

Rwanda Medical Journal

Cover Journal



Register Login

ENHANCED BY Google

Q

HOME JOURNALS HOW TO USE AJOL RESOURCES SUPPORTERS



Rwanda Medical Journal

Journal / Rwanda Medical Journal / Vol. 80 No. 3 (2023) / Articles

OPEN ACCESS

Current Archives About

Published: 2023-10-13

Articles

[Endoscopic removal of a tablespoon retained in the stomach - A case report](#)

E Rutaganda, N. Umugwaneza, F. Shikama, V. Ndayiragije, D. Nyampinga, Z. Ingabire, S. Mukanumviye, P. Ngabitsinze, S. Kibuka, J. M. Nyayisenga, J. Ndahayo, F. Ngabonziza, B. Seminaga, V. Dusabejambo

DOI: [10.4314/rmj.v80i3.1](https://doi.org/10.4314/rmj.v80i3.1)

Dewan Redaksi

Editorial Team

CHIEF EDITOR

Leon Mutesa, MD, PhD, Professor, Center for Human Genetics, University of Rwanda

DEPUTY CHIEF EDITORS

Peter Cartledge, MD, BSc, MBChB, MRCPCH, PCME, MSc, Assistant Professor, Yale University, Rwanda Human Resources for Health (HRH) Program, University Teaching Hospital of Kigali, Rwanda

Jean Paul Rwabihama, MD, PhD, Professor at the University of Rwanda, Kigali, Rwanda, Paris-Est Creteil University, Paris, France

Joseph Mucumbitsi, MD, MPH, Honorary Associate Professor, Department of Pediatrics, King Faisal Hospital, Kigali, Rwanda

MEDICAL EDITOR & EDITORIAL ASSISTANT

Christian Nsanzabaganwa, MD, Rwanda Military Hospital, Rwanda

Fidele Byiringiro, MD, MCS (ECSA), MMed Department of Surgery, Rwanda Military Hospital, Rwanda

DESKTOP PUBLISHER

Christian Nsanzabaganwa, MD, Rwanda Military Hospital, Rwanda

COMMUNICATIONS MANAGER

Oscar Mwizerwa, MD, Department of pediatrics, University of Rwanda

ASSOCIATE EDITORS

Cameron Page MD, Internal Medicine, Brooklyn, New York, USA

Chantal Ingabire, Ph.D, Social & Community Health, College of Medicine and Health Sciences, University of Rwanda, Kigali

EDITORIAL BOARD MEMBERS

Jesse Raiten MD, Anesthesiology, Critical Care, Perioperative Medicine, University of Pennsylvania, Anesthesiology and Critical Care Department, Philadelphia, USA

Paulin Banguti, MD, Anesthesiology, Critical Care, Cardiac Anesthesiologist, College of Medicine and Health Sciences, University of Rwanda, Kigali

Brian Swan DDS, MPH, Dentistry, Cambridge Health Alliance, Cambridge, MA. USA

Eleana Stoufi DDS, MSc, PhD, Dentistry, Oral Medicine & Oral Pathology, Harvard School of Dental Medicine, HRH, Rwanda

Ladan Basiri MA, DMD, Dentistry, Washington DC, USA

Amelia Pousson MD, MPH, Emergency medicine, CHUK, Kigali, Rwanda

Giles Cattermole BM BCh FRCER DTM&H, Emergency medicine & Medical ethics, King's College Hospital NHS Trust, London UK

Katie Cartledge BSc, MBChB, DRCOG, DFRH, RCGP, Family medicine & Medical education, International dispensary & UR, Kigali Rwanda

Navin Kumar Devaraj MD MMed, Department of Family Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, MALAYSIA.

Claude Mambo Muvunyi, MD, PhD, Microbiology & Infectious Diseases, College of Medicine and Health Sciences, University of Rwanda, Kigali

Aline Uwimana, MD, MPH, Tropical & Infectious Diseases, Rwanda Biomedical Center

Krs Bujarski MD, Internal medicine & Neurology, Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire, USA

Norbert Lameire MD, Nephrology, Internal Medicine, Retired professor of medicine University of Gent, Belgium

Joseph H Friedman MD, Neurology, Providence, Boston, USA

Florence Masaisa, MD, PhD, Hematology & Internal Medicine, College of Medicine and Health Sciences

University of Rwanda, Kigali

Dirk J van Leeuwen MD, PhD, FAASLD, Gastroenterology and Hepatology, The Geisel School of Medicine at Dartmouth College, Hanover NH, USA

Tim Walker MBBS(Hons), FRACP, MPHTM, Gastroenterology, Internal medicine & Tropical medicine. Department of Medicine, Calvary Mater Hospital, Newcastle, Australia.

Linda Baxter CNM, MSN. Clinical Educator Nurse-Midwifery, Great Barrington MA, USA

Geldine Chironda BSc, MSc, PhD, General Nursing, nephrology & Public Health, Human Resources for Health, Rwanda

Maria Kidner APRN, DNP, FNP-BC, FAANP, Clinical Educator & nursing, Essentia Health, Fargo, North Dakota, USA

Renee Pyburn PMHNP-BC, RN, APRN, MSN. PMHNP at Samaritan Behavioral Health, Watertown, NY USA.

Sheila Shaibu BEd, MSc, PhD, General Nursing

Stephen Rulisa, MD, PhD, Reproductive Health, College of Medicine and Health Sciences, University of Rwanda, Kigali

Lucy Baxter BSc MBChB FRCOphth, Ophthalmology, Moorfields Eye Hospital, London, UK

Douglas Blackall MD, MPH, Pathology, St Louis University, Missouri, USA

Justin Wane, MD, MMed, Pathology, King Faisal Hospital, Rwanda

Craig J Conard MD, MPH, FAAP, Pediatric Hospitalist, Ochsner Health System, New Orleans, LA, USA

Janvier Hitayezu MD, MMed, Pediatrics and critical care, Department of Pediatrics, School of Medicine and Pharmacy, University Teaching Hospital of Kigali, Kigali, Rwanda.

Natalie McCall MD, MPH, FAAP, Pediatric emergency medicine, Yale University, Human Resources for Health, Rwanda

Tanya Rogo MD, MPH&TM, Pediatric infectious diseases, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

Stefan Jansen, MSc, PhD, Psychology & Mental Health, College of Medicine and Health Sciences, University of Rwanda, Kigali

Vedaste Ndahindwa, MD, MSc, Biostatistics & Public health, School Public Health, University of Rwanda, Kigali

Jean Claude Byiringiro, MD, MMed, FCS(ECSA), Surgery (Orthopedics), Surgery (Education), College of Medicine and Health Sciences, University of Rwanda, Kigali

Michael Sinclair MD, Cardiothoracic surgery, CHUB, Butare, Rwanda

David Skavadhil MD MPH, Surgery (general), Maine Medical Center, Portland Maine USA

Georges Ntakiyiruta, MD, MMed, FCS(ECSA), General Surgery, College of Medicine and Health Sciences
University of Rwanda, Kigali

Type-editors

Our type-editors are native English speakers with an interest in developing and

advancing research activities in Rwanda. They support authors who submit manuscripts to improve the quality of the written language used.

Shazaib Ahmed, University of Cambridge, UK
Sean Batenhorst, University of Wyoming, USA
Haley Sessions, University of Wyoming, USA
Hannah King, Wheaton College, USA
Matthew Cardillo, Wheaton College, USA
Himani Jayasinghe, University of Sheffield, England
Mary Evelyn Howard, Wheaton College, USA
Tristan Bohlman, University of Wyoming, USA

Daftar Isi

Published: 2023-10-13

Articles

[Endoscopic removal of a tablespoon retained in the stomach - A case report](#)

E Rutaganda, N. Umugwaneza, F. Shikama, V. Ndayiragije, D. Nyampinga, Z. Ingabire, S. Mukanumviye, P. Ngabitsinze, S. Kibuka, J. M. Nyayisenga, J. Ndahayo, F. Ngabonziza, B. Seminega, V. Dusabejambo

DOI: [10.4314/rmj.v80i3.1](#)

↓ DOWNLOAD PDF

[Use of pain assessment tools and associated factors among nurses caring for hospitalized children in District Hospital in Rwanda](#)

T Mukaziboneye, P. Uwimana, G. Gakende
65-72

DOI: [10.4314/rmj.v80i3.9](#)

↓ DOWNLOAD PDF

[Novel breast cancer risk factors in Nigeria: the findings of Nigerian breast cancer risk factor study](#)

Samuel O. Azubuike, Dori-Michelle Beeler

24-33

DOI: [10.4314/rmj.v80i3.4](#)

[↓ DOWNLOAD PDF](#)

[Managing medical emergencies: Sustainable pre-hospital medical education in Rwanda](#)

A. Shustak, J. M. Uwitonze, J. Kabagema, M. Wojick, B. Asay, A. Louka, L. Wolfe, T. Dushime, S. Jayaraman

34-41

DOI: [10.4314/rmj.v80i3.5](#)

[↓ DOWNLOAD PDF](#)

[Comparative study of fetal weight estimation by various methods at term and its correlation with the actual birth weight](#)

Anjali Singh, Anjani K. Srivastava, Ankit Singh

8-14

DOI: [10.4314/rmj.v80i3.2](#)

[↓ DOWNLOAD PDF](#)

[Evaluation of immunological response to hepatitis B vaccine among healthcare workers at Rwanda Military Hospital](#)

C. Muhinda, J. Kabahizi, K. Bazatsinda, N. Dukuze, C. Nsanzabaganwa, G. Murenzi, M. Yotebieng, B. Bagaya, L. Mutesa

57-64

DOI: [10.4314/rmj.v80i3.8](#)

[↓ DOWNLOAD PDF](#)

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

Alvina, Pusparini

16-23

DOI: [10.4314/rmj.v80i3.3](#)

[↓ DOWNLOAD PDF](#)

[A UV Spectrophotometric method for simultaneous determination of Ibuprofen and Paracetamol concentration in suspensions](#)

E. Mugwiza, I. Hahirwa, T. Umumarungu

49-56

DOI: [10.4314/rmj.v80i3.7](#)

[Tuberculosis burden and its predictors among people living with HIV/AIDS at a Nigerian treatment centre: a prospective study](#)

S. A. Olowookere, O. F. Awopeju, F. O. Fehintola, O. Arije, T. O. Ojo, O. I. Obe-Adeoye, O. A. Aina, M. O. Gbadamosi, E. G. Adepoju
42-48

DOI: [10.4314/rmj.v80i3.6](https://doi.org/10.4314/rmj.v80i3.6)

[↓ DOWNLOAD PDF](#)

[Prevalence of anxiety and depression and associated factors among firefighters: a cross-sectional study in Turkey](#)

I. Çelebi, E. Gökkaya
73-81

DOI: [10.4314/rmj.v80i3.10](https://doi.org/10.4314/rmj.v80i3.10)

[↓ DOWNLOAD PDF](#)

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

Authors: Alvina^{1,*}; Pusparini¹

Affiliations: ¹Department of Clinical Pathology, Universitas Trisakti, Jakarta, Indonesia

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).

Received: 09th March 2023; **Initial decision given:** 4th April 2023; **Revised manuscript received:** 13th July 2023; **Accepted:** 26th August 2023. **Copyright:** © The Author(s). This is an Open Access article distributed under the terms of the Creative Commons Attribution License (CC BY-NC-ND) ([click here](https://creativecommons.org/licenses/by-nc-nd/4.0/)) which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. **Publisher:** Rwanda Biomedical Centre (RBC)/Rwanda Health Communication Center, P. O. Box 4586, Kigali. ISSN: 2079-097X (print); 2410-8626 (online)

Citation for this article: Alvina; P. Pusparini. 25-hydroxy vitamin d concentrations negatively correlated with HbA1c in type 2 diabetes mellitus patients: a cross-sectional study in Mampang, South Jakarta. Rwanda Medical Journal, Vol. 80, no. 3, p. 16-23, 2023. <https://dx.doi.org/10.4314/rmj.v80i3.3>

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.

REFERENCES

1. Kharroubi, A.T.; Darwish, H.M. Diabetes mellitus: The epidemic of the century. *World J Diabetes* 2015, 6, 850-867, doi:10.4239/wjd.v6.i6.850.
2. American Diabetes Association Professional Practice Committee. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2022. *Diabetes Care* 2022, 45(Suppl 1), S17-S38, doi:10.2337/dc22-S002
3. Rehman, K.; Akash, M.S. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J Biomed Sci* 2016, 23, 87, doi: 10.1186/s12929-016-0303-y.
4. Das, G. Vitamin D and type 2 diabetes. *Practical Diabetes* 2017, 34, 19-24. URL: <https://wileymicrositebuilder.com/practicaldiabetes/wp-content/uploads/sites/29/2017/01/Rev-Das-lsw.pdf>
5. Yu, J.R.; Lee, S.A.; Lee, J.G.; Seong, G.M.; Ko, S.J.; Koh, G.; et al. Serum vitamin d status and its relationship to metabolic parameters in patients with type 2 diabetes mellitus. *Chonnam Med J* 2012, 48, 108-115, doi: 10.4068/cmj.2012.48.2.108.
6. Aaty, T.A.A.; Magallaa, M.H.Z.; Moneim, H.A.; Ismail, H.M.; Genena, D.M.; Frugina, R. Serum vitamin D level in type 2 diabetic subjects: Relation to glycemic control, insulin resistance and proinflammatory markers. *Journal of High Institute of Public Health* 2017, 47, 62-68, doi:10.21608/JHIPH.2017.19966.
7. Mihardja, L.; Soetrismo, U.; Soegondo, S. Prevalence and clinical profile of diabetes mellitus in productive aged urban Indonesians. *J Diabetes Investig* 2014, 5, 507-512, doi: 10.1111/jdi.12177.
8. Azlin, A.; Ganie, R.A.; Syafril, S. The difference of vitamin D levels between controlled and uncontrolled type 2 diabetes mellitus patients. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 26, 18-22, doi:10.24293/ijcpml.v26i1.1407.
9. Tetap produktif, cegah dan atasi DM melitus. Pusat Data dan Informasi Kementerian Kesehatan Republik Indonesia 2020. ISSN 2442-7659. URL: <https://www.kemkes.go.id/downloads/resources/download/pusdatin/infodatin/Infodatin%202020%20Diabetes%20Melitus.pdf>
10. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004, 27, 1047-1053, doi: 10.2337/diacare.27.5.1047.
11. Sharan, A.; Mukhopadhyay, S.; Majumdar, J.; Ghosh, B.; Sengupta, S. A study of prevalence of vitamin D deficiency in new onset type 2 diabetes mellitus in a tertiary care hospital of Eastern India. *JACM* 2018, 19, 106-111. URL: https://www.jiacm.in/journals/2018/april_june_2018/Journal%2070%20Page%20106-111%20Original%20Article.pdf
12. Holick, M.F. Vitamin D deficiency. *N Engl J Med* 2007, 357, 266-281, doi: 10.1056/NEJMra070553.
13. Pusparini.; Meriyanti, L.T.; Sudharma, N.I. Increased matrix metalloproteinase-9 in male elderly with low 25-hydroxy-vitamin D. *Univ Med* 2016, 35, 171-180, doi:10.18051/UnivMed.2016.v35.171-180.
14. Keumala, D.; Alrasyid, H.; NurIndrawaty, L.; Zulkifli, L. Occurrence of vitamin D deficiency among women in North Sumatera, Indonesia. *Mal J Nutr* 2014, 20, 63-70. URL: https://www.researchgate.net/publication/285524669_Occurrence_of_vitamin_D_deficiency_among_women_in_North_Sumatera_Indonesia#fullTextFileContent
15. Arjana, A.Z.; Devita, N.; Nurmasitoh, T.; Fidianingsih, I.; Dewi, M.; Khoiriyah, U. High proportion of vitamin D deficiency in male adolescents in Yogyakarta Indonesia. In *Proceedings of the 4th International Conference on Sustainable Innovation 2020—Health Science and Nursing (ICoSIHSN 2020)*, March 2021; Atlantis Press B.V, 2021; volume 33, pp. 54-58, doi:10.2991/ahsr.k.210115.011.
16. Pusparini. Defisiensi vitamin D terhadap penyakit. *Indonesian Journal Of Clinical Pathology and Medical Laboratory* 2014, 21, 90-95, doi:10.24293/ijcpml.v21i1.1265.
17. Rimahardika, R.; Subagio, H.W.; Wijayanti, H.S. Asupan vitamin D dan paparan sinar matahari pada orang yang bekerja di dalam ruangan dan di luar ruangan. *Journal Of Nutrition College* 2017, 6, 333-342, doi:10.14710/jnc.v6i4.18785.
18. Asegaonkar, S.B. Vitamin D and type 2 diabetes mellitus: Indian perspectives. *J Diabetic Complications Med* 2016, 1, 1-4, doi:10.4172/2475-3211.1000110.
19. Holick, M.F.; Binkley, N.C.; Bischoff-Ferrari, H.A.; Gordon, C.M.; Hanley, D.A.; Heaney, R.P.; et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical

