Masood and Iqbal, as cited by Keumala et al. [14] the inability to buy vitamin D-containing foods results in the occurrence of vitamin D deficiency. Salmon and cod liver oil are highly expensive vitamin D-containing foods on the market. It should be noted that around 69% of our subjects wore covered or long-sleeved clothing for 12-24 hours, such as to prevent sunlight from penetrating the body through the skin's surface. According to Holick, adequate vitamin D can be obtained by twice weekly sunlight exposure of the arms and legs for 5-30 minutes between 10 AM and 3 PM (as determined by weather and skin color) [12]. Similar results were found between our study and Keumala's, in that deficiency of vitamin D correlates with indoor occupations and activities, less vitamin D-containing foods in the diet, and sunbathing of ≤ 1 hour daily [14].

Mahmodnia's team [20] reported a strong positive correlation of vitamin D and age. The age variable is a dominant factor that causes vitamin D deficiency, which is correlated with low outdoor activities, resulting in low exposure to sunlight. In addition, there are changes in the skin, namely a reduction in the content of 7-dehydroxycholesterol, the precursor of vitamin D3 [21]. The study of Jha et al. [22] also showed low vitamin D concentrations with increasing age. The reduced synthesis of vitamin D with increasing age, in addition to being due to reduced 7-dehydroxycholesterol concentrations, is also caused by reduced oral absorption of vitamin D.

In our study, an important difference was found in the vitamin D levels of men and women, This may have been the result of differences in physical activity and lifestyle between males and females, where the activities of the males were presumably carried out to a greater extent out of doors, in comparison to those of the females, such that the males were more frequently exposed to sunlight. Lifestyle, in this case, the style of clothing, also affects vitamin D concentrations. The majority of the female respondents in this study wore covered clothing (Muslim clothing with headscarves), causing a reduction in the exposure of the body to sunlight, such that vitamin D synthesis is reduced. Another factor that may have caused the contrasting vitamin D levels in men and women is the larger amount of body fat in women than in men. Vitamin D is soluble in fats, while body fat absorbs and stores vitamin D, which may reduce circulatory vitamin D concentrations in females [23,24].

As stated above, our study found that vitamin D had a strong negative relationship with HbA1c but not with FBG. This agrees with Mehta's report of a significant negative correlation ($r = -0.1205$) of HbA1c with vitamin D, where decreased vitamin D level is connected with increased HbA1c level [25]. Our study also agrees with Jha et al. [22], which reported an inverse relationship between vitamin D and HbA1c. However, our study results differ from those of Suguna's group [26] from Bengaluru, showing no strong relationship of HbA1c with vitamin D concentrations in T2DM patients ($r = -0.109$ at $p = 0.05$).

Research findings of Raumulfaro [24] revealed no association between vitamin D and HbA1c ($r = 0.214$, $P = 0.153$) on the one hand and vitamin D and blood glucose ($r = 0.195$, $p = 0.193$) on the other. The non-significant correlation of FBG with vitamin D may have been among other causes

due to body fat percentage. A person with a high body fat percentage may have reduced vitamin D concentrations, as a large amount of body fat retains vitamin D, thereby diminishing circulatory vitamin D, leading to low serum levels. Consumption of glucocorticoids and blood-pressure-lowering medications may also influence vitamin D concentrations. The use of glucocorticoids may interfere with osteoblastic and osteoclastic functions, such that it can reduce vitamin D concentrations. Furthermore, sport increases the level of this vitamin, as reported in the study by Fernandez [24,27–29]. The study conducted by Sakung [30] found that physical activity heightens tissue sensitivity to insulin and absorption of glucose, thereby reducing blood glucose concentrations.

One study showed that vitamin D is negatively correlated with HbA1c, but not with FBG, thereby showing that adequate vitamin D in T2DM patients improves HbA1c but not FBG. This may have been due to differences in the level of glycemic control of HbA1c and FBG, where HbA1c represents the mean glucose level over the last 60 to 90 days., whereas serum glucose concentration is determined after an overnight fast. The FBG level is determined by basal insulin secretion, whereas HbA1c is determined by basal and post-prandial insulin secretion. The significant connection of vitamin D with HbA1c was presumably due to the effect of vitamin D on post-prandial but not on basal insulin release. Post-prandial vitamin D increase is connected with calcium absorption, leading to increased intracellular calcium, which mediates post-prandial insulin release, thereby improving HbA1c concentrations without affecting FBG concentrations [31–33].

No significant differences were found in HbA1c and blood glucose parameters between males and females in this study. Vitamin D was also not strongly correlated with HbA1c or with blood glucose after adjustment for gender subgroups (data not shown), possibly because of the disproportionately larger number of females.

According to Kautzky-Willer et al. [34] the gender differences for HbA1c cannot be related to the makeup of the body, because there are various factors affecting HbA1c level, among others, genetic, age-related, ethnic, and environmental factors.

Fasting blood glucose is influenced mainly by the release of glucose in the liver [34,35]. Measurement of random glucose concentration, such as post-prandial glucose, may improve glycemic control in females, particularly when there are differences between HbA1c and fasting glucose [34,36].

The American Diabetes Association recommends HbA1c instead of FBG for the diagnosis of diabetes mellitus. HbA1c constitutes an invaluable monitor of blood glucose control because it represents the glucose level accumulated over the last 60 to 90 days. HbA1c is also highly correlated with the risk of diabetic sequelae over a prolonged period of time [32].

Vitamin D is important for glucose metabolism, stimulating insulin release from pancreatic beta cells and increasing intracellular calcium and insulin sensitivity in peripheral muscles and adipocytes [18]. Vitamin D also stimulates insulin receptor expression and peroxisome proliferator-activated receptor delta (PPAR-δ) activation involved in the metabolism of fatty acids and the transport of glucose in the muscles, where these compounds will be converted into ATP as an energy source. Vitamin D deficiency may affect calcium concentrations in cells that are sensitive to insulin, thus leading to peripheral insulin resistance that reduces glucose transport to the muscles. In addition, vitamin D deficiency indirectly influences insulin resistance via the renin-angiotensin-aldosterone system, where angiotensin blocks vascular and skeletal muscle insulin, thus interfering with energy needs [37].

Maintaining a normal vitamin D concentration may help glucose homeostasis, while in individuals with high HbA1c, screening for vitamin D may be performed [25].

The weak points of the present study were its cross-sectional study design, which prevented delving into the relationship of vitamin D versus HbA1c or glucose, and the fact that it did not use healthy individuals as controls; and thirdly, this study performed only single blood glucose determinations that did not reflect glycemic variability. Further studies need to be conducted on vitamin D supplementation in the form of a ` randomized, double-blind, placebo-controlled trial to determine the effects of vitamin D on HbA1c and FBG.

CONCLUSION

This study found a strong negative relationship of vitamin D with HbA1c, but not with FBG. Additional

research is required using healthy controls and performing serial blood glucose determinations.

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