Association between 25-hydroxyvitamin D and hemoglobin A1c levels in patients with type 2 diabetic kidney disease

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Received: 10 August 2018
Accepted: 19 September 2018
Published: 18 August 2020

The Egyptian Journal of Internal Medicine
2019, 31:573–579

Introduction
Vitamin D is suggested to influence glucose homeostasis. An inverse relationship between serum 25-hydroxyvitamin D (25(OH)D) and glycemic control in non-chronic kidney disease (CKD) patients with type 2 diabetes was reported. We aimed to examine this association among type 2 diabetes patients with CKD.
Objectives
To examine the relation between plasma 25-hydroxyvitamin D_3 ($25(OH)D_3$) levels and glycemic state in diabetic patients at various stages of CKD.
Patients and methods
A total of 70 participants (40 men and 30 women) with a mean age of 65.3±11.5 years suffering from type 2 diabetes mellitus with various stages of CKD were recruited. Blood for glycated hemoglobin (HbA1c), serum $25(OH)D_3$, renal profile, and estimated glomerular filtration rate was drawn at enrollment. Correlation and regression analyses were carried out to assess the relationship of serum 25(OH)D, HbA1c, and other metabolic traits.
Results
Our study shows the following results:
Most of the participants are urban with age range from 50 to 70 years.
Forty percent of the participants are with good glycemic control, 30% with moderate control, and 30% with bad control.
Fifty percent of the patients were at CKD stage 3. Stage 5 patients differed significantly from stages 1 to 4 patients where they were younger, with lowest mean HbA1C value and a much higher mean 25(OH)D level (around twice of stage 1 patients).
Half of the cases are vitamin D deficient, nearly a third of them are insufficient, and about 20% are sufficient.
The level of $25(OH)D_3$ correlates inversely with the level of HbA1C irrespective of estimated glomerular filtration rate or the age of the patients.
Conclusion
The present study reported a significant inverse relationship between serum 25 $(OH)D_3$ and HbA1c levels in type 2 diabetics with suboptimal glycemic control and concomitant different stages of CKD.

Keywords:
chronic kidney disease, glycated hemoglobin, type 2 diabetes, vitamin D_3

Egypt J Intern Med 31:573–579

1110-7782

Introduction

Type 2 diabetes mellitus consists of an array of dysfunctions characterized by hyperglycemia and resulting from a combination of resistance to insulin action, inadequate insulin secretion, and excessive or inappropriate glucagon secretion (see the image below [1]). The top 10 countries in number of people with diabetes are currently India, China, the USA, Indonesia, Japan, Pakistan, Russia, Brazil, Italy, and Bangladesh. The greatest percentage increase in rates of diabetes will occur in Africa over the next 20 years. Unfortunately, at least 80% of people in Africa with diabetes are undiagnosed, and many in their 30s–60s will die from diabetes [2]. Currently, diabetic kidney disease is the leading cause of chronic kidney disease (CKD) in the USA and other western societies. It is also one of the most significant long-term complications in terms of morbidity and mortality for individual patients with diabetes. Diabetes is responsible for 30–40% of all end-stage renal disease cases in the USA [3]. Vitamin D is a group of fat-soluble compounds with a four-ringed cholesterol backbone. First described by Whistler and Glisson in the mid-1600s, vitamin D is now recognized as a prohormone. Vitamin D exists in two major forms: vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). The precursor for vitamin D_2 is a

plant sterol ergosterol. Vitamin D_2 can be synthesized by ultraviolet irradiation of ergosterol from yeast. Similarly, vitamin D_3 is synthesized in the body when sunlight (ultraviolet B, wavelength 280–315 nm) photoisomerizes 7-dehydroxycholesterol found in the skin. Vitamin D_3 is also found in animal-based foods (e.g. fatty fish, liver, milk, eggs) in the liver. Vitamin D is hydroxylated by the enzyme 25-hydroxylase (CYP2R1) to become 25-hydroxyvitamin D (25(OH)D). 25(OH) D is the major circulating form of vitamin D. From the liver, 25(OH)D is transported to the kidneys via the carrier proteins [4]. Insulin resistance is a recognized precursor for the development of type 2 diabetes. Vitamin D may have a beneficial effect on insulin action either directly, by stimulating the expression of insulin receptors thereby enhancing insulin responsiveness for glucose transport (Mathieu and Gysemans, [5]), or indirectly via its role in regulating extracellular calcium ensuring normal calcium influx through cell membranes and adequate intracellular cytosolic calcium ($Ca2^+$)i pool. Calcium is essential for insulin-mediated intracellular processes in insulin-responsive tissues such as the skeletal muscle and adipose tissue with a very narrow range of ($Ca2^+$)i needed for optimal insulin-mediated functions. Associations between low vitamin D level and decreased insulin sensitivity have been reported in cross-sectional studies [6]. Vitamin D may improve insulin sensitivity and promote beta-cell survival by directly modulating the generation and effects of cytokines. There are very limited and conflicting data from human studies that have directly examined the relationship between vitamin D or calcium status and systemic inflammation in relation to type 2 diabetes [7].