- practice guideline. *J Clin Endocrinol Metab* 2011, 96, 1911-1930, doi: 10.1210/jc.2011-0385.
20. Mahmoodnia, L.; Tamadon, M.R.; Sadoughi, M.; Beigrezaei, S. Vitamin D status and its relationship with age in type 2 diabetic patients. *Journal of Parathyroid Disease* 2017, 5, 45-48, doi:10.15171/jpd.2017.05.
21. Hidayat, R.; Setiati, S.; Soewondo, P. The association between vitamin D deficiency and type 2 diabetes mellitus in elderly patients. *Acta Med Indones* 2010, 42, 123-129. URL: <https://pubmed.ncbi.nlm.nih.gov/20724765/>
22. Jha, S.C.; Kumar, H.; Faisal, S.Y. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *Academia Journal of Medicine* 2020, 3, 4-10, doi:10.47008/ajm.2020.3.1.2.
23. Al-Horani, H.; Dayyih, W.A.; Mallah, E.; Hamad, M.; Mima, M.; Awad, R.; et al. Nationality, gender, age, and body mass index influences on vitamin D concentration among elderly patients and young Iraqi and Jordanian in Jordan. *Biochem Res Int* 2016, 2016, 1-8, doi: 10.1155/2016/8920503.
24. Akbar, A.R.; Kurnia, E.; Hendrianingtyas, M.; Nugroho, H. Correlation between fasting blood glucose and Hba1c with vitamin D in diabetes mellitus. *Pakistan Journal of Medical Health Sciences* 2019, 13, 1093-1096. URL: https://pjmhonline.com/2019/oct_dec/pdf/1093.pdf
25. Mehta, N.; Shah, S.; Shah, P.P.; Prajapati, V. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *GCSMC J Med Sci* 2016, 5, 42-46. URL: <https://gcsmc.org/assets/pdf/journal/journal-1227/original-article/correlation-between-vitamin-d-and-hba1c-in-type-2-diabetic-patients201810051242459072370.pdf>
26. Suguna, S.; Kusumadevi, M.S. Relationship between vitamin D and HbA1c levels in patients with type 2 diabetes mellitus of Bengaluru City. *International Journal of Physiology* 2019, 7, 104-108, doi:10.5958/2320-608x.2019.00149.5.
27. Zhang, M.; Li, P.; Zhu, Y.; Chang, H.; Wang, X.; Liu, W.; et al. Higher visceral fat area increases the risk of vitamin D insufficiency and deficiency in Chinese adults. *Nutr Metab (Lond)* 2015, 12, 50, doi: 10.1186/s12986-015-0046-x.
28. Fernandes, M.R.; Barreto, W.D.R.J. Association between physical activity and vitamin D: A narrative literature review. *Rev Assoc Med Bras* 2017, 63, 550-556, doi: 10.1590/1806-9282.63.06.550.
29. Grober, U.; Kisters, K. Influence of drugs on vitamin D and calcium metabolism. *Dermatoendocrinol* 2012, 4, 158-166, doi: 10.4161/derm.20731.
30. Sakung, J.M.; Sirajuddin, S.; Zulkifli, A.; Rahman, S.A.; Sudargo, T. Physical activity is associated with lower blood glucose level in high school teachers in Palu, Indonesia. *Int J Community Med Public Health* 2018, 5, 3176-3179, doi:10.18203/2394-6040.ijcmph20183047.
31. Alkhatatbeh, M.J.; Abdul-Razzak, K.K. Association between serum 25-hydroxyvitamin D, hemoglobin A1c and fasting blood glucose levels in adults with diabetes mellitus. *Biomed Rep* 2018, 9, 523-530, doi: 10.3892/br.2018.1159.
32. Sherwani, S.I.; Khan, H.A.; Ekhzaimy, A.; Masood, A.; Sakharkar, M.K. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomark Insights* 2016, 11, 95-104, doi: 10.4137/BMI.S38440.
33. Monnier, L.; Colette, C. Contributions of fasting and post-prandial glucose to hemoglobin A1c. *Endocr Pract* 2006, 12 Suppl 1, 42-46, doi: 10.4158/EP.12.S1.42.
34. Kautzky-Willer, A.; Kosi, L.; Lin, J.; Mihaljevic, R. Gender-based differences in glycaemic control and hypoglycaemia prevalence in patients with type 2 diabetes: results from patient-level pooled data of six randomized controlled trials. *Diabetes Obes Metab* 2015, 17, 533-540, doi: 10.1111/dom.12449.
35. Tabak, A.G.; Herder, C.; Rathmann, W.; Brunner, E.J.; Kivimaki, M. Prediabetes: a high-risk state for diabetes development. *Lancet* 2012, 379, 2279-2290, doi: 10.1016/S0140-6736(12)60283-9.
36. Ehrhardt, N.M.; Chellappa, M.; Walker, M.S.; Fonda, S.J.; Vigersky, R.A. The effect of real-time continuous glucose monitoring on glycemic control in patients with type 2 diabetes mellitus. *J Diabetes Sci Technol* 2011, 5, 668-675, doi: 10.1177/193229681100500320.
37. Sanda, A.; Bahrin, U.; Pakasi, R.D.N.; Aman, A.M. Analysis of vitamin D in patients with type 2 diabetes mellitus. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 25, 150-154, doi: 10.24293/ijcpml.v25i2.1360.

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

By Alvina Alvina

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

Authors: Alvina^{1,*}; Pusparini¹

Affiliations: ¹Department of Clinical Pathology, Universitas Trisakti, Jakarta, Indonesia

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 ineffective 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: dcalvina@trisakti.ac.id; **2** **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).

: 09th March 2023; : 4th 2023; : 13th July 2023; : 26th August 2023.

Citation for this article: Alvina; P. Pusparini. 25-hydroxy vitamin d concentrations negatively correlated with HbA1c in type 2 diabetes mellitus patients: a cross-sectional study in Mampang, South Jakarta. Rwanda Medical Journal, Vol. 80, no. 3, p. 16-23, 2023. <https://dx.doi.org/10.4314/rmj.v80i3.3>

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 may increase insulin secretion, skeletal muscle glucose, and lipid metabolism and reduce systemic inflammation [4]. 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 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 with 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)

⁴ 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 [4].

The median 25(OH)D in this study was 11.2 ng/mL, corresponding to vitamin D deficiency in 86% of patients [7]. 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) [20] 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 Mahmood 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.

REFERENCES

1. Kharroubi, A.T.; Darwish, H.M. Diabetes mellitus: The epidemic of the century. *World J Diabetes* 2015, 6, 850-867, doi:10.4239/wjd.v6.i6.850.
2. American Diabetes Association Professional Practice Committee. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2022. *Diabetes Care* 2022, 45(Suppl 1), S17-S38, doi:10.2337/dc22-S002
3. Rehman, K.; Akash, M.S. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J Biomed Sci* 2016, 23, 87, doi: 10.1186/s12929-016-0303-y.
4. Das, G. Vitamin D and type 2 diabetes. *Practical Diabetes* 2017, 34, 19-24. URL: <https://wileymicrositebuilder.com/practicaldiabetes/wp-content/uploads/sites/29/2017/01/Rev-Das-lsw.pdf>
5. Yu, J.R.; Lee, S.A.; Lee, J.G.; Seong, G.M.; Ko, S.J.; Koh, G.; et al. Serum vitamin d status and its relationship to metabolic parameters in patients with type 2 diabetes mellitus. *Chonnam Med J* 2012, 48, 108-115, doi: 10.4068/cmj.2012.48.2.108.
6. Aaty, T.A.A.; Magallaa, M.H.Z.; Moneim, H.A.; Ismail, H.M.; Genena, D.M.; Frugina, R. Serum vitamin D level in type 2 diabetic subjects: Relation to glycemic control, insulin resistance and proinflammatory markers. *Journal of High Institute of Public Health* 2017, 47, 62-68, doi:10.21608/JHIPH.2017.19966.
7. Mihardja, L.; Soetrisno, U.; Soegondo, S. Prevalence and clinical profile of diabetes mellitus in productive aged urban Indonesians. *J Diabetes Investig* 2014, 5, 507-512, doi: 10.1111/jdi.12177.
8. Azlin, A.; Ganie, R.A.; Syafril, S. The difference of vitamin D levels between controlled and uncontrolled type 2 diabetes mellitus patients. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 26, 18-22, doi:10.24293/ijcpml.v26i1.1407.
9. Tetap produktif, cegah dan atasi DM melitus. Pusat Data dan Informasi Kementrian Kesehatan Republik Indonesia 2020. ISSN 2442-7659. URL: <https://www.kemkes.go.id/downloads/resources/download/pusdatin/infodatin/Infodatin%202020%20Diabetes%20Melitus.pdf>
10. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004, 27, 1047-1053, doi: 10.2337/diacare.27.5.1047.
11. Sharan, A.; Mukhopadhyay, S.; Majumdar, J.; Ghosh, B.; Sengupta, S. A study of prevalence of vitamin D deficiency in new onset type 2 diabetes mellitus in a tertiary care hospital of Eastern India. *JIACM* 2018, 19, 106-111. URL: https://www.jiacm.in/journals/2018/april_june_2018/Journal%2070%20Page%20106-111%20Original%20Article.pdf
12. Holick, M.F. Vitamin D deficiency. *N Engl J Med* 2007, 357, 266-281, doi: 10.1056/NEJMr070553.
13. Pusparini.; Meriyanti, L.T.; Sudharma, N.I. Increased matrix metalloproteinase-9 in male elderly with low 25-hydroxy-vitamin D. *Univ Med* 2016, 35, 171-180, doi:10.18051/UnivMed.2016.v35.171-180.
14. Keumala, D.; Alrasyid, H.; NurIndrawaty, L.; Zulkifli, L. Occurrence of vitamin D deficiency among women in North Sumatera, Indonesia. *Mal J Nutr* 2014, 20, 63-70. URL: https://www.researchgate.net/publication/285524669_Occurrence_of_vitamin_D_deficiency_among_women_in_North_Sumatera_Indonesia#fullTextFileContent
15. Arjana, A.Z.; Devita, N.; Nurmasitoh, T.; Fidiansih, I.; Dewi, M.; Khoiriyah, U. High proportion of vitamin D deficiency in male adolescents in Yogyakarta Indonesia. In *Proceedings of the 4th International Conference on Sustainable Innovation 2020—Health Science and Nursing (ICoSIHSN 2020)*, March 2021; Atlantis Press B.V, 2021; volume 33, pp. 54-58, doi:10.2991/ahsr.k.210115.011.
16. Pusparini. Defisiensi vitamin D terhadap penyakit. *Indonesian Journal Of Clinical Pathology and Medical Laboratory* 2014, 21, 90-95, doi:10.24293/ijcpml.v21i1.1265.
17. Rimahardika, R.; Subagio, H.W.; Wijayanti, H.S. Asupan vitamin D dan paparan sinar matahari pada orang yang bekerja di dalam ruangan dan di luar ruangan. *Journal Of Nutrition College* 2017, 6, 333-342, doi:10.14710/jnc.v6i4.18785.
18. Asegaonkar, S.B. Vitamin D and type 2 diabetes mellitus: Indian perspectives. *J Diabetic Complications Med* 2016, 1, 1-4, doi:10.4172/2475-3211.1000110.
19. Holick, M.F.; Binkley, N.C.; Bischoff-Ferrari, H.A.; Gordon, C.M.; Hanley, D.A.; Heaney, R.P.; et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical

- practice guideline. *J Clin Endocrinol Metab* 2011, 96, 1911-1930, doi: 10.1210/jc.2011-0385.
20. Mahmoodnia, L.; Tamadon, M.R.; Sadoughi, M.; Beigrezaei, S. Vitamin D status and its relationship with age in type 2 diabetic patients. *Journal of Parathyroid Disease* 2017, 5, 45-48, doi:10.15171/jpd.2017.05.
21. Hidayat, R.; Setiati, S.; Soewondo, P. The association between vitamin D deficiency and type 2 diabetes mellitus in elderly patients. *Acta Med Indones* 2010, 42, 123-129. URL: <https://pubmed.ncbi.nlm.nih.gov/20724765/>
22. Jha, S.C.; Kumar, H.; Faisal, S.Y. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *Academia Journal of Medicine* 2020, 3, 4-10, doi:10.47008/ajm.2020.3.1.2.
23. Al-Horani, H.; Dayyih, W.A.; Mallah, E.; Hamad, M.; Mima, M.; Awad, R.; et al. Nationality, gender, age, and body mass index influences on vitamin D concentration among elderly patients and young Iraqi and Jordanian in Jordan. *Biochem Res Int* 2016, 2016, 1-8, doi: 10.1155/2016/8920503.
24. Akbar, A.R.; Kurnia, E.; Hendrianingtyas, M.; Nugroho, H. Correlation between fasting blood glucose and Hba1c with vitamin D in diabetes mellitus. *Pakistan Journal of Medical Health Sciences* 2019, 13, 1093-1096. URL: https://pjmhsonline.com/2019/oct_dec/pdf/1093.pdf
25. Mehta, N.; Shah, S.; Shah, P.P.; Prajapati, V. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *GCSMC J Med Sci* 2016, 5, 42-46. URL: <https://gcsmc.org/assets/pdf/journal/journal-1227/original-article/correlation-between-vitamin-d-and-hba1c-in-type-2-diabetic-patients201810051242459072370.pdf>
26. Suguna, S.; Kusumadevi, M.S. Relationship between vitamin D and HbA1c levels in patients with type 2 diabetes mellitus of Bengaluru City. *International Journal of Physiology* 2019, 7, 104-108, doi:10.5958/2320-608x.2019.00149.5.
27. Zhang, M.; Li, P.; Zhu, Y.; Chang, H.; Wang, X.; Liu, W.; et al. Higher visceral fat area increases the risk of vitamin D insufficiency and deficiency in Chinese adults. *Nutr Metab (Lond)* 2015, 12, 50, doi: 10.1186/s12986-015-0046-x.
28. Fernandes, M.R.; Barreto, W.D.R.J. Association between physical activity and vitamin D: A narrative literature review. *Rev Assoc Med Bras* 2017, 63, 550-556, doi: 10.1590/1806-9282.63.06.550.
29. Grober, U.; Kisters, K. Influence of drugs on vitamin D and calcium metabolism. *Dermatoendocrinol* 2012, 4, 158-166, doi: 10.4161/derm.20731.
30. Sakung, J.M.; Sirajuddin, S.; Zulkifli, A.; Rahman, S.A.; Sudargo, T. Physical activity is associated with lower blood glucose level in high school teachers in Palu, Indonesia. *Int J Community Med Public Health* 2018, 5, 3176-3179, doi:10.18203/2394-6040.ijcmph20183047.
31. Alkhatatbeh, M.J.; Abdul-Razzak, K.K. Association between serum 25-hydroxyvitamin D, hemoglobin A1c and fasting blood glucose levels in adults with diabetes mellitus. *Biomed Rep* 2018, 9, 523-530, doi: 10.3892/br.2018.1159.
32. Sherwani, S.I.; Khan, H.A.; Ekhzaimy, A.; Masood, A.; Sakharkar, M.K. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomark Insights* 2016, 11, 95-104, doi: 10.4137/BMI.S38440.
33. Monnier, L.; Colette, C. Contributions of fasting and post-prandial glucose to hemoglobin A1c. *Endocr Pract* 2006, 12 Suppl 1, 42-46, doi: 10.4158/EP12.S1.42.
34. Kautzky-Willer, A.; Kosi, L.; Lin, J.; Mihaljevic, R. Gender-based differences in glycaemic control and hypoglycaemia prevalence in patients with type 2 diabetes: results from patient-level pooled data of six randomized controlled trials. *Diabetes Obes Metab* 2015, 17, 533-540, doi: 10.1111/dom.12449.
35. Tabak, A.G.; Herder, C.; Rathmann, W.; Brunner, E.J.; Kivimaki, M. Prediabetes: a high-risk state for diabetes development. *Lancet* 2012, 379, 2279-2290, doi: 10.1016/S0140-6736(12)60283-9.
36. Ehrhardt, N.M.; Chellappa, M.; Walker, M.S.; Fonda, S.J.; Vigersky, R.A. The effect of real-time continuous glucose monitoring on glycemic control in patients with type 2 diabetes mellitus. *J Diabetes Sci Technol* 2011, 5, 668-675, doi: 10.1177/193229681100500320.
37. Sanda, A.; Bahrin, U.; Pakasi, R.D.N.; Aman, A.M. Analysis of vitamin D in patients with type 2 diabetes mellitus. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 25, 150-154, doi: 10.24293/ijcpml.v25i2.1360.

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

ORIGINALITY REPORT

16%

SIMILARITY INDEX

PRIMARY SOURCES

1	hdl.handle.net Internet	91 words — 2%
2	research.tees.ac.uk Internet	71 words — 2%
3	pdfs.semanticscholar.org Internet	66 words — 2%
4	www.mdpi.com Internet	44 words — 1%
5	inabj.org Internet	42 words — 1%
6	www.researchgate.net Internet	42 words — 1%
7	vitamindwiki.com Internet	21 words — 1%
8	ppj.phypha.ir Internet	17 words — < 1%
9	www.ncbi.nlm.nih.gov Internet	16 words — < 1%
10	daraluzbirthcenter.org	

15 words — < 1%

-
- 11** Nathalie Bergeron, Patty W. Siri-Tarino, George A. Bray, Ronald M. Krauss. "Nutrition and Cardiometabolic Health", CRC Press, 2017
Publications
14 words — < 1%
-
- 12** services.kfu.edu.sa
Internet
14 words — < 1%
-
- 13** investigacion.unirioja.es
Internet
13 words — < 1%
-
- 14** www.ijhsr.org
Internet
12 words — < 1%
-
- 15** jddtonline.info
Internet
11 words — < 1%
-
- 16** koreascience.kr
Internet
11 words — < 1%
-
- 17** www.medrxiv.org
Internet
11 words — < 1%
-
- 18** chip-seq.info
Internet
10 words — < 1%
-
- 19** www.degruyter.com
Internet
10 words — < 1%
-
- 20** www.dissertation.npmcn.edu.ng
Internet
10 words — < 1%
-
- 21** George A. Bray, Claude Bouchard. "Handbook of Obesity – Volume 1 – Epidemiology, Etiology, and Physiopathology", CRC Press, 2023
Publications
9 words — < 1%

22	digitalknowledge.cput.ac.za Internet	9 words — < 1%
23	Emmanuel Opara. "NUTRITION and DIABETES – Pathophysiology and Management", CRC Press, 2005 Publications	8 words — < 1%
24	Mulchand S. Patel, Jens H. Nielsen. "Fetal and Early Postnatal Programming and Its Influence on Adult Health", CRC Press, 2017 Publications	8 words — < 1%
25	e-journal.unair.ac.id Internet	8 words — < 1%
26	jurnal.ugm.ac.id Internet	8 words — < 1%
27	wjahr.com Internet	8 words — < 1%
28	www.sciencegate.app Internet	8 words — < 1%
29	www.neliti.com Internet	4 words — < 1%

EXCLUDE QUOTES ON
EXCLUDE BIBLIOGRAPHY ON

EXCLUDE SOURCES OFF
EXCLUDE MATCHES OFF

**BUKTI KORESPONDENSI SYARAT KHUSUS
ARTIKEL JURNAL INTERNASIONAL TERINDEKS SCOPUS Q4**

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

Jurnal : Rwanda Medical Journal, volume 80, no. 3, (2023),Agustus: 16 – 23.

Penulis : Alvina,Pusparini.

No	Perihal	Tanggal
1	Bukti konfirmasi submit artikel dan artikel yang disubmit	4 April 2023
2	Bukti konfirmasi review dan hasil review pertama	4 April 2023
3	Bukti konfirmasi submit revisi pertama, respon kepada reviewer, dan artikel yang diresubmit	8 April 2023
4	Bukti konfirmasi review dan hasil review kedua	30 Juni 2023
5	Bukti konfirmasi submit revisi kedua, respon kepada reviewer, dan artikel yang diresubmit	2 Juli 2023
6	Bukti konfirmasi proofreading dan hasil proofreading	26 Agustus 2023
7	Bukti konfirmasi perbaikan proofreading dan hasil perbaikan proofreading	28 Agustus 2023
8	Bukti konfirmasi publikasi artikel dan artikel yang dipublikasi	6 Oktober 2023

1. BUKTI KONFIRMASI SUBMIT ARTIKEL
DAN ARTIKEL YANG DISUBMIT
(4 APRIL 2023)



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

Modifications on submission

2 messages

Rwanda Medical Journal <rwandamedicaljournal@gmail.com>
To: dr.alvina@trisakti.ac.id, pusparini@trisakti.ac.id

Tue, Apr 4, 2023 at 9:01 PM

Dear authors,

Thank you so much for sending us your manuscript for consideration at the RMJ. Your article is of interest to us.

Here are suggested changes on your manuscript

Please can you:

- Respond to the comments added
- Use the attached checklist for improving your write-up
- Use a reference manager such as Zotero to automatically add your references and make them live and we also encourage you to add article's DOI wherever available.

I very much look forward to receiving your manuscript. You do not need to submit this to the official website, rather just send it back to this email address.

--

Kind regards

Editorial team

Rwanda Medical Journal

www.rwandamedicaljournal.org
[@rwandamedicaljo](mailto:rwandamedicaljournal@gmail.com)

2 attachments

 **RMJ New article checklist updated.pdf**
374K

 **OP.23.29 V2.docx**
64K

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

Sat, Apr 8, 2023 at 10:18 AM

To: Rwanda Medical Journal <rwandamedicaljournal@gmail.com>

Dear,
Editorial Team Rwanda Medical Journal

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,
Alvina,MD

[Quoted text hidden]

2 attachments

 **Revision OP.23.29 V2.docx**
94K

 **RMJ New article checklist updated_Alvina_OP.23.29V2.pdf**
316K

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°C 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%, 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 HbA_{1c}, but not with FBG. This agrees with Mehta's [25] report of a significant negative correlation ($r = -0.1205$) of HbA_{1c} with vitamin D, where decreased vitamin D level is connected with increased HbA_{1c} level. Our study also agrees with Jha's study [22] that reported an inverse relationship between vitamin D and HbA_{1c}. However, our study results differ from those of Suguna's group [26] from Bengaluru, showing no strong relationship of HbA_{1c} 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 HbA_{1c} ($r = 0.214$ 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.

ACKNOWLEDGMENT

We thank the physicians and staff at the Public Health Center Kecamatan Mampang, South Jakarta.

REFERENCES

1. Kharroubi AT, Darwish HM. Diabetes mellitus: The epidemic of the century. *World J Diabetes*. 2015;6(6):850-67, doi:10.4239/wjd.v6.i6.850
2. American Diabetes Association Professional Practice Committee. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2022. *Diabetes Care*. 2022;45(Suppl 1):S17-S38, doi:10.2337/dc22-S002
3. Rehman K, Akash MS. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J Biomed Sci*. 2016;23(1):87, doi: 10.1186/s12929-016-0303-y.
4. Das G. Vitamin D and type 2 diabetes. *Practical Diabetes*. 2017;34(1):19-24.
5. Yu JR, Lee SA, Lee JG, Seong GM, Ko SJ, Koh G, et al. Serum vitamin d status and its relationship to metabolic parameters in patients with type 2 diabetes mellitus. *Chonnam Med J*. 2012;48(2):108-15, doi: 10.4068/cmj.2012.48.2.108.
6. Aaty TAA, Magallaa MHZ, Moneim HA, Ismail HM, Genena DM, Frugina R. Serum vitamin D level in type 2 diabetic subjects: Relation to glycemic control, insulin resistance and proinflammatory markers. *Journal of High Institute of Public Health*. 2017;47(2):62-8, doi:10.21608/JHIPH.2017.19966.
7. Mihardja L, Soetrisno U, Soegondo S. Prevalence and clinical profile of diabetes mellitus in productive aged urban Indonesians. *J Diabetes Investig*. 2014;5(5):507-12, doi: 10.1111/jdi.12177.
8. Azlin A, Ganie RA, Syafril S. The difference of vitamin D levels between controlled and uncontrolled type 2 diabetes mellitus patients. *Indonesian Journal of Clinical Pathology and Medical Laboratory*. 2019;26(1):18-22, doi:10.24293/ijcpml.v26i1.1407
9. Tetap produktif, cegah dan atasi DM melitus. Pusat Data dan Informasi Kementerian Kesehatan Republik Indonesia 2020. ISSN 2442-7659.
10. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004;27(5):1047-53, doi: 10.2337/diacare.27.5.1047.
11. Sharan A, Mukhopadhyay S, Majumdar J, Ghosh B, Sengupta S. A study of prevalence of vitamin D deficiency in new onset type 2 diabetes mellitus in a tertiary care hospital of Eastern India. *JACM*. 2018;19(2):106-11.
12. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266-81, doi: 10.1056/NEJMr070553.
13. Pusparini, Meriyanti LT, Sudharma NI. Increased matrix metalloproteinase-9 in male elderly with low 25-hydroxy-vitamin D. *UnivMed*. 2016;35(3):171-80, doi:10.18051/UnivMed.2016.v35.171-180
14. Keumala D, Alrasyid H, NurIndrawaty L, Zulkifli L. Occurrence of vitamin D deficiency among women in North Sumatera, Indonesia. *Mal J Nutr*. 2014;20(1):63-70.
15. Arjana AZ, Devita N, Nurmasitoh T, Fidiansih I, Dewi M, Khoiriyah U. High proportion of vitamin D deficiency in male adolescents in Yogyakarta Indonesia. International Conference on Sustainable Innovation 2020–Health Science and Nursing (ICoSIHSN 2020): Atlantis Press B.V.; 2020. p. 54-8.
16. Pusparini. Defisiensi vitamin D terhadap penyakit. *Indonesian Journal Of Clinical Pathology and Medical Laboratory*. 2014;21(1):90-5, doi:10.24293/ijcpml.v21i1.1265.

17. Rimahardika R, Subagio HW, Wijayanti HS. Asupan vitamin D dan paparan sinar matahari pada orang yang bekerja di dalam ruangan dan di luar ruangan. *Journal Of Nutrition College*. 2017;6(4):333-42, doi:10.14710/jnc.v6i4.18785.
18. Asegaonkar SB. Vitamin D and type 2 diabetes mellitus: Indian perspectives. *J Diabetic Complications Med*. 2016;1(3):1-4, doi:10.4172/2475-3211.1000110
19. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96(7):1911-30, doi: 10.1210/jc.2011-0385.
20. Mahmoodnia L, Tamadon MR, Sadoughi M, Beigrezaei S. Vitamin D status and its relationship with age in type 2 diabetic patients. *Journal of Parathyroid Disease*. 2017;5(2):45-8, doi:10.15171/jpd.2017.05.
21. Hidayat R, Setiati S, Soewondo P. The association between vitamin D deficiency and type 2 diabetes mellitus in elderly patients. *Acta Med Indones*. 2010;42(3):123-9.
22. Jha SC, Kumar H, Faisal SY. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *Academia Journal of Medicine*. 2020;3(1):4-10, doi:10.47008/ajm.2020.3.1.2
23. Al-Horani H, Dayyih WA, Mallah E, Hamad M, Mima M, Awad R, et al. Nationality, gender, age, and body mass index influences on vitamin D concentration among elderly patients and young Iraqi and Jordanian in Jordan. *Biochem Res Int*. 2016;2016:8920503, doi: 10.1155/2016/8920503.
24. Akbar AR, Kurnia E, Hendrianingtyas M, Nugroho H. Correlation between fasting blood glucose and Hba1c with vitamin D in diabetes mellitus. *Pakistan Journal of Medical Health Sciences*. 2019;13(4):1093-6.
25. Mehta N, Shah S, Shah PP, Prajapati V. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *GCSMC J Med Sci*. 2016;5(1):42-6.
26. Suguna S, Kusumadevi MS. Relationship between vitamin D and HbA1c levels in patients with type 2 diabetes mellitus of Bengaluru City. *International Journal of Physiology*. 2019;7(4):104-8, doi:10.5958/2320-608x.2019.00149.5.
27. Zhang M, Li P, Zhu Y, Chang H, Wang X, Liu W, et al. Higher visceral fat area increases the risk of vitamin D insufficiency and deficiency in Chinese adults. *Nutr Metab (Lond)*. 2015;12:50, doi: 10.1186/s12986-015-0046-x.
28. Fernandes MR, Barreto WDRJ. Association between physical activity and vitamin D: A narrative literature review. *Rev Assoc Med Bras (1992)*. 2017;63(6):550-6, doi: 10.1590/1806-9282.63.06.550.
29. Grober U, Kisters K. Influence of drugs on vitamin D and calcium metabolism. *Dermatoendocrinol*. 2012;4(2):158-66, doi: 10.4161/derm.20731.
30. Sakung JM, Sirajuddin S, Zulkifli A, Rahman SA, Sudargo T. Physical activity is associated with lower blood glucose level in high school teachers in Palu, Indonesia. *Int J Community Med Public Health*. 2018;5(8):3176-9, doi:10.18203/2394-6040.ijcmph20183047.
31. Alkhatatbeh MJ, Abdul-Razzak KK. Association between serum 25-hydroxyvitamin D, hemoglobin A1c and fasting blood glucose levels in adults with diabetes mellitus. *Biomed Rep*. 2018;9(6):523-30, doi: 10.3892/br.2018.1159.

32. Sherwani SI, Khan HA, Ekhzaimey A, Masood A, Sakharkar MK. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomark Insights*. 2016;11:95-104, doi: 10.4137/BMI.S38440.
33. Monnier L, Colette C. Contributions of fasting and postprandial glucose to hemoglobin A1c. *Endocr Pract*. 2006;12 Suppl 1:42-6, doi: 10.4158/EP.12.S1.42.
34. Kautzky-Willer A, Kosi L, Lin J, Mihaljevic R. Gender-based differences in glycaemic control and hypoglycaemia prevalence in patients with type 2 diabetes: results from patient-level pooled data of six randomized controlled trials. *Diabetes Obes Metab*. 2015;17(6):533-40, doi: 10.1111/dom.12449.
35. Tabak AG, Herder C, Rathmann W, Brunner EJ, Kivimaki M. Prediabetes: a high-risk state for diabetes development. *Lancet*. 2012;379(9833):2279-90, doi: 10.1016/S0140-6736(12)60283-9.
36. Ehrhardt NM, Chellappa M, Walker MS, Fonda SJ, Vigersky RA. The effect of real-time continuous glucose monitoring on glycemic control in patients with type 2 diabetes mellitus. *J Diabetes Sci Technol*. 2011;5(3):668-75, doi: 10.1177/193229681100500320.
37. Sanda A, Bahrin U, Pakasi RDN, Aman AM. Analysis of vitamin D in patients with type 2 diabetes mellitus. *Indonesian Journal of Clinical Pathology and Medical Laboratory*. 2019;25(2):150-4, doi: 10.24293/ijcpml.v25i2.1360.

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 (μ g/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)

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. Horizontal 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)	√

PLEASE MAKE SURE ALL SURE YOUR REFERENCING IS CORRECTLY FORMATED. HERE IS THE CORRECT WAY (USING VANCOUVER) AND COMMON MISTAKES SEEN.

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.

Reference bibliography

1. Yang, Y.J. An Overview of Current Physical Activity Recommendations in Primary Care. *Korean J Fam Med* **2019**, *40*, 135–142, doi:10.4082/kjfm.19.0038.
2. Al-Khathami, A.D.; Ogbeide, D.O. Prevalence of Mental Illness among Saudi Adult Primary-Care Patients in Central Saudi Arabia. *Saudi Med J* **2002**, *23*, 721–724.
3. Al-Zalabani, A.H.; Al-Hamdan, N.A.; Saeed, A.A. The Prevalence of Physical Activity and Its Socioeconomic Correlates in Kingdom of Saudi Arabia: A Cross-Sectional Population-Based National Survey. *Journal of Taibah University Medical Sciences* **2015**, *10*, 208–215, doi:10.1016/j.jtumed.2014.11.001.
4. Tollefson, M.M.; Van Houten, H.K.; Asante, D.; Yao, X.; Maradit Kremers, H. Association of Psoriasis With Comorbidity Development in Children With Psoriasis. *JAMA Dermatol* **2018**, *154*, 286, doi:10.1001/jamadermatol.2017.5417.
5. Gunawan, P.I.; Suryaningtyas, W. Hemispherectomy with Corpus Callosotomy in Pediatric Lennox Gastaut Syndrome Associated Encephalomalacia Cyst: The First Case in Indonesia. *rmj* **2022**, *79*, 5–8, doi:10.4314/rmj.v79i3.1.
6. Tuyishimire, B.; Irere, H.; Rutagarama, F.; Ndatinya, A.; Karangwa, O.R.; Gasana, A.; Nsanzabaganwa, C.; Mutesa, L. Management Challenges of Disorders of Sex Development- Case Series. *rmj* **2022**, *79*, 9–13, doi:10.4314/rmj.v79i3.9.
7. Musabwasoni, M.G.S.; Ngoga, E.; Sitini, B.; Muganda, J.; Kanyamanza, E.; Nyiringango, E.; Tengera, O.; Uwimana, M.C.; Muganwa, K.; Bazirere, O.; et al. Lived Experience of Healthcare Professionals Providing Safe Abortion in Rwanda. *rmj* **2022**, *79*, 70–77, doi:10.4314/rmj.v79i3.8.