Patients and methods

Selection of study patients

The study was conducted on 70 patients [40 (57.1%) men and 30 (42.9%) women] with mean±SD age 65.3 ±11.5 years previously diagnosed as type 2 diabetes mellitus attending the outpatient clinic of Al-Azhar University Hospitals (Assuit branch). Patients and controls were subjected to the following: full history taking, including age, sex, and antidiabetic medications; clinical examination, with emphasis on blood pressure, neurological, and cardiac examination electrocardiography; and abdominal ultrasonography, for detection of kidney abnormalities and electrocardiography to detect and exclude cardiac abnormality. Laboratory investigations includes complete blood count, complete urine analysis, glycated hemoglobin (HbA1c), blood urea and serum creatinine levels, estimated glomerular filtration rate (eGFR), basal metabolic rate, and serum level of vitamin D (25-hydroxyvitamin D_3 25(OH)D_3).

Exclusion criteria

Patients with liver or heart failure, malignancies, infectious diseases, previous gastrointestinal surgery, gastrointestinal diseases such as ulcerative colitis, Crohn's disease, celiac disease, pancreatitis, or on any medications that affect vitamin D levels (corticosteroids, calcium, and/or vitamin D supplements and anti-epileptics) were excluded.

Ethical considerations

(1) All steps of the study were explained clearly to the patient and/or to the caregiver before participation.
(2) A written consent was being obtained from all the participants.

Analytical methods

Creatinine levels were measured using a colorimetric assay. Levels of 25(OH)D were determined using chemiluminometric immunoassays. The new 25(OH) D enzyme-linked immunosorbent assay kit is designed for the determination of 25(OH)D in human serum or plasma samples. In the first analysis step, the calibrators and patient samples are diluted with biotin-labeled 25 (OH)D and added to microplate wells coated with monoclonal anti-25(OH) vitamin antibodies. During incubation, an unknown amount of 25(OH) vitamin competes for the antibody-binding sites in the microplate wells plate. Unbound 25(OH)D is removed by washing. For the detection of bound biotin-labeled 25(OH)D, a second incubation is performed using the peroxidase substrate tetramethylbenzidine, the bound peroxidase promotes a color reaction. The color intensity is inversely proportional to the 25(OH)D concentration. An ion-exchange chromatography method (Variant II Turbo; Bio-Rad, Hercules, California, USA) was used to assay HbA1c, according to the manufacturer's instructions. eGFR was estimated according to the modification of diet in the renal disease equation, which includes four variables. As regard eGFR was measured according to the equation (ml/min per 1.73 m^2)=175×(serum creatinine)−1.154×(age)−0.203×(0.742 if female) (conventional units).

Data analyses

Every participant was categorized according to HbA1c, to be with good glycemic control (HbA1c≤7%), with moderate glycemic control (HbA1c 7.1–9%), or with poor glycemic control (HbA1c >9%). Also we divided the participants according to vitamin D into three

groups. Group I includes patients with serum 25(OH) D less than 20 ng/ml, named as the 'deficient group.' Group II includes patients with serum 25(OH)D of 21–29 ng/ml, named as the 'insufficient group.' And finally group III includes patients with serum 25(OH) D more than 30 ng/ml, named as the 'sufficient group' [8].

Regarding the eGFR, the patients were divided into five stages as follows:

Stage 1 CKD: eGFR more than 90 ml/min.
Stage 2 CKD: eGFR 60–89 ml/min.
Stage 3 CKD: eGFR 30–59 ml/min.
Stage 4 CKD: eGFR 15–29 ml/min.
Stage 5 CKD: eGFR less than 15 ml/min.
A multivariate analysis was performed to establish whether or not there was a relationship between vitamin D status and HbA1c levels after adjusting the renal status.