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

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.

Commented [P2]: Please revise and correct grammar, punctuation and text spacing issues throughout the manuscript. Reformat your articles indicated in the attached checklist

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°C 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%, 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 females 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 HbA_{1c}, but not with FBG. This agrees with Mehta's [25] report of a significant negative correlation ($r = -0.1205$) of HbA_{1c}

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~~ 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~~ ~~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.

ACKNOWLEDGMENT

We thank the physicians and staff at the Public Health Center Kecamatan Mampang, South Jakarta.

REFERENCES

1. Kharroubi AT, Darwish HM. Diabetes mellitus: The epidemic of the century. *World J Diabetes*. 2015;6(6):850-67, doi:10.4239/wjd.v6.i6.850
2. American Diabetes Association Professional Practice Committee. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2022. *Diabetes Care*. 2022;45(Suppl 1):S17-S38, doi:10.2337/dc22-S002
3. Rehman K, Akash MS. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J Biomed Sci*. 2016;23(1):87, doi: 10.1186/s12929-016-0303-y.
4. Das G. Vitamin D and type 2 diabetes. *Practical Diabetes*. 2017;34(1):19-24.
5. Yu JR, Lee SA, Lee JG, Seong GM, Ko SJ, Koh G, et al. Serum vitamin d status and its relationship to metabolic parameters in patients with type 2 diabetes mellitus. *Chonnam Med J*. 2012;48(2):108-15, doi: 10.4068/cmj.2012.48.2.108.
6. Aaty TAA, Magallaa MHZ, Moneim HA, Ismail HM, Genena DM, Frugina R. Serum vitamin D level in type 2 diabetic subjects: Relation to glycemic control, insulin resistance and proinflammatory markers. *Journal of High Institute of Public Health*. 2017;47(2):62-8, doi:10.21608/JHIPH.2017.19966.
7. Mihardja L, Soetrisno U, Soegondo S. Prevalence and clinical profile of diabetes mellitus in productive aged urban Indonesians. *J Diabetes Investig*. 2014;5(5):507-12, doi: 10.1111/jdi.12177.
8. Azlin A, Ganie RA, Syafril S. The difference of vitamin D levels between controlled and uncontrolled type 2 diabetes mellitus patients. *Indonesian Journal of Clinical Pathology and Medical Laboratory*. 2019;26(1):18-22, doi:10.24293/ijcpml.v26i1.1407
9. Tetap produktif, cegah dan atasi DM melitus. Pusat Data dan Informasi Kementerian Kesehatan Republik Indonesia 2020. ISSN 2442-7659.
10. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004;27(5):1047-53, doi: 10.2337/diacare.27.5.1047.
11. Sharan A, Mukhopadhyay S, Majumdar J, Ghosh B, Sengupta S. A study of prevalence of vitamin D deficiency in new onset type 2 diabetes mellitus in a tertiary care hospital of Eastern India. *JACM*. 2018;19(2):106-11.
12. Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357(3):266-81, doi: 10.1056/NEJMra070553.

13. Pusparini, Meriyanti LT, Sudharma NI. Increased matrix metalloproteinase-9 in male elderly with low 25-hydroxy-vitamin D. *UnivMed*. 2016;35(3):171-80, doi:10.18051/UnivMed.2016.v35.171-180
14. Keumala D, Alrasyid H, NurIndrawaty L, Zulkifli L. Occurence of vitamin D deficiency among women in North Sumatera, Indonesia. *Mal J Nutr*. 2014;20(1):63-70.
15. Arjana AZ, Devita N, Nurmasitoh T, Fidiansih I, Dewi M, Khoiriyah U. High proportion of vitamin D deficiency in male adolescents in Yogyakarta Indonesia. International Conference on Sustainable Innovation 2020–Health Science and Nursing (ICoSIHSN 2020): Atlantis Press B.V.; 2020. p. 54-8.
16. Pusparini. Defisiensi vitamin D terhadap penyakit. *Indonesian Journal Of Clinical Pathology and Medical Laboratory*. 2014;21(1):90-5, doi:10.24293/ijcpml.v21i1.1265.
17. Rimahardika R, Subagio HW, Wijayanti HS. Asupan vitamin D dan paparan sinar matahari pada orang yang bekerja di dalam ruangan dan di luar ruangan. *Journal Of Nutrition College*. 2017;6(4):333-42, doi:10.14710/jnc.v6i4.18785.
18. Asegaonkar SB. Vitamin D and type 2 diabetes mellitus: Indian perspectives. *J Diabetic Complications Med*. 2016;1(3):1-4, doi:10.4172/2475-3211.1000110
19. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96(7):1911-30, doi: 10.1210/jc.2011-0385.
20. Mahmoodnia L, Tamadon MR, Sadoughi M, Beigrezaei S. Vitamin D status and its relationship with age in type 2 diabetic patients. *Journal of Parathyroid Disease*. 2017;5(2):45-8, doi:10.15171/jpd.2017.05.
21. Hidayat R, Setiati S, Soewondo P. The association between vitamin D deficiency and type 2 diabetes mellitus in elderly patients. *Acta Med Indones*. 2010;42(3):123-9.
22. Jha SC, Kumar H, Faisal SY. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *Academia Journal of Medicine*. 2020;3(1):4-10, doi:10.47008/ajm.2020.3.1.2
23. Al-Horani H, Dayyih WA, Mallah E, Hamad M, Mima M, Awad R, et al. Nationality, gender, age, and body mass index influences on vitamin D concentration among elderly patients and young Iraqi and Jordanian in Jordan. *Biochem Res Int*. 2016;2016:8920503, doi: 10.1155/2016/8920503.
24. Akbar AR, Kurnia E, Hendriantingtyas M, Nugroho H. Correlation between fasting blood glucose and Hba1c with vitamin D in diabetes mellitus. *Pakistan Journal of Medical Health Sciences*. 2019;13(4):1093-6.
25. Mehta N, Shah S, Shah PP, Prajapati V. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *GCSMC J Med Sci*. 2016;5(1):42-6.
26. Suguna S, Kusumadevi MS. Relationship between vitamin D and HbA1c levels in patients with type 2 diabetes mellitus of Bengaluru City. *International Journal of Physiology*. 2019;7(4):104-8, doi:10.5958/2320-608x.2019.00149.5.
27. Zhang M, Li P, Zhu Y, Chang H, Wang X, Liu W, et al. Higher visceral fat area increases the risk of vitamin D insufficiency and deficiency in Chinese adults. *Nutr Metab (Lond)*. 2015;12:50, doi: 10.1186/s12986-015-0046-x.

28. Fernandes MR, Barreto WDRJ. Association between physical activity and vitamin D: A narrative literature review. *Rev Assoc Med Bras (1992)*. 2017;63(6):550-6, doi: 10.1590/1806-9282.63.06.550.
29. Grober U, Kisters K. Influence of drugs on vitamin D and calcium metabolism. *Dermatoendocrinol*. 2012;4(2):158-66, doi: 10.4161/derm.20731.
30. Sakung JM, Sirajuddin S, Zulkifli A, Rahman SA, Sudargo T. Physical activity is associated with lower blood glucose level in high school teachers in Palu, Indonesia. *Int J Community Med Public Health*. 2018;5(8):3176-9, doi:10.18203/2394-6040.ijcmph20183047.
31. Alkhatatbeh MJ, Abdul-Razzak KK. Association between serum 25-hydroxyvitamin D, hemoglobin A1c and fasting blood glucose levels in adults with diabetes mellitus. *Biomed Rep*. 2018;9(6):523-30, doi: 10.3892/br.2018.1159.
32. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomed Insights*. 2016;11:95-104, doi: 10.4137/BMI.S38440.
33. Monnier L, Colette C. Contributions of fasting and postprandial glucose to hemoglobin A1c. *Endocr Pract*. 2006;12 Suppl 1:42-6, doi: 10.4158/EP.12.S1.42.
34. Kautzky-Willer A, Kosi L, Lin J, Mihaljevic R. Gender-based differences in glycaemic control and hypoglycaemia prevalence in patients with type 2 diabetes: results from patient-level pooled data of six randomized controlled trials. *Diabetes Obes Metab*. 2015;17(6):533-40, doi: 10.1111/dom.12449.
35. Tabak AG, Herder C, Rathmann W, Brunner EJ, Kivimaki M. Prediabetes: a high-risk state for diabetes development. *Lancet*. 2012;379(9833):2279-90, doi: 10.1016/S0140-6736(12)60283-9.
36. Ehrhardt NM, Chellappa M, Walker MS, Fonda SJ, Vigersky RA. The effect of real-time continuous glucose monitoring on glycemic control in patients with type 2 diabetes mellitus. *J Diabetes Sci Technol*. 2011;5(3):668-75, doi: 10.1177/193229681100500320.
37. Sanda A, Bahrin U, Pakasi RDN, Aman AM. Analysis of vitamin D in patients with type 2 diabetes mellitus. *Indonesian Journal of Clinical Pathology and Medical Laboratory*. 2019;25(2):150-4, doi: 10.24293/ijcpml.v25i2.1360.

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 (μ g/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

3. Bukti konfirmasi submit revisi pertama,
respon kepada reviewer, dan artikel yang
diresubmit
(8 APRIL 2023)

 **OP.23.29 V2.docx**
64K

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

Sat, Apr 8, 2023 at 10:18 AM

To: Rwanda Medical Journal <rwandamedicaljournal@gmail.com>

Dear,
Editorial Team Rwanda Medical Journal

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,
Alvina,MD

[Quoted text hidden]

2 attachments

 **Revision OP.23.29 V2.docx**
94K

 **RMJ New article checklist updated_Alvina_OP.23.29V2.pdf**
316K

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°C 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%, 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 HbA_{1c}, but not with FBG. This agrees with Mehta's [25] report of a significant negative correlation ($r = -0.1205$) of HbA_{1c} with vitamin D, where decreased vitamin D level is connected with increased HbA_{1c} level. Our study also agrees with Jha's study [22] that reported an inverse relationship between vitamin D and HbA_{1c}. 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.

ACKNOWLEDGMENT

We thank the physicians and staff at the Public Health Center Kecamatan Mampang, South Jakarta.

REFERENCES

1. Kharroubi, A.T.; Darwish, H.M. Diabetes mellitus: The epidemic of the century. *World J Diabetes* 2015, 6, 850-867, doi:10.4239/wjd.v6.i6.850.
2. American Diabetes Association Professional Practice Committee. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2022. *Diabetes Care* 2022, 45(Suppl 1), S17-S38, doi:10.2337/dc22-S002
3. Rehman, K.; Akash, M.S. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J Biomed Sci* 2016, 23, 87, doi: 10.1186/s12929-016-0303-y.
4. Das, G. Vitamin D and type 2 diabetes. *Practical Diabetes* 2017, 34, 19-24.
5. Yu, J.R.; Lee, S.A.; Lee, J.G.; Seong, G.M.; Ko, S.J.; Koh, G.; et al. Serum vitamin d status and its relationship to metabolic parameters in patients with type 2 diabetes mellitus. *Chonnam Med J* 2012, 48, 108-115, doi: 10.4068/cmj.2012.48.2.108.
6. Aaty, T.A.A.; Magallaa, M.H.Z.; Moneim, H.A.; Ismail, H.M.; Genena, D.M.; Frugina, R. Serum vitamin D level in type 2 diabetic subjects: Relation to glycemic control, insulin resistance and proinflammatory markers. *Journal of High Institute of Public Health* 2017, 47, 62-68, doi:10.21608/JHIPH.2017.19966.
7. Mihardja, L.; Soetrisno, U.; Soegondo, S. Prevalence and clinical profile of diabetes mellitus in productive aged urban Indonesians. *J Diabetes Investig* 2014, 5, 507-512, doi: 10.1111/jdi.12177.
8. Azlin, A.; Ganie, R.A.; Syafril, S. The difference of vitamin D levels between controlled and uncontrolled type 2 diabetes mellitus patients. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 26, 18-22, doi:10.24293/ijcpml.v26i1.1407.
9. Tetap produktif, cegah dan atasi DM melitus. Pusat Data dan Informasi Kementrian Kesehatan Republik Indonesia 2020. ISSN 2442-7659.
10. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004, 27, 1047-1053, doi: 10.2337/diacare.27.5.1047.
11. Sharan, A.; Mukhopadhyay, S.; Majumdar, J.; Ghosh, B.; Sengupta, S. A study of prevalence of vitamin D deficiency in new onset type 2 diabetes mellitus in a tertiary care hospital of Eastern India. *JACM* 2018, 19, 106-111.
12. Holick, M.F. Vitamin D deficiency. *N Engl J Med* 2007, 357, 266-281, doi: 10.1056/NEJMra070553.

13. Pusparini.; Meriyanti, L.T.; Sudharma, N.I. Increased matrix metalloproteinase-9 in male elderly with low 25-hydroxy-vitamin D. *Univ Med* 2016, 35, 171-180, doi:10.18051/UnivMed.2016.v35.171-180.
14. Keumala, D.; Alrasyid, H.; NurIndrawaty, L.; Zulkifli, L. Occurrence of vitamin D deficiency among women in North Sumatera, Indonesia. *Mal J Nutr* 2014, 20, 63-70.
15. Arjana, A.Z.; Devita, N.; Nurmasitoh, T.; Fidiansih, I.; Dewi, M.; Khoiriyah, U. High proportion of vitamin D deficiency in male adolescents in Yogyakarta Indonesia. In Proceedings of the 4th International Conference on Sustainable Innovation 2020—Health Science and Nursing (ICoSIHSN 2020), March 2021; Atlantis Press B.V, 2021; volume 33, pp. 54-58.
16. Pusparini. Defisiensi vitamin D terhadap penyakit. *Indonesian Journal Of Clinical Pathology and Medical Laboratory* 2014, 21, 90-95, doi:10.24293/ijcpml.v21i1.1265.
17. Rimahardika, R.; Subagio, H.W.; Wijayanti, H.S. Asupan vitamin D dan paparan sinar matahari pada orang yang bekerja di dalam ruangan dan di luar ruangan. *Journal Of Nutrition College* 2017, 6, 333-342, doi:10.14710/jnc.v6i4.18785.
18. Asegaonkar, S.B. Vitamin D and type 2 diabetes mellitus: Indian perspectives. *J Diabetic Complications Med* 2016, 1, 1-4, doi:10.4172/2475-3211.1000110.
19. Holick, M.F.; Binkley, N.C.; Bischoff-Ferrari, H.A.; Gordon, C.M.; Hanley, D.A.; Heaney, R.P.; et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011, 96, 1911-1930, doi: 10.1210/jc.2011-0385.
20. Mahmoodnia, L.; Tamadon, M.R.; Sadoughi, M.; Beigrezaei, S. Vitamin D status and its relationship with age in type 2 diabetic patients. *Journal of Parathyroid Disease* 2017, 5, 45-48, doi:10.15171/jpd.2017.05.
21. Hidayat, R.; Setiati, S.; Soewondo, P. The association between vitamin D deficiency and type 2 diabetes mellitus in elderly patients. *Acta Med Indones* 2010, 42, 123-129.
22. Jha, S.C.; Kumar, H.; Faisal, S.Y. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *Academia Journal of Medicine* 2020, 3, 4-10, doi:10.47008/ajm.2020.3.1.2.
23. Al-Horani, H.; Dayyih, W.A.; Mallah, E.; Hamad, M.; Mima, M.; Awad, R.; et al. Nationality, gender, age, and body mass index influences on vitamin D concentration among elderly patients and young Iraqi and Jordanian in Jordan. *Biochem Res Int* 2016, 2016, 1-8, doi: 10.1155/2016/8920503.
24. Akbar, A.R.; Kurnia, E.; Hendrianingtyas, M.; Nugroho, H. Correlation between fasting blood glucose and Hba1c with vitamin D in diabetes mellitus. *Pakistan Journal of Medical Health Sciences* 2019, 13, 1093-1096.
25. Mehta, N.; Shah, S.; Shah, P.P.; Prajapati, V. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *GCSMC J Med Sci* 2016, 5, 42-46.
26. Suguna, S.; Kusumadevi, M.S. Relationship between vitamin D and HbA1c levels in patients with type 2 diabetes mellitus of Bengaluru City. *International Journal of Physiology* 2019, 7, 104-108, doi:10.5958/2320-608x.2019.00149.5.

27. Zhang, M.; Li, P.; Zhu, Y.; Chang, H.; Wang, X.; Liu, W.; et al. Higher visceral fat area increases the risk of vitamin D insufficiency and deficiency in Chinese adults. *Nutr Metab (Lond)* 2015, 12, 50, doi: 10.1186/s12986-015-0046-x.
28. Fernandes, M.R.; Barreto, W.D.R.J. Association between physical activity and vitamin D: A narrative literature review. *Rev Assoc Med Bras* 2017, 63, 550-556, doi: 10.1590/1806-9282.63.06.550.
29. Grober, U.; Kisters, K. Influence of drugs on vitamin D and calcium metabolism. *Dermatoendocrinol* 2012, 4, 158-166, doi: 10.4161/derm.20731.
30. Sakung, J.M.; Sirajuddin, S.; Zulkifli, A.; Rahman, S.A.; Sudargo, T. Physical activity is associated with lower blood glucose level in high school teachers in Palu, Indonesia. *Int J Community Med Public Health* 2018, 5, 3176-3179, doi:10.18203/2394-6040.ijcmph20183047.
31. Alkhatatbeh, M.J.; Abdul-Razzak, K.K. Association between serum 25-hydroxyvitamin D, hemoglobin A1c and fasting blood glucose levels in adults with diabetes mellitus. *Biomed Rep* 2018, 9, 523-530, doi: 10.3892/br.2018.1159.
32. Sherwani, S.I.; Khan, H.A.; Ekhzaimy, A.; Masood, A.; Sakharkar, M.K. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomark Insights* 2016, 11, 95-104, doi: 10.4137/BMI.S38440.
33. Monnier, L.; Colette, C. Contributions of fasting and postprandial glucose to hemoglobin A1c. *Endocr Pract* 2006, 12 Suppl 1, 42-46, doi: 10.4158/EP.12.S1.42.
34. Kautzky-Willer, A.; Kosi, L.; Lin, J.; Mihaljevic, R. Gender-based differences in glycaemic control and hypoglycaemia prevalence in patients with type 2 diabetes: results from patient-level pooled data of six randomized controlled trials. *Diabetes Obes Metab* 2015, 17, 533-540, doi: 10.1111/dom.12449.
35. Tabak, A.G.; Herder, C.; Rathmann, W.; Brunner, E.J.; Kivimaki, M. Prediabetes: a high-risk state for diabetes development. *Lancet* 2012, 379, 2279-2290, doi: 10.1016/S0140-6736(12)60283-9.
36. Ehrhardt, N.M.; Chellappa, M.; Walker, M.S.; Fonda, S.J.; Vigersky, R.A. The effect of real-time continuous glucose monitoring on glycemic control in patients with type 2 diabetes mellitus. *J Diabetes Sci Technol* 2011, 5, 668-675, doi: 10.1177/193229681100500320.
37. Sanda, A.; Bahrin, U.; Pakasi, R.D.N.; Aman, A.M. Analysis of vitamin D in patients with type 2 diabetes mellitus. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 25, 150-154, doi: 10.24293/ijcpml.v25i2.1360.

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)



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

SUGGESTED MINOR CHANGES BEFORE ACCEPTANCE

3 messages

Rwanda Medical Journal <rwandamedicaljournal@gmail.com>
To: Alvina fk <dr.alvina@trisakti.ac.id>

Fri, Jun 30, 2023 at 12:31 PM

Dear **Author**,

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

Thank you for considering the RMJ.

--

Kind regards

Editorial team

Rwanda Medical Journal

www.rwandamedicaljournal.org
@rwandamedicaljo

 **Revision OP.23.29 R1-SE.docx**
101K

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

Sun, Jul 2, 2023 at 10:36 PM

To: Rwanda Medical Journal <rwandamedicaljournal@gmail.com>

Dear
Editorial Team Rwanda Medical Journal

Thank you for the input to my manuscripts. I have corrected the manuscripts. Hopefully my manuscripts can be accepted.

Thank you
Best regards,
Alvina, MD

[Quoted text hidden]



Alvina(1)_Revision OP.23.29 R1-SE.docx
96K

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

Sun, Aug 20, 2023 at 10:04 AM

To: Rwanda Medical Journal <rwandamedicaljournal@gmail.com>

Dear
Editorial Team Rwanda Medical Journal

I have sent a repair of my manuscript via email on July 2, 2023. Can I get information about the progress of my manuscript?

Hopefully my manuscripts can be accepted.

Thank you

Best regards,

Alvina, MD

[Quoted text hidden]

25(OH)D-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] 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 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 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]: Add a line or two for clinical implications of these results

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°C 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.

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 Mihadja, 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

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%, 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.

Commented [P5]: Discussion further your limitations, especially selection biases and add recommendations for future studies that fix all limitations

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

We thank the physicians and staff at the Public Health Center Kecamatan Mampang, South Jakarta.

REFERENCES

1. Kharroubi, A.T.; Darwish, H.M. Diabetes mellitus: The epidemic of the century. *World J Diabetes* 2015, 6, 850-867, doi:10.4239/wjd.v6.i6.850.
2. American Diabetes Association Professional Practice Committee. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2022. *Diabetes Care* 2022, 45(Suppl 1), S17-S38, doi:10.2337/dc22-S002
3. Rehman, K.; Akash, M.S. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J Biomed Sci* 2016, 23, 87, doi: 10.1186/s12929-016-0303-y.
4. Das, G. Vitamin D and type 2 diabetes. *Practical Diabetes* 2017, 34, 19-24.
5. Yu, J.R.; Lee, S.A.; Lee, J.G.; Seong, G.M.; Ko, S.J.; Koh, G.; et al. Serum vitamin d status and its relationship to metabolic parameters in patients with type 2 diabetes mellitus. *Chonnam Med J* 2012, 48, 108-115, doi: 10.4068/cmj.2012.48.2.108.
6. Aaty, T.A.A.; Magallaa, M.H.Z.; Moneim, H.A.; Ismail, H.M.; Genena, D.M.; Frugina, R. Serum vitamin D level in type 2 diabetic subjects: Relation to glycemic control, insulin resistance and proinflammatory markers. *Journal of High Institute of Public Health* 2017, 47, 62-68, doi:10.21608/JHIPH.2017.19966.
7. Mihardja, L.; Soetrisno, U.; Soegondo, S. Prevalence and clinical profile of diabetes mellitus in productive aged urban Indonesians. *J Diabetes Investig* 2014, 5, 507-512, doi: 10.1111/jdi.12177.
8. Azlin, A.; Ganie, R.A.; Syafril, S. The difference of vitamin D levels between controlled and uncontrolled type 2 diabetes mellitus patients. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 26, 18-22, doi:10.24293/ijcpml.v26i1.1407.
9. Tetap produktif, cegah dan atasi DM melitus. Pusat Data dan Informasi Kementerian Kesehatan Republik Indonesia 2020. ISSN 2442-7659.
10. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004, 27, 1047-1053, doi: 10.2337/diacare.27.5.1047.
11. Sharan, A.; Mukhopadhyay, S.; Majumdar, J.; Ghosh, B.; Sengupta, S. A study of prevalence of vitamin D deficiency in new onset type 2 diabetes mellitus in a tertiary care hospital of Eastern India. *JACM* 2018, 19, 106-111.
12. Holick, M.F. Vitamin D deficiency. *N Engl J Med* 2007, 357, 266-281, doi: 10.1056/NEJMra070553.

Commented [P6]: For all references, please add the DOI and if unavailable, add their URL links

13. Pusparini.; Meriyanti, L.T.; Sudharma, N.I. Increased matrix metalloproteinase-9 in male elderly with low 25-hydroxy-vitamin D. *Univ Med* 2016, 35, 171-180, doi:10.18051/UnivMed.2016.v35.171-180.
14. Keumala, D.; Alrasyid, H.; Nurlndrawaty, L.; Zulkifli, L. Occurence of vitamin D deficiency among women in North Sumatera, Indonesia. *Mal J Nutr* 2014, 20, 63-70.
15. Arjana, A.Z.; Devita, N.; Nurmasitoh, T.; Fidianingsih, I.; Dewi, M.; Khoiriyah, U. High proportion of vitamin D deficiency in male adolescents in Yogyakarta Indonesia. In Proceedings of the 4th International Conference on Sustainable Innovation 2020–Health Science and Nursing (ICoSIHSN 2020), March 2021; Atlantis Press B.V, 2021; volume 33, pp. 54-58.
16. Pusparini. Defisiensi vitamin D terhadap penyakit. *Indonesian Journal Of Clinical Pathology and Medical Laboratory* 2014, 21, 90-95, doi:10.24293/ijcpml.v21i1.1265.
17. Rimahardika, R.; Subagio, H.W.; Wijayanti, H.S. Asupan vitamin D dan paparan sinar matahari pada orang yang bekerja di dalam ruangan dan di luar ruangan. *Journal Of Nutrition College* 2017, 6, 333-342, doi:10.14710/jnc.v6i4.18785.
18. Asegaonkar, S.B. Vitamin D and type 2 diabetes mellitus: Indian perspectives. *J Diabetic Complications Med* 2016, 1, 1-4, doi:10.4172/2475-3211.1000110.
19. Holick, M.F.; Binkley, N.C.; Bischoff-Ferrari, H.A.; Gordon, C.M.; Hanley, D.A.; Heaney, R.P.; et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011, 96, 1911-1930, doi: 10.1210/jc.2011-0385.
20. Mahmoodnia, L.; Tamadon, M.R.; Sadoughi, M.; Beigrezaei, S. Vitamin D status and its relationship with age in type 2 diabetic patients. *Journal of Parathyroid Disease* 2017, 5, 45-48, doi:10.15171/jpd.2017.05.
21. Hidayat, R.; Setiati, S.; Soewondo, P. The association between vitamin D deficiency and type 2 diabetes mellitus in elderly patients. *Acta Med Indones* 2010, 42, 123-129.
22. Jha, S.C.; Kumar, H.; Faisal, S.Y. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *Academia Journal of Medicine* 2020, 3, 4-10, doi:10.47008/ajm.2020.3.1.2.
23. Al-Horani, H.; Dayyih, W.A.; Mallah, E.; Hamad, M.; Mima, M.; Awad, R.; et al. Nationality, gender, age, and body mass index influences on vitamin D concentration among elderly patients and young Iraqi and Jordanian in Jordan. *Biochem Res Int* 2016, 2016, 1-8, doi: 10.1155/2016/8920503.
24. Akbar, A.R.; Kurnia, E.; Hendrianingtyas, M.; Nugroho, H. Correlation between fasting blood glucose and Hba1c with vitamin D in diabetes mellitus. *Pakistan Journal of Medical Health Sciences* 2019, 13, 1093-1096.
25. Mehta, N.; Shah, S.; Shah, P.P.; Prajapati, V. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *GCSMC J Med Sci* 2016, 5, 42-46.
26. Suguna, S.; Kusumadevi, M.S. Relationship between vitamin D and HbA1c levels in patients with type 2 diabetes mellitus of Bengaluru City. *International Journal of Physiology* 2019, 7, 104-108, doi:10.5958/2320-608x.2019.00149.5.

27. Zhang, M.; Li, P.; Zhu, Y.; Chang, H.; Wang, X.; Liu, W.; et al. Higher visceral fat area increases the risk of vitamin D insufficiency and deficiency in Chinese adults. *Nutr Metab (Lond)* 2015, 12, 50, doi: 10.1186/s12986-015-0046-x.
28. Fernandes, M.R.; Barreto, W.D.R.J. Association between physical activity and vitamin D: A narrative literature review. *Rev Assoc Med Bras* 2017, 63, 550-556, doi: 10.1590/1806-9282.63.06.550.
29. Grober, U.; Kisters, K. Influence of drugs on vitamin D and calcium metabolism. *Dermatoendocrinol* 2012, 4, 158-166, doi: 10.4161/derm.20731.
30. Sakung, J.M.; Sirajuddin, S.; Zulkifli, A.; Rahman, S.A.; Sudargo, T. Physical activity is associated with lower blood glucose level in high school teachers in Palu, Indonesia. *Int J Community Med Public Health* 2018, 5, 3176-3179, doi:10.18203/2394-6040.ijcmph20183047.
31. Alkhatatbeh, M.J.; Abdul-Razzak, K.K. Association between serum 25-hydroxyvitamin D, hemoglobin A1c and fasting blood glucose levels in adults with diabetes mellitus. *Biomed Rep* 2018, 9, 523-530, doi: 10.3892/br.2018.1159.
32. Sherwani, S.I.; Khan, H.A.; Ekhzaimy, A.; Masood, A.; Sakharkar, M.K. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomark Insights* 2016, 11, 95-104, doi: 10.4137/BMI.S38440.
33. Monnier, L.; Colette, C. Contributions of fasting and postprandial glucose to hemoglobin A1c. *Endocr Pract* 2006, 12 Suppl 1, 42-46, doi: 10.4158/EP.12.S1.42.
34. Kautzky-Willer, A.; Kosi, L.; Lin, J.; Mihaljevic, R. Gender-based differences in glycaemic control and hypoglycaemia prevalence in patients with type 2 diabetes: results from patient-level pooled data of six randomized controlled trials. *Diabetes Obes Metab* 2015, 17, 533-540, doi: 10.1111/dom.12449.
35. Tabak, A.G.; Herder, C.; Rathmann, W.; Brunner, E.J.; Kivimaki, M. Prediabetes: a high-risk state for diabetes development. *Lancet* 2012, 379, 2279-2290, doi: 10.1016/S0140-6736(12)60283-9.
36. Ehrhardt, N.M.; Chellappa, M.; Walker, M.S.; Fonda, S.J.; Vigersky, R.A. The effect of real-time continuous glucose monitoring on glycemic control in patients with type 2 diabetes mellitus. *J Diabetes Sci Technol* 2011, 5, 668-675, doi: 10.1177/193229681100500320.
37. Sanda, A.; Bahrin, U.; Pakasi, R.D.N.; Aman, A.M. Analysis of vitamin D in patients with type 2 diabetes mellitus. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 25, 150-154, doi: 10.24293/ijcpml.v25i2.1360.

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 (μ g/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

5. Bukti konfirmasi submit revisi kedua, respon kepada reviewer, dan artikel yang diresubmit (2 Juli 2023)



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

SUGGESTED MINOR CHANGES BEFORE ACCEPTANCE

3 messages

Rwanda Medical Journal <rwandamedicaljournal@gmail.com>
To: Alvina fk <dr.alvina@trisakti.ac.id>

Fri, Jun 30, 2023 at 12:31 PM

Dear **Author**,

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

Thank you for considering the RMJ.

--

Kind regards

Editorial team

Rwanda Medical Journal

www.rwandamedicaljournal.org
@rwandamedicaljo

 **Revision OP.23.29 R1-SE.docx**
101K

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

Sun, Jul 2, 2023 at 10:36 PM

To: Rwanda Medical Journal <rwandamedicaljournal@gmail.com>

Dear
Editorial Team Rwanda Medical Journal

Thank you for the input to my manuscripts. I have corrected the manuscripts. Hopefully my manuscripts can be accepted.

Thank you
Best regards,
Alvina, MD

[Quoted text hidden]



Alvina(1)_Revision OP.23.29 R1-SE.docx
96K

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

Sun, Aug 20, 2023 at 10:04 AM

To: Rwanda Medical Journal <rwandamedicaljournal@gmail.com>

Dear
Editorial Team Rwanda Medical Journal

I have sent a repair of my manuscript via email on July 2, 2023. Can I get information about the progress of my manuscript?

Hopefully my manuscripts can be accepted.

Thank you

Best regards,

Alvina, MD

[Quoted text hidden]

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°C 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)
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 (%)

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*
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

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%, 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 HbA_{1c}, but not with FBG. This agrees with Mehta's [25] report of a significant negative correlation ($r = -0.1205$) of HbA_{1c} with vitamin D, where decreased vitamin D level is connected with increased HbA_{1c} 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.

ACKNOWLEDGMENT

We thank the physicians and staff at the Public Health Center Kecamatan Mampang, South Jakarta.