Statistical analysis

All data were being collected, tabulated, and statistically analyzed using the statistical package for the social sciences, version 23.0 for Windows (IBM; Armonk, New York, USA). Data are presented as the mean±SD, frequency, and percentage. Continuous variables were compared by the Student's *t* test (two-tailed) and one-way analysis of variance test for parametric data with Bonferroni's post-hoc test to detect the differences between subgroups. Pearson's correlations were used to study the correlation between the studied variables. The level of significance was accepted if the *P* value less than 0.05.

Results

The study was conducted on 70 diabetic patients with various degrees of CKD attending to Al-Azhar Assuit University Hospital, 40 (57.1%) men and 30 (42.9%) women with the age range of 54–76 years and mean ±SD of 65.3±11.5 years. The range of duration of diabetes mellitus in the studied patients was from 10 to 23 years with a mean±SD of 16.6±3.5 years; 80% of patients included in this study were from rural background. The patients enrolled in this study did not receive 1, 25-dihydroxyvitamin D_3 (or one of its active analogs). The anthropometric measures among our patients are shown in Table 1.

As regards the degree of glycemic control among the studied patients, 41.4% were with good glycemic control, 30% were with moderate glycemic control, and 28.6% were with poor control as shown in Table 2.

Table 1 Anthropometric measures of the studied patients

	Result	
	Range	Mean±SD
Weight (kg)	68–101	78.6±9.3
Height (cm)	165–178	170.3±3.9
BMI (kg/m^2)	22.5–32.6	27.1±3.4

Table 2 Classification of the studied cases according to glycated hemoglobin level

	N=70 [*n* (%)]
HbA1c level	
Good glycemic control (HbA1c≤7%)	29 (41.4)
Moderate glycemic control (HbA1c 7.1–9%)	21 (30.0)
Poor glycemic control (HbA1c>9%)	20 (28.6)

HbA1c, glycated hemoglobin.

Table 3 Classification of the studied cases according to chronic kidney disease stages

	N=70 [*n* (%)]
CKD stages	
Stage 1	3 (4.2)
Stage 2	13 (18.5)
Stage 3	35 (50.0)
Stage 4	7 (10.0)
Stage 5	12 (17.2)

CKD, chronic kidney disease.

The patients were categorized according to the degree of kidney affection into five stages as shown in Table 3. Where half of the cases of the study are of stage 3 CKD, around 20% are of stage 2, another 20% lies on stages 4 and 5, while a small proportion (3%) lies in the stage 1 category (Table 3).

As regards vitamin D status in the studied patients, the study revealed that around half (45.7%) of the cases are vitamin D deficient less than 20 ng/dl; 34.3% of them are sufficient (>30 ng/dl); and 20% are insufficient (20–29 ng/dl) as shown in Table 4.

HbA1c correlates inversely with the level of 25(OH)D, irrespective of the eGFR or age of the patients ($P<0.001$) (Table 5).

On the other hand, comparison of demographic and laboratory data of the studied cases in relation to vitamin D show that glycemic control decreased significantly with vitamin D deficiency (Tables 6 and 7).

Fifty percent of the patients were at CKD stage 3. Stage 5 patients differed significantly from stages 1 to 4 patients: they were younger, with lowest mean HbA1C

Table 4 Classification of the studied cases according to vitamin D status

	N=70 [*n* (%)]
Vitamin D status	
Sufficiency (25(OH)D >30 ng/dl)	24 (34.3)
Insufficiency (25(OH)D 20–29 ng/dl)	14 (20.0)
Deficiency (25(OH)D<20 ng/dl)	32 (45.7)

25(OH)D, 25-hydroxyvitamin D.

Table 6 Comparison of demographic and laboratory data of the studied cases according to glycated hemoglobin level

Parameters	Sufficiency (*N*=24)	Insufficiency (*N*=14)	Deficiency (*N*=32)	*P* value
Age (years)	65.6±10.9	63.4±11.6	65.3±11.3	0.42 NS
eGFR (ml/min per1.73 m^2)	38.3±23.1	42.5±22.4	49.6±23.8	<0.001
HbA1c (%)	6.7±1.0	7.3±1.5	9.3±2.0	<0.001
25(OH)D (ng/dl)	45.9±14.9	24.8±2.7	10.7±4.8	<0.001

Values are presented as mean±SD. 25(OH)D, 25-hydroxyvitamin D; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; NS, nonsignificant. *F* test (analysis of variance) was used, statistically significant difference, *P* value less than 0.05.

value, a much higher mean 25(OH)D level (around twice of stage 1 patients) (Figs 1–3).

Discussion

Our present study revealed a significant, negative correlation between 25(OH)D levels and HbA1c values. However, the presence of a correlation does not necessarily mean that vitamin D has a positive impact on glucose homeostasis. Indeed, various factors may influence the relationship between vitamin D and HbA1c. Although the analysis of variance revealed a significant difference in age between the CKD stage subgroups ($P<0.001$) most of the study population was middle aged or elderly (under 70 years). Most of the patients had moderate to severe CKD (with around 77% at stages 3–5). On average, CKD stage 5 patients were younger than other CKD groups (e.g. almost 9 years younger than the CKD stage 4 group), suggesting that the onset of CKD had occurred earlier in life in the CKD stage 5 patients. It is noteworthy that the mean HbA1c value was lower in CKD stage 5 patients than in CKD stages 1–4 patients (6.3 and 7.6%, respectively). This difference must be considered in the light of 25(OH)D levels: the mean 25(OH)D values in the population as a whole and in the CKD stages 1–4 patients were around 24 mmol/l (corresponding to deficiency), whereas the value in CKD stage patients was almost twice as high (40.6 mmol/l, corresponding to insufficiency). In other words, the CKD stage 5 patients had a particular profile, with an HbA1c value below 6.5% and the absence of vitamin D deficiency. The results are satisfying from a clinical viewpoint and may reflect the need to monitor vitamin D status in patients with diabetic CKD for early preventive treatment of secondary hyperparathyroidism. A retrospective analysis among a French type 2 diabetes cohort conducted on 245 patients previously diagnosed to be with type 2 diabetes mellitus with concomitant stages 1–5 CKD identified a negative linear relationship between serum 25(OH)D and HbA1c levels, even after multivariate adjustments [9]. A similar result was noted in Malaysian retrospective analysis among 100 patients with type 2 diabetes with stages 3–4 CKD, which showed also an inverse linear relationship between the level of plasma 25(OH)D and HbA1C percentage [10]. Conversely, in a cross-sectional Canadian study conducted on 60 patients, most of them (90%) were of type 2 diabetics and all of them were CKD (stages 1–4); the study showed no correlation between the level of HbA1c and 25(OH)D which may be attributed to the small sample size [11].

Table 5 Comparison of demographic and laboratory data of the studied cases according to glycated hemoglobin level

Parameters	HbA1c ≤7% (*N*=29)	HbA1c 7.1–9% (*N*=21)	HbA1c >9% (*N*=20)	*P* value
Age (years)	55.3±5.3	60.2±4.6	68.3±2.9	<0.001
eGFR (ml/min per 1.73 m^2)	37.3±2.3	45.3±11.	52.3±18.6	<0.001
HbA1c (%)	6.7±1.2	7.9±1.23	9.8±1.8	<0.001
25(OH)D (ng/dl)	42.3±11.3	28.7±3.6	12.3±5.6	<0.001

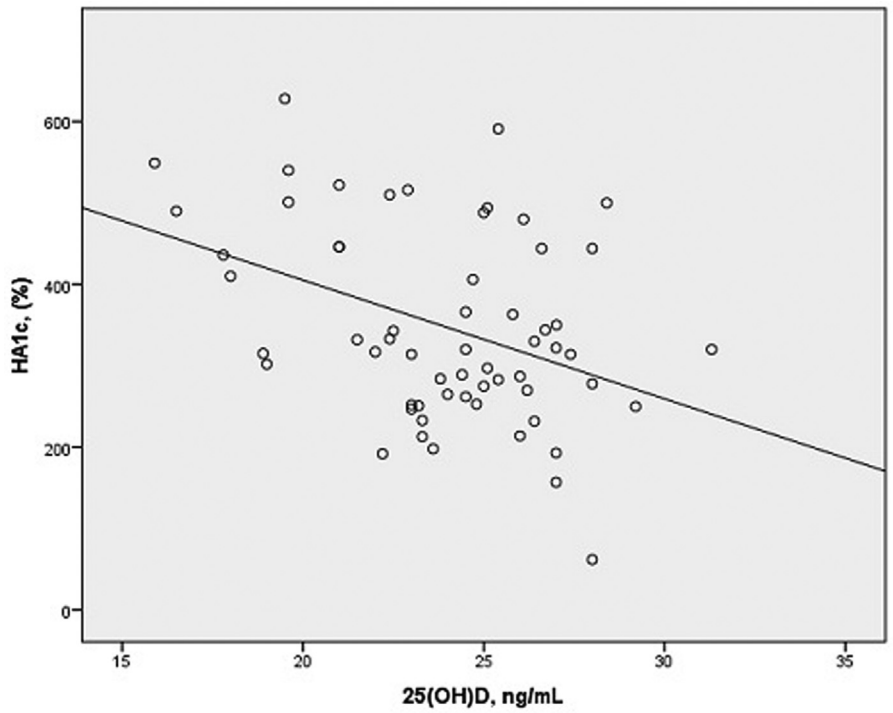
Values are presented as mean±SD. 25(OH)D, 25-hydroxyvitamin D; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin. *F* test (analysis of variance), was used, statistically significant difference, *P* value less than 0.05.