REFERENCES

1. Kharroubi, A.T.; Darwish, H.M. Diabetes mellitus: The epidemic of the century. *World J Diabetes* 2015, 6, 850-867, doi:10.4239/wjd.v6.i6.850.
2. American Diabetes Association Professional Practice Committee. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2022. *Diabetes Care* 2022, 45(Suppl 1), S17-S38, doi:10.2337/dc22-S002
3. Rehman, K.; Akash, M.S. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J Biomed Sci* 2016, 23, 87, doi: 10.1186/s12929-016-0303-y.
4. Das, G. Vitamin D and type 2 diabetes. *Practical Diabetes* 2017, 34, 19-24. URL: <https://wileymicrositebuilder.com/practicaldiabetes/wp-content/uploads/sites/29/2017/01/Rev-Das-lsw.pdf>
5. Yu, J.R.; Lee, S.A.; Lee, J.G.; Seong, G.M.; Ko, S.J.; Koh, G.; et al. Serum vitamin d status and its relationship to metabolic parameters in patients with type 2 diabetes mellitus. *Chonnam Med J* 2012, 48, 108-115, doi: 10.4068/cmj.2012.48.2.108.
6. Aaty, T.A.A.; Magallaa, M.H.Z.; Moneim, H.A.; Ismail, H.M.; Genena, D.M.; Frugina, R. Serum vitamin D level in type 2 diabetic subjects: Relation to glycemic control, insulin resistance and proinflammatory markers. *Journal of High Institute of Public Health* 2017, 47, 62-68, doi:10.21608/JHIPH.2017.19966.
7. Mihardja, L.; Soetrisno, U.; Soegondo, S. Prevalence and clinical profile of diabetes mellitus in productive aged urban Indonesians. *J Diabetes Investig* 2014, 5, 507-512, doi: 10.1111/jdi.12177.
8. Azlin, A.; Ganie, R.A.; Syafril, S. The difference of vitamin D levels between controlled and uncontrolled type 2 diabetes mellitus patients. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 26, 18-22, doi:10.24293/ijcpml.v26i1.1407.
9. Tetap produktif, cegah dan atasi DM melitus. Pusat Data dan Informasi Kementerian Kesehatan Republik Indonesia 2020. ISSN 2442-7659. URL: <https://www.kemkes.go.id/downloads/resources/download/pusdatin/infodatin/Infodatin%202020%20Diabetes%20Melitus.pdf>
10. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004, 27, 1047-1053, doi: 10.2337/diacare.27.5.1047.

11. Sharan, A.; Mukhopadhyay, S.; Majumdar, J.; Ghosh, B.; Sengupta, S. A study of prevalence of vitamin D deficiency in new onset type 2 diabetes mellitus in a tertiary care hospital of Eastern India. *J IACM* 2018, 19, 106-111. URL: https://www.jiacm.in/journals/2018/april_june_2018/Journal%2070%20Page%20106-111%20Original%20Article.pdf
12. Holick, M.F. Vitamin D deficiency. *N Engl J Med* 2007, 357, 266-281, doi: 10.1056/NEJMra070553.
13. Pusparini.; Meriyanti, L.T.; Sudharma, N.I. Increased matrix metalloproteinase-9 in male elderly with low 25-hydroxy-vitamin D. *Univ Med* 2016, 35, 171-180, doi:10.18051/UnivMed.2016.v35.171-180.
14. Keumala, D.; Alrasyid, H.; Nurlndrawaty, L.; Zulkifli, L. Occurrence of vitamin D deficiency among women in North Sumatera, Indonesia. *Mal J Nutr* 2014, 20, 63-70. URL: https://www.researchgate.net/publication/285524669_Occurrence_of_vitamin_D_deficiency_among_women_in_North_Sumatera_Indonesia#fullTextFileContent
15. Arjana, A.Z.; Devita, N.; Nurmasitoh, T.; Fidianingsih, I.; Dewi, M.; Khoiriyah, U. High proportion of vitamin D deficiency in male adolescents in Yogyakarta Indonesia. In Proceedings of the 4th International Conference on Sustainable Innovation 2020–Health Science and Nursing (ICoSIHSN 2020), March 2021; Atlantis Press B.V, 2021; volume 33, pp. 54-58, doi:10.2991/ahsr.k.210115.011.
16. Pusparini. Defisiensi vitamin D terhadap penyakit. *Indonesian Journal Of Clinical Pathology and Medical Laboratory* 2014, 21, 90-95, doi:10.24293/ijcpml.v21i1.1265.
17. Rimahardika, R.; Subagio, H.W.; Wijayanti, H.S. Asupan vitamin D dan paparan sinar matahari pada orang yang bekerja di dalam ruangan dan di luar ruangan. *Journal Of Nutrition College* 2017, 6, 333-342, doi:10.14710/jnc.v6i4.18785.
18. Asegaonkar, S.B. Vitamin D and type 2 diabetes mellitus: Indian perspectives. *J Diabetic Complications Med* 2016, 1, 1-4, doi:10.4172/2475-3211.1000110.
19. Holick, M.F.; Binkley, N.C.; Bischoff-Ferrari, H.A.; Gordon, C.M.; Hanley, D.A.; Heaney, R.P.; et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011, 96, 1911-1930, doi: 10.1210/jc.2011-0385.
20. Mahmoodnia, L.; Tamadon, M.R.; Sadoughi, M.; Beigrezaei, S. Vitamin D status and its relationship with age in type 2 diabetic patients. *Journal of Parathyroid Disease* 2017, 5, 45-48, doi:10.15171/jpd.2017.05.
21. Hidayat, R.; Setiati, S.; Soewondo, P. The association between vitamin D deficiency and type 2 diabetes mellitus in elderly patients. *Acta Med Indones* 2010, 42, 123-129. URL: <https://pubmed.ncbi.nlm.nih.gov/20724765/>
22. Jha, S.C.; Kumar, H.; Faisal, S.Y. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *Academia Journal of Medicine* 2020, 3, 4-10, doi:10.47008/ajm.2020.3.1.2.
23. Al-Horani, H.; Dayyih, W.A.; Mallah, E.; Hamad, M.; Mima, M.; Awad, R.; et al. Nationality, gender, age, and body mass index influences on vitamin D concentration among elderly patients and young Iraqi and Jordanian in Jordan. *Biochem Res Int* 2016, 2016, 1-8, doi: 10.1155/2016/8920503.

24. Akbar, A.R.; Kurnia, E.; Hendrianingtyas, M.; Nugroho, H. Correlation between fasting blood glucose and HbA1c with vitamin D in diabetes mellitus. *Pakistan Journal of Medical Health Sciences* 2019, 13, 1093-1096. URL: https://pjmhsonline.com/2019/oct_dec/pdf/1093.pdf
25. Mehta, N.; Shah, S.; Shah, P.P.; Prajapati, V. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *GCSMC J Med Sci* 2016, 5, 42-46. URL: <https://gcsmc.org/assets/pdf/journal/journal-1227/original-article/correlation-between-vitamin-d-and-hba1c-in-type-2-diabetic-patients201810051242459072370.pdf>
26. Suguna, S.; Kusumadevi, M.S. Relationship between vitamin D and HbA1c levels in patients with type 2 diabetes mellitus of Bengaluru City. *International Journal of Physiology* 2019, 7, 104-108, doi:10.5958/2320-608x.2019.00149.5.
27. Zhang, M.; Li, P.; Zhu, Y.; Chang, H.; Wang, X.; Liu, W.; et al. Higher visceral fat area increases the risk of vitamin D insufficiency and deficiency in Chinese adults. *Nutr Metab (Lond)* 2015, 12, 50, doi: 10.1186/s12986-015-0046-x.
28. Fernandes, M.R.; Barreto, W.D.R.J. Association between physical activity and vitamin D: A narrative literature review. *Rev Assoc Med Bras* 2017, 63, 550-556, doi: 10.1590/1806-9282.63.06.550.
29. Grober, U.; Kisters, K. Influence of drugs on vitamin D and calcium metabolism. *Dermatoendocrinol* 2012, 4, 158-166, doi: 10.4161/derm.20731.
30. Sakung, J.M.; Sirajuddin, S.; Zulkifli, A.; Rahman, S.A.; Sudargo, T. Physical activity is associated with lower blood glucose level in high school teachers in Palu, Indonesia. *Int J Community Med Public Health* 2018, 5, 3176-3179, doi:10.18203/2394-6040.ijcmph20183047.
31. Alkhatatbeh, M.J.; Abdul-Razzak, K.K. Association between serum 25-hydroxyvitamin D, hemoglobin A1c and fasting blood glucose levels in adults with diabetes mellitus. *Biomed Rep* 2018, 9, 523-530, doi: 10.3892/br.2018.1159.
32. Sherwani, S.I.; Khan, H.A.; Ekhzaimy, A.; Masood, A.; Sakharkar, M.K. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomark Insights* 2016, 11, 95-104, doi: 10.4137/BMI.S38440.
33. Monnier, L.; Colette, C. Contributions of fasting and postprandial glucose to hemoglobin A1c. *Endocr Pract* 2006, 12 Suppl 1, 42-46, doi: 10.4158/EP.12.S1.42.
34. Kautzky-Willer, A.; Kosi, L.; Lin, J.; Mihaljevic, R. Gender-based differences in glycaemic control and hypoglycaemia prevalence in patients with type 2 diabetes: results from patient-level pooled data of six randomized controlled trials. *Diabetes Obes Metab* 2015, 17, 533-540, doi: 10.1111/dom.12449.
35. Tabak, A.G.; Herder, C.; Rathmann, W.; Brunner, E.J.; Kivimaki, M. Prediabetes: a high-risk state for diabetes development. *Lancet* 2012, 379, 2279-2290, doi: 10.1016/S0140-6736(12)60283-9.
36. Ehrhardt, N.M.; Chellappa, M.; Walker, M.S.; Fonda, S.J.; Vigersky, R.A. The effect of real-time continuous glucose monitoring on glycemic control in patients with type 2 diabetes mellitus. *J Diabetes Sci Technol* 2011, 5, 668-675, doi: 10.1177/193229681100500320.
37. Sanda, A.; Bahrun, U.; Pakasi, R.D.N.; Aman, A.M. Analysis of vitamin D in patients with type 2 diabetes mellitus. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 25, 150-154, doi: 10.24293/ijcpml.v25i2.1360.

6. Bukti konfirmasi proofreading
dan hasil proofreading
(26 Agustus 2023)



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

Proofread before publication

5 messages

Rwanda Medical Journal <rwandamedicaljournal@gmail.com>
To: Alvina fk <dr.alvina@trisakti.ac.id>

Sat, Aug 26, 2023 at 7:50 PM

Dear colleague

Re:**Reference code: OP.23.29**

PLAN: Review revisions made in attached manuscript and proofread line by line.

Please submit full names of all authors

I would like to thank you for your submission to the Rwanda Medical Journal (RMJ).

We have accepted with minor revision (by the journal). We have therefore made these changes to your manuscript and attach them to this letter as PDF

If you have any objections to the minor revisions we have made we need to have a response from you promptly. This is in order for us to send your manuscript to the desktop publishers promptly for publication.

May I again thank you for considering the RMJ for the submission and publication of your work. We encourage you to consider the RMJ for any future academic submissions.

Yours sincerely

--

Kind regards

Editorial team

Rwanda Medical Journalwww.rwandamedicaljournal.org

@rwandamedicaljo

 **OP.23.29.pdf**
415K

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

Mon, Aug 28, 2023 at 7:45 AM

To: Rwanda Medical Journal <rwandamedicaljournal@gmail.com>

Dear
Editorial team RMJ

Thank you my manuscript has been accepted at RMJ.

There are some minor errors in the PDF, for example:

- 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

Full names of all authors: Alvina; Pusparini

Attached PDF marked/highlight for typo.

Thank you
Best regards
Alvina,MD

[Quoted text hidden]

 **Marked typo_OP.23.29.pdf**
404K

Rwanda Medical Journal <rwandamedicaljournal@gmail.com>

Sun, Sep 3, 2023 at 3:23 PM

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

Dear Alvina,

Here is the corrected version. Do the authors have only one name for each? Please give us all names for authors if they exist.

Thank you

[Quoted text hidden]

[Quoted text hidden]



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

Authors: Alvina^{1*}; P. Pusparini¹

Affiliations: ¹Department of Clinical Pathology, Universitas Trisakti, Jakarta, Indonesia

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].

***Corresponding author:** Alvina, Department of Clinical Pathology, Universitas Trisakti, Jakarta, Indonesia, email: dr.alvina@trisakti.ac.id; **Potential Conflicts of Interest (Col):** 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).

Received: 09th March 2023; **Initial decision given:** 4th April 2023; **Revised manuscript received:** 13th July 2023; **Accepted:** 26th August 2023.

Copyright: © The Author(s). This is an Open Access article distributed under the terms of the Creative Commons Attribution License (CC BY-NC-ND) ([click here](https://creativecommons.org/licenses/by-nc-nd/4.0/)) which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. **Publisher:** Rwanda Biomedical Centre (RBC)/Rwanda Health Communication Center, P. O. Box 4586, Kigali. ISSN: 2079-097X (print); 2410-8626 (online)

Citation for this article: Alvina; P. Pusparini. 25-hydroxy vitamin d concentrations negatively correlated with HbA1c in type 2 diabetes mellitus patients: a cross-sectional study in Mampang, South Jakarta. Rwanda Medical Journal, Vol. 80, no. 3, p. 16-23, 2023. <https://dx.doi.org/10.4314/rmj.v80i3.3>

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.

REFERENCES

1. Kharroubi, A.T.; Darwish, H.M. Diabetes mellitus: The epidemic of the century. *World J Diabetes* 2015, 6, 850-867, doi:10.4239/wjd.v6.i6.850.
2. American Diabetes Association Professional Practice Committee. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2022. *Diabetes Care* 2022, 45(Suppl 1), S17-S38, doi:10.2337/dc22-S002
3. Rehman, K.; Akash, M.S. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J Biomed Sci* 2016, 23, 87, doi: 10.1186/s12929-016-0303-y.
4. Das, G. Vitamin D and type 2 diabetes. *Practical Diabetes* 2017, 34, 19-24. URL: <https://wileymicrositebuilder.com/practicaldiabetes/wp-content/uploads/sites/29/2017/01/Rev-Das-lsw.pdf>
5. Yu, J.R.; Lee, S.A.; Lee, J.G.; Seong, G.M.; Ko, S.J.; Koh, G.; et al. Serum vitamin d status and its relationship to metabolic parameters in patients with type 2 diabetes mellitus. *Chonnam Med J* 2012, 48, 108-115, doi: 10.4068/cmj.2012.48.2.108.
6. Aaty, T.A.A.; Magallaa, M.H.Z.; Moneim, H.A.; Ismail, H.M.; Genena, D.M.; Frugina, R. Serum vitamin D level in type 2 diabetic subjects: Relation to glycemic control, insulin resistance and proinflammatory markers. *Journal of High Institute of Public Health* 2017, 47, 62-68, doi:10.21608/JHIPH.2017.19966.
7. Mihardja, L.; Soetrisno, U.; Soegondo, S. Prevalence and clinical profile of diabetes mellitus in productive aged urban Indonesians. *J Diabetes Investig* 2014, 5, 507-512, doi: 10.1111/jdi.12177.
8. Azlin, A.; Ganie, R.A.; Syafril, S. The difference of vitamin D levels between controlled and uncontrolled type 2 diabetes mellitus patients. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 26, 18-22, doi:10.24293/ijcpml.v26i1.1407.
9. Tetap produktif, cegah dan atasi DM melitus. Pusat Data dan Informasi Kementerian Kesehatan Republik Indonesia 2020. ISSN 2442-7659. URL: <https://www.kemkes.go.id/downloads/resources/download/pusdatin/infodatin/Infodatin%202020%20Diabetes%20Melitus.pdf>
10. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004, 27, 1047-1053, doi: 10.2337/diacare.27.5.1047.
11. Sharan, A.; Mukhopadhyay, S.; Majumdar, J.; Ghosh, B.; Sengupta, S. A study of prevalence of vitamin D deficiency in new onset type 2 diabetes mellitus in a tertiary care hospital of Eastern India. *JACM* 2018, 19, 106-111. URL: https://www.jiacm.in/journals/2018/april_june_2018/Journal%2070%20Page%20106-111%20Original%20Article.pdf
12. Holick, M.F. Vitamin D deficiency. *N Engl J Med* 2007, 357, 266-281, doi: 10.1056/NEJMra070553.
13. Pusparini.; Meriyanti, L.T.; Sudharma, N.I. Increased matrix metalloproteinase-9 in male elderly with low 25-hydroxy-vitamin D. *Univ Med* 2016, 35, 171-180, doi:10.18051/UnivMed.2016.v35.171-180.
14. Keumala, D.; Alrasyid, H.; Nurlndrawaty, L.; Zulkifli, L. Occurrence of vitamin D deficiency among women in North Sumatera, Indonesia. *Mal J Nutr* 2014, 20, 63-70. URL: https://www.researchgate.net/publication/285524669_Occurrence_of_vitamin_D_deficiency_among_women_in_North_Sumatera_Indonesia#fullTextFileContent
15. Arjana, A.Z.; Devita, N.; Nurmasitoh, T.; Fidianingsih, I.; Dewi, M.; Khoiriyah, U. High proportion of vitamin D deficiency in male adolescents in Yogyakarta Indonesia. In *Proceedings of the 4th International Conference on Sustainable Innovation 2020—Health Science and Nursing (ICoSIHSN 2020)*, March 2021; Atlantis Press B.V, 2021; volume 33, pp. 54-58, doi:10.2991/ahsr.k.210115.011.
16. Pusparini. Defisiensi vitamin D terhadap penyakit. *Indonesian Journal Of Clinical Pathology and Medical Laboratory* 2014, 21, 90-95, doi:10.24293/ijcpml.v21i1.1265.
17. Rimahardika, R.; Subagio, H.W.; Wijayanti, H.S. Asupan vitamin D dan paparan sinar matahari pada orang yang bekerja di dalam ruangan dan di luar ruangan. *Journal Of Nutrition College* 2017, 6, 333-342, doi:10.14710/jnc.v6i4.18785.
18. Asegaonkar, S.B. Vitamin D and type 2 diabetes mellitus: Indian perspectives. *J Diabetic Complications Med* 2016, 1, 1-4, doi:10.4172/2475-3211.1000110.
19. Holick, M.F.; Binkley, N.C.; Bischoff-Ferrari, H.A.; Gordon, C.M.; Hanley, D.A.; Heaney, R.P.; et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical

- practice guideline. *J Clin Endocrinol Metab* 2011, 96, 1911-1930, doi: 10.1210/jc.2011-0385.
20. Mahmoodnia, L.; Tamadon, M.R.; Sadoughi, M.; Beigrezaei, S. Vitamin D status and its relationship with age in type 2 diabetic patients. *Journal of Parathyroid Disease* 2017, 5, 45-48, doi:10.15171/jpd.2017.05.
21. Hidayat, R.; Setiati, S.; Soewondo, P. The association between vitamin D deficiency and type 2 diabetes mellitus in elderly patients. *Acta Med Indones* 2010, 42, 123-129. URL: <https://pubmed.ncbi.nlm.nih.gov/20724765/>
22. Jha, S.C.; Kumar, H.; Faisal, S.Y. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *Academia Journal of Medicine* 2020, 3, 4-10, doi:10.47008/ajm.2020.3.1.2.
23. Al-Horani, H.; Dayyih, W.A.; Mallah, E.; Hamad, M.; Mima, M.; Awad, R.; et al. Nationality, gender, age, and body mass index influences on vitamin D concentration among elderly patients and young Iraqi and Jordanian in Jordan. *Biochem Res Int* 2016, 2016, 1-8, doi: 10.1155/2016/8920503.
24. Akbar, A.R.; Kurnia, E.; Hendrianingtyas, M.; Nugroho, H. Correlation between fasting blood glucose and Hba1c with vitamin D in diabetes mellitus. *Pakistan Journal of Medical Health Sciences* 2019, 13, 1093-1096. URL: https://pjmsonline.com/2019/oct_dec/pdf/1093.pdf
25. Mehta, N.; Shah, S.; Shah, P.P.; Prajapati, V. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *GCSMC J Med Sci* 2016, 5, 42-46. URL: <https://gcsmc.org/assets/pdf/journal/journal-1227/original-article/correlation-between-vitamin-d-and-hba1c-in-type-2-diabetic-patients201810051242459072370.pdf>
26. Suguna, S.; Kusumadevi, M.S. Relationship between vitamin D and HbA1c levels in patients with type 2 diabetes mellitus of Bengaluru City. *International Journal of Physiology* 2019, 7, 104-108, doi:10.5958/2320-608x.2019.00149.5.
27. Zhang, M.; Li, P.; Zhu, Y.; Chang, H.; Wang, X.; Liu, W.; et al. Higher visceral fat area increases the risk of vitamin D insufficiency and deficiency in Chinese adults. *Nutr Metab (Lond)* 2015, 12, 50, doi: 10.1186/s12986-015-0046-x.
28. Fernandes, M.R.; Barreto, W.D.R.J. Association between physical activity and vitamin D: A narrative literature review. *Rev Assoc Med Bras* 2017, 63, 550-556, doi: 10.1590/1806-9282.63.06.550.
29. Grober, U.; Kisters, K. Influence of drugs on vitamin D and calcium metabolism. *Dermatoendocrinol* 2012, 4, 158-166, doi: 10.4161/derm.20731.
30. Sakung, J.M.; Sirajuddin, S.; Zulkifli, A.; Rahman, S.A.; Sudargo, T. Physical activity is associated with lower blood glucose level in high school teachers in Palu, Indonesia. *Int J Community Med Public Health* 2018, 5, 3176-3179, doi:10.18203/2394-6040.ijcmph20183047.
31. Alkhatatbeh, M.J.; Abdul-Razzak, K.K. Association between serum 25-hydroxyvitamin D, hemoglobin A1c and fasting blood glucose levels in adults with diabetes mellitus. *Biomed Rep* 2018, 9, 523-530, doi: 10.3892/br.2018.1159.
32. Sherwani, S.I.; Khan, H.A.; Ekhzaimy, A.; Masood, A.; Sakharkar, M.K. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomark Insights* 2016, 11, 95-104, doi: 10.4137/BMI.S38440.
33. Monnier, L.; Colette, C. Contributions of fasting and post-prandial glucose to hemoglobin A1c. *Endocr Pract* 2006, 12 Suppl 1, 42-46, doi: 10.4158/EP.12.S1.42.
34. Kautzky-Willer, A.; Kosi, L.; Lin, J.; Mihaljevic, R. Gender-based differences in glycaemic control and hypoglycaemia prevalence in patients with type 2 diabetes: results from patient-level pooled data of six randomized controlled trials. *Diabetes Obes Metab* 2015, 17, 533-540, doi: 10.1111/dom.12449.
35. Tabak, A.G.; Herder, C.; Rathmann, W.; Brunner, E.J.; Kivimaki, M. Prediabetes: a high-risk state for diabetes development. *Lancet* 2012, 379, 2279-2290, doi: 10.1016/S0140-6736(12)60283-9.
36. Ehrhardt, N.M.; Chellappa, M.; Walker, M.S.; Fonda, S.J.; Vigersky, R.A. The effect of real-time continuous glucose monitoring on glycemic control in patients with type 2 diabetes mellitus. *J Diabetes Sci Technol* 2011, 5, 668-675, doi: 10.1177/193229681100500320.
37. Sanda, A.; Bahrin, U.; Pakasi, R.D.N.; Aman, A.M. Analysis of vitamin D in patients with type 2 diabetes mellitus. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 25, 150-154, doi: 10.24293/ijcpml.v25i2.1360.

7. Bukti konfirmasi perbaikan proofreading
dan hasil perbaikan proofreading
(28 Agustus 2023)

 **OP.23.29.pdf**
415K

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

Mon, Aug 28, 2023 at 7:45 AM

To: Rwanda Medical Journal <rwandamedicaljournal@gmail.com>

Dear
Editorial team RMJ

Thank you my manuscript has been accepted at RMJ.

There are some minor errors in the PDF, for example:

- 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

Full names of all authors: Alvina; Pusparini

Attached PDF marked/highlight for typo.

Thank you
Best regards
Alvina,MD

[Quoted text hidden]

 **Marked typo_OP.23.29.pdf**
404K

Rwanda Medical Journal <rwandamedicaljournal@gmail.com>

Sun, Sep 3, 2023 at 3:23 PM

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

Dear Alvina,

Here is the corrected version. Do the authors have only one name for each? Please give us all names for authors if they exist.

Thank you

[Quoted text hidden]

[Quoted text hidden]



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

Authors: Alvina^{1,*}; P. Pusparini¹

Affiliations: ¹Department of Clinical Pathology, Universitas Trisakti, Jakarta, Indonesia

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].

***Corresponding author:** Alvina, Department of Clinical Pathology, Universitas Trisakti, Jakarta, Indonesia, email: dr.alvina@trisakti.ac.id; **Potential Conflicts of Interest (Col):** 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).

Received: 09th March 2023; **Initial decision given:** 4th April 2023; **Revised manuscript received:** 13th July 2023; **Accepted:** 26th August 2023.

Copyright: © The Author(s). This is an Open Access article distributed under the terms of the Creative Commons Attribution License (CC BY-NC-ND) ([click here](https://creativecommons.org/licenses/by-nc-nd/4.0/)) which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. **Publisher:** Rwanda Biomedical Centre (RBC)/Rwanda Health Communication Center, P. O. Box 4586, Kigali. ISSN: 2079-097X (print); 2410-8626 (online)

Citation for this article: Alvina; P. Pusparini. 25-hydroxy vitamin d concentrations negatively correlated with HbA1c in type 2 diabetes mellitus patients: a cross-sectional study in Mampang, South Jakarta. Rwanda Medical Journal, Vol. 80, no. 3, p. 16-23, 2023. <https://dx.doi.org/10.4314/rmj.v80i3.3>

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.

REFERENCES

1. Kharroubi, A.T.; Darwish, H.M. Diabetes mellitus: The epidemic of the century. *World J Diabetes* 2015, 6, 850-867, doi:10.4239/wjd.v6.i6.850.
2. American Diabetes Association Professional Practice Committee. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2022. *Diabetes Care* 2022, 45(Suppl 1), S17-S38, doi:10.2337/dc22-S002
3. Rehman, K.; Akash, M.S. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J Biomed Sci* 2016, 23, 87, doi: 10.1186/s12929-016-0303-y.
4. Das, G. Vitamin D and type 2 diabetes. *Practical Diabetes* 2017, 34, 19-24. URL: <https://wileymicrositebuilder.com/practicaldiabetes/wp-content/uploads/sites/29/2017/01/Rev-Das-lsw.pdf>
5. Yu, J.R.; Lee, S.A.; Lee, J.G.; Seong, G.M.; Ko, S.J.; Koh, G.; et al. Serum vitamin d status and its relationship to metabolic parameters in patients with type 2 diabetes mellitus. *Chonnam Med J* 2012, 48, 108-115, doi: 10.4068/cmj.2012.48.2.108.
6. Aaty, T.A.A.; Magallaa, M.H.Z.; Moneim, H.A.; Ismail, H.M.; Genena, D.M.; Frugina, R. Serum vitamin D level in type 2 diabetic subjects: Relation to glycemic control, insulin resistance and proinflammatory markers. *Journal of High Institute of Public Health* 2017, 47, 62-68, doi:10.21608/JHIPH.2017.19966.
7. Mihardja, L.; Soetrisno, U.; Soegondo, S. Prevalence and clinical profile of diabetes mellitus in productive aged urban Indonesians. *J Diabetes Investig* 2014, 5, 507-512, doi: 10.1111/jdi.12177.
8. Azlin, A.; Ganie, R.A.; Syafril, S. The difference of vitamin D levels between controlled and uncontrolled type 2 diabetes mellitus patients. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 26, 18-22, doi:10.24293/ijcpml.v26i1.1407.
9. Tetap produktif, cegah dan atasi DM melitus. Pusat Data dan Informasi Kementerian Kesehatan Republik Indonesia 2020. ISSN 2442-7659. URL: <https://www.kemkes.go.id/downloads/resources/download/pusdatin/infodatin/Infodatin%202020%20Diabetes%20Melitus.pdf>
10. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004, 27, 1047-1053, doi: 10.2337/diacare.27.5.1047.
11. Sharan, A.; Mukhopadhyay, S.; Majumdar, J.; Ghosh, B.; Sengupta, S. A study of prevalence of vitamin D deficiency in new onset type 2 diabetes mellitus in a tertiary care hospital of Eastern India. *JACM* 2018, 19, 106-111. URL: https://www.jiacm.in/journals/2018/april_june_2018/Journal%2070%20Page%20106-111%20Original%20Article.pdf
12. Holick, M.F. Vitamin D deficiency. *N Engl J Med* 2007, 357, 266-281, doi: 10.1056/NEJMra070553.
13. Pusparini.; Meriyanti, L.T.; Sudharma, N.I. Increased matrix metalloproteinase-9 in male elderly with low 25-hydroxy-vitamin D. *Univ Med* 2016, 35, 171-180, doi:10.18051/UnivMed.2016.v35.171-180.
14. Keumala, D.; Alrasyid, H.; Nurlndrawaty, L.; Zulkifli, L. Occurrence of vitamin D deficiency among women in North Sumatera, Indonesia. *Mal J Nutr* 2014, 20, 63-70. URL: https://www.researchgate.net/publication/285524669_Occurrence_of_vitamin_D_deficiency_among_women_in_North_Sumatera_Indonesia#fullTextFileContent
15. Arjana, A.Z.; Devita, N.; Nurmasitoh, T.; Fidianingsih, I.; Dewi, M.; Khoiriyah, U. High proportion of vitamin D deficiency in male adolescents in Yogyakarta Indonesia. In *Proceedings of the 4th International Conference on Sustainable Innovation 2020—Health Science and Nursing (ICoSIHSN 2020)*, March 2021; Atlantis Press B.V, 2021; volume 33, pp. 54-58, doi:10.2991/ahsr.k.210115.011.
16. Pusparini. Defisiensi vitamin D terhadap penyakit. *Indonesian Journal Of Clinical Pathology and Medical Laboratory* 2014, 21, 90-95, doi:10.24293/ijcpml.v21i1.1265.
17. Rimahardika, R.; Subagio, H.W.; Wijayanti, H.S. Asupan vitamin D dan paparan sinar matahari pada orang yang bekerja di dalam ruangan dan di luar ruangan. *Journal Of Nutrition College* 2017, 6, 333-342, doi:10.14710/jnc.v6i4.18785.
18. Asegaonkar, S.B. Vitamin D and type 2 diabetes mellitus: Indian perspectives. *J Diabetic Complications Med* 2016, 1, 1-4, doi:10.4172/2475-3211.1000110.
19. Holick, M.F.; Binkley, N.C.; Bischoff-Ferrari, H.A.; Gordon, C.M.; Hanley, D.A.; Heaney, R.P.; et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical

- practice guideline. *J Clin Endocrinol Metab* 2011, 96, 1911-1930, doi: 10.1210/jc.2011-0385.
20. Mahmoodnia, L.; Tamadon, M.R.; Sadoughi, M.; Beigrezaei, S. Vitamin D status and its relationship with age in type 2 diabetic patients. *Journal of Parathyroid Disease* 2017, 5, 45-48, doi:10.15171/jpd.2017.05.
21. Hidayat, R.; Setiati, S.; Soewondo, P. The association between vitamin D deficiency and type 2 diabetes mellitus in elderly patients. *Acta Med Indones* 2010, 42, 123-129. URL: <https://pubmed.ncbi.nlm.nih.gov/20724765/>
22. Jha, S.C.; Kumar, H.; Faisal, S.Y. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *Academia Journal of Medicine* 2020, 3, 4-10, doi:10.47008/ajm.2020.3.1.2.
23. Al-Horani, H.; Dayyih, W.A.; Mallah, E.; Hamad, M.; Mima, M.; Awad, R.; et al. Nationality, gender, age, and body mass index influences on vitamin D concentration among elderly patients and young Iraqi and Jordanian in Jordan. *Biochem Res Int* 2016, 2016, 1-8, doi: 10.1155/2016/8920503.
24. Akbar, A.R.; Kurnia, E.; Hendrianingtyas, M.; Nugroho, H. Correlation between fasting blood glucose and Hba1c with vitamin D in diabetes mellitus. *Pakistan Journal of Medical Health Sciences* 2019, 13, 1093-1096. URL: https://pjmsonline.com/2019/oct_dec/pdf/1093.pdf
25. Mehta, N.; Shah, S.; Shah, P.P.; Prajapati, V. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *GCSMC J Med Sci* 2016, 5, 42-46. URL: <https://gcsmc.org/assets/pdf/journal/journal-1227/original-article/correlation-between-vitamin-d-and-hba1c-in-type-2-diabetic-patients201810051242459072370.pdf>
26. Suguna, S.; Kusumadevi, M.S. Relationship between vitamin D and HbA1c levels in patients with type 2 diabetes mellitus of Bengaluru City. *International Journal of Physiology* 2019, 7, 104-108, doi:10.5958/2320-608x.2019.00149.5.
27. Zhang, M.; Li, P.; Zhu, Y.; Chang, H.; Wang, X.; Liu, W.; et al. Higher visceral fat area increases the risk of vitamin D insufficiency and deficiency in Chinese adults. *Nutr Metab (Lond)* 2015, 12, 50, doi: 10.1186/s12986-015-0046-x.
28. Fernandes, M.R.; Barreto, W.D.R.J. Association between physical activity and vitamin D: A narrative literature review. *Rev Assoc Med Bras* 2017, 63, 550-556, doi: 10.1590/1806-9282.63.06.550.
29. Grober, U.; Kisters, K. Influence of drugs on vitamin D and calcium metabolism. *Dermatoendocrinol* 2012, 4, 158-166, doi: 10.4161/derm.20731.
30. Sakung, J.M.; Sirajuddin, S.; Zulkifli, A.; Rahman, S.A.; Sudargo, T. Physical activity is associated with lower blood glucose level in high school teachers in Palu, Indonesia. *Int J Community Med Public Health* 2018, 5, 3176-3179, doi:10.18203/2394-6040.ijcmph20183047.
31. Alkhatatbeh, M.J.; Abdul-Razzak, K.K. Association between serum 25-hydroxyvitamin D, hemoglobin A1c and fasting blood glucose levels in adults with diabetes mellitus. *Biomed Rep* 2018, 9, 523-530, doi: 10.3892/br.2018.1159.
32. Sherwani, S.I.; Khan, H.A.; Ekhzaimy, A.; Masood, A.; Sakharkar, M.K. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomark Insights* 2016, 11, 95-104, doi: 10.4137/BMI.S38440.
33. Monnier, L.; Colette, C. Contributions of fasting and post-prandial glucose to hemoglobin A1c. *Endocr Pract* 2006, 12 Suppl 1, 42-46, doi: 10.4158/EP.12.S1.42.
34. Kautzky-Willer, A.; Kosi, L.; Lin, J.; Mihaljevic, R. Gender-based differences in glycaemic control and hypoglycaemia prevalence in patients with type 2 diabetes: results from patient-level pooled data of six randomized controlled trials. *Diabetes Obes Metab* 2015, 17, 533-540, doi: 10.1111/dom.12449.
35. Tabak, A.G.; Herder, C.; Rathmann, W.; Brunner, E.J.; Kivimaki, M. Prediabetes: a high-risk state for diabetes development. *Lancet* 2012, 379, 2279-2290, doi: 10.1016/S0140-6736(12)60283-9.
36. Ehrhardt, N.M.; Chellappa, M.; Walker, M.S.; Fonda, S.J.; Vigersky, R.A. The effect of real-time continuous glucose monitoring on glycemic control in patients with type 2 diabetes mellitus. *J Diabetes Sci Technol* 2011, 5, 668-675, doi: 10.1177/193229681100500320.
37. Sanda, A.; Bahrin, U.; Pakasi, R.D.N.; Aman, A.M. Analysis of vitamin D in patients with type 2 diabetes mellitus. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 25, 150-154, doi: 10.24293/ijcpml.v25i2.1360.

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



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

Congratulations - Your article was published in the RMJ Vol. 80 (3) Issue

4 messages

Rwanda Medical Journal <rwandamedicaljournal@gmail.com>
To: Alvina fk <dr.alvina@trisakti.ac.id>

Fri, Oct 6, 2023 at 12:47 AM

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

Your article is available at <https://www.rwandamedicaljournal.org/previous-issues.html> and will soon be available on all publisher platforms (SCOPUS, AJOL, Bioline, DOAJ) where the RMJ is indexed.

Thank you for considering the RMJ for your publication, and we look forward to your future submissions.

Thank you

Sincerely,

--

Kind regards

Editorial team

Rwanda Medical Journal

www.rwandamedicaljournal.org
@rwandamedicaljo

Alvina fk <dr.alvina@trisakti.ac.id>
To: Rwanda Medical Journal <rwandamedicaljournal@gmail.com>

Fri, Oct 6, 2023 at 10:27 PM

Dear
Editorial Team RMJ

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

[Quoted text hidden]

Rwanda Medical Journal <rwandamedicaljournal@gmail.com>
To: Alvina fk <dr.alvina@trisakti.ac.id>

Fri, Oct 6, 2023 at 11:15 PM

Dear Alvina,

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

Thank you for considering the RMJ

[Quoted text hidden]

[Quoted text hidden]



UNIVERSITAS TRISAKTI

"Is a one stop learning for sustainable development"

Kampus A, Jl. Kyai Tapa No.1, Grogol

Jakarta Barat 11440 - INDONESIA

www.trisakti.ac.id

(t) +62-21.566 3232, (f) +62-21.567 3001

[Quoted text hidden]

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.

Thank you
Best regards,
Alvina,MD

[Quoted text hidden]



OP.23.29 Revision.pdf

551K

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

Authors: Alvina^{1,*}; Pusparini¹

Affiliations: ¹Department of Clinical Pathology, Universitas Trisakti, Jakarta, Indonesia

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 (Col):** 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).

Received: 09th March 2023; **Initial decision given:** 4th April 2023; **Revised manuscript received:** 13th July 2023; **Accepted:** 26th August 2023.

Copyright: © The Author(s). This is an Open Access article distributed under the terms of the Creative Commons Attribution License (CC BY-NC-ND) ([click here](https://creativecommons.org/licenses/by-nc-nd/4.0/)) which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. **Publisher:** Rwanda Biomedical Centre (RBC)/Rwanda Health Communication Center, P. O. Box 4586, Kigali. ISSN: 2079-097X (print); 2410-8626 (online)

Citation for this article: Alvina; P. Pusparini. 25-hydroxy vitamin d concentrations negatively correlated with HbA1c in type 2 diabetes mellitus patients: a cross-sectional study in Mampang, South Jakarta. Rwanda Medical Journal, Vol. 80, no. 3, p. 16-23, 2023. <https://dx.doi.org/10.4314/rmj.v80i3.3>

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.

REFERENCES

1. Kharroubi, A.T.; Darwish, H.M. Diabetes mellitus: The epidemic of the century. *World J Diabetes* 2015, 6, 850-867, doi:10.4239/wjd.v6.i6.850.
2. American Diabetes Association Professional Practice Committee. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2022. *Diabetes Care* 2022, 45(Suppl 1), S17-S38, doi:10.2337/dc22-S002
3. Rehman, K.; Akash, M.S. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J Biomed Sci* 2016, 23, 87, doi: 10.1186/s12929-016-0303-y.
4. Das, G. Vitamin D and type 2 diabetes. *Practical Diabetes* 2017, 34, 19-24. URL: <https://wileymicrositebuilder.com/practicaldiabetes/wp-content/uploads/sites/29/2017/01/Rev-Das-lsw.pdf>
5. Yu, J.R.; Lee, S.A.; Lee, J.G.; Seong, G.M.; Ko, S.J.; Koh, G.; et al. Serum vitamin d status and its relationship to metabolic parameters in patients with type 2 diabetes mellitus. *Chonnam Med J* 2012, 48, 108-115, doi: 10.4068/cmj.2012.48.2.108.
6. Aaty, T.A.A.; Magallaa, M.H.Z.; Moneim, H.A.; Ismail, H.M.; Genena, D.M.; Frugina, R. Serum vitamin D level in type 2 diabetic subjects: Relation to glycemic control, insulin resistance and proinflammatory markers. *Journal of High Institute of Public Health* 2017, 47, 62-68, doi:10.21608/JHIPH.2017.19966.
7. Mihardja, L.; Soetrisno, U.; Soegondo, S. Prevalence and clinical profile of diabetes mellitus in productive aged urban Indonesians. *J Diabetes Investig* 2014, 5, 507-512, doi: 10.1111/jdi.12177.
8. Azlin, A.; Ganie, R.A.; Syafril, S. The difference of vitamin D levels between controlled and uncontrolled type 2 diabetes mellitus patients. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 26, 18-22, doi:10.24293/ijcpml.v26i1.1407.
9. Tetap produktif, cegah dan atasi DM melitus. Pusat Data dan Informasi Kementerian Kesehatan Republik Indonesia 2020. ISSN 2442-7659. URL: <https://www.kemkes.go.id/downloads/resources/download/pusdatin/infodatin/Infodatin%202020%20Diabetes%20Melitus.pdf>
10. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004, 27, 1047-1053, doi: 10.2337/diacare.27.5.1047.
11. Sharan, A.; Mukhopadhyay, S.; Majumdar, J.; Ghosh, B.; Sengupta, S. A study of prevalence of vitamin D deficiency in new onset type 2 diabetes mellitus in a tertiary care hospital of Eastern India. *JACM* 2018, 19, 106-111. URL: https://www.jiacm.in/journals/2018/april_june_2018/Journal%2070%20Page%20106-111%20Original%20Article.pdf
12. Holick, M.F. Vitamin D deficiency. *N Engl J Med* 2007, 357, 266-281, doi: 10.1056/NEJMra070553.
13. Pusparini.; Meriyanti, L.T.; Sudharma, N.I. Increased matrix metalloproteinase-9 in male elderly with low 25-hydroxy-vitamin D. *Univ Med* 2016, 35, 171-180, doi:10.18051/UnivMed.2016.v35.171-180.
14. Keumala, D.; Alrasyid, H.; Nurlndrawaty, L.; Zulkifli, L. Occurrence of vitamin D deficiency among women in North Sumatera, Indonesia. *Mal J Nutr* 2014, 20, 63-70. URL: https://www.researchgate.net/publication/285524669_Occurrence_of_vitamin_D_deficiency_among_women_in_North_Sumatera_Indonesia#fullTextFileContent
15. Arjana, A.Z.; Devita, N.; Nurmasitoh, T.; Fidianingsih, I.; Dewi, M.; Khoiriyah, U. High proportion of vitamin D deficiency in male adolescents in Yogyakarta Indonesia. In *Proceedings of the 4th International Conference on Sustainable Innovation 2020—Health Science and Nursing (ICoSIHSN 2020)*, March 2021; Atlantis Press B.V, 2021; volume 33, pp. 54-58, doi:10.2991/ahsr.k.210115.011.
16. Pusparini. Defisiensi vitamin D terhadap penyakit. *Indonesian Journal Of Clinical Pathology and Medical Laboratory* 2014, 21, 90-95, doi:10.24293/ijcpml.v21i1.1265.
17. Rimahardika, R.; Subagio, H.W.; Wijayanti, H.S. Asupan vitamin D dan paparan sinar matahari pada orang yang bekerja di dalam ruangan dan di luar ruangan. *Journal Of Nutrition College* 2017, 6, 333-342, doi:10.14710/jnc.v6i4.18785.
18. Asegaonkar, S.B. Vitamin D and type 2 diabetes mellitus: Indian perspectives. *J Diabetic Complications Med* 2016, 1, 1-4, doi:10.4172/2475-3211.1000110.
19. Holick, M.F.; Binkley, N.C.; Bischoff-Ferrari, H.A.; Gordon, C.M.; Hanley, D.A.; Heaney, R.P.; et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical

- practice guideline. *J Clin Endocrinol Metab* 2011, 96, 1911-1930, doi: 10.1210/jc.2011-0385.
20. Mahmoodnia, L.; Tamadon, M.R.; Sadoughi, M.; Beigrezaei, S. Vitamin D status and its relationship with age in type 2 diabetic patients. *Journal of Parathyroid Disease* 2017, 5, 45-48, doi:10.15171/jpd.2017.05.
21. Hidayat, R.; Setiati, S.; Soewondo, P. The association between vitamin D deficiency and type 2 diabetes mellitus in elderly patients. *Acta Med Indones* 2010, 42, 123-129. URL: <https://pubmed.ncbi.nlm.nih.gov/20724765/>
22. Jha, S.C.; Kumar, H.; Faisal, S.Y. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *Academia Journal of Medicine* 2020, 3, 4-10, doi:10.47008/ajm.2020.3.1.2.
23. Al-Horani, H.; Dayyih, W.A.; Mallah, E.; Hamad, M.; Mima, M.; Awad, R.; et al. Nationality, gender, age, and body mass index influences on vitamin D concentration among elderly patients and young Iraqi and Jordanian in Jordan. *Biochem Res Int* 2016, 2016, 1-8, doi: 10.1155/2016/8920503.
24. Akbar, A.R.; Kurnia, E.; Hendrianingtyas, M.; Nugroho, H. Correlation between fasting blood glucose and Hba1c with vitamin D in diabetes mellitus. *Pakistan Journal of Medical Health Sciences* 2019, 13, 1093-1096. URL: https://pjmsonline.com/2019/oct_dec/pdf/1093.pdf
25. Mehta, N.; Shah, S.; Shah, P.P.; Prajapati, V. Correlation between vitamin D and HbA1c in type 2 diabetic patients. *GCSMC J Med Sci* 2016, 5, 42-46. URL: <https://gcsmc.org/assets/pdf/journal/journal-1227/original-article/correlation-between-vitamin-d-and-hba1c-in-type-2-diabetic-patients201810051242459072370.pdf>
26. Suguna, S.; Kusumadevi, M.S. Relationship between vitamin D and HbA1c levels in patients with type 2 diabetes mellitus of Bengaluru City. *International Journal of Physiology* 2019, 7, 104-108, doi:10.5958/2320-608x.2019.00149.5.
27. Zhang, M.; Li, P.; Zhu, Y.; Chang, H.; Wang, X.; Liu, W.; et al. Higher visceral fat area increases the risk of vitamin D insufficiency and deficiency in Chinese adults. *Nutr Metab (Lond)* 2015, 12, 50, doi: 10.1186/s12986-015-0046-x.
28. Fernandes, M.R.; Barreto, W.D.R.J. Association between physical activity and vitamin D: A narrative literature review. *Rev Assoc Med Bras* 2017, 63, 550-556, doi: 10.1590/1806-9282.63.06.550.
29. Grober, U.; Kisters, K. Influence of drugs on vitamin D and calcium metabolism. *Dermatoendocrinol* 2012, 4, 158-166, doi: 10.4161/derm.20731.
30. Sakung, J.M.; Sirajuddin, S.; Zulkifli, A.; Rahman, S.A.; Sudargo, T. Physical activity is associated with lower blood glucose level in high school teachers in Palu, Indonesia. *Int J Community Med Public Health* 2018, 5, 3176-3179, doi:10.18203/2394-6040.ijcmph20183047.
31. Alkhatatbeh, M.J.; Abdul-Razzak, K.K. Association between serum 25-hydroxyvitamin D, hemoglobin A1c and fasting blood glucose levels in adults with diabetes mellitus. *Biomed Rep* 2018, 9, 523-530, doi: 10.3892/br.2018.1159.
32. Sherwani, S.I.; Khan, H.A.; Ekhzaimy, A.; Masood, A.; Sakharkar, M.K. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomark Insights* 2016, 11, 95-104, doi: 10.4137/BMI.S38440.
33. Monnier, L.; Colette, C. Contributions of fasting and post-prandial glucose to hemoglobin A1c. *Endocr Pract* 2006, 12 Suppl 1, 42-46, doi: 10.4158/EP.12.S1.42.
34. Kautzky-Willer, A.; Kosi, L.; Lin, J.; Mihaljevic, R. Gender-based differences in glycaemic control and hypoglycaemia prevalence in patients with type 2 diabetes: results from patient-level pooled data of six randomized controlled trials. *Diabetes Obes Metab* 2015, 17, 533-540, doi: 10.1111/dom.12449.
35. Tabak, A.G.; Herder, C.; Rathmann, W.; Brunner, E.J.; Kivimaki, M. Prediabetes: a high-risk state for diabetes development. *Lancet* 2012, 379, 2279-2290, doi: 10.1016/S0140-6736(12)60283-9.
36. Ehrhardt, N.M.; Chellappa, M.; Walker, M.S.; Fonda, S.J.; Vigersky, R.A. The effect of real-time continuous glucose monitoring on glycemic control in patients with type 2 diabetes mellitus. *J Diabetes Sci Technol* 2011, 5, 668-675, doi: 10.1177/193229681100500320.
37. Sanda, A.; Bahrin, U.; Pakasi, R.D.N.; Aman, A.M. Analysis of vitamin D in patients with type 2 diabetes mellitus. *Indonesian Journal of Clinical Pathology and Medical Laboratory* 2019, 25, 150-154, doi: 10.24293/ijcpml.v25i2.1360.