Table 7 Comparison of demographic and laboratory data of the studied cases according to chronic kidney disease stages (*N*=70)

Parameters	Stage 1 (*N*=3)	Stage 2 (*N*=13)	Stage 3 (*N*=35)	Stage 4 (*N*=7)	Stage 5 (*N*=12)	*P* value
Age (years)	59.9 ±12.6	63.4 ±12.0	66.8 ±10.1	66.3 ±2.0	57.8 ±10.9	<0.001
eGFR (ml/min per1.73 m^2)	103.9 ±11.1	72.0 ±8.7	42.9 ±8.3	22.3 ±4.3	9.3 ±3.1	<0.001
HbA1c (%)	7.5 ±1.6	7.9 ±1.9	7.8 ±1.9	7.4 ±1.4	6.3 ±1.2	<0.001
25(OH)D (ng/dl)	19.7 ±15.9	22.7 ±16.3	23.6 ±16.8	28.7 ±19	40.6 ±21.6	<0.001

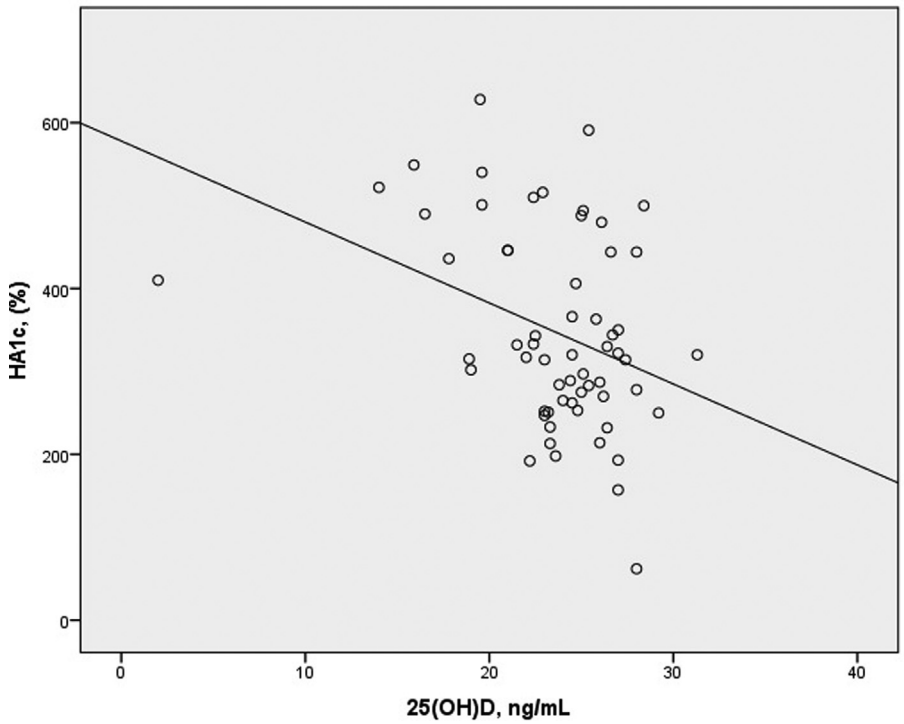
Values are presented as mean±SD. 25(OH)D, 25-hydroxyvitamin D; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin. *F* test (analysis of variance), was used, statistically significant difference, *P* value less than 0.05.

Figure 1



Comparison between HbA1c levels of the studied cases and chronic kidney disease stages. 25(OH)D, 25-hydroxyvitamin D; HbA1c, glycated hemoglobin.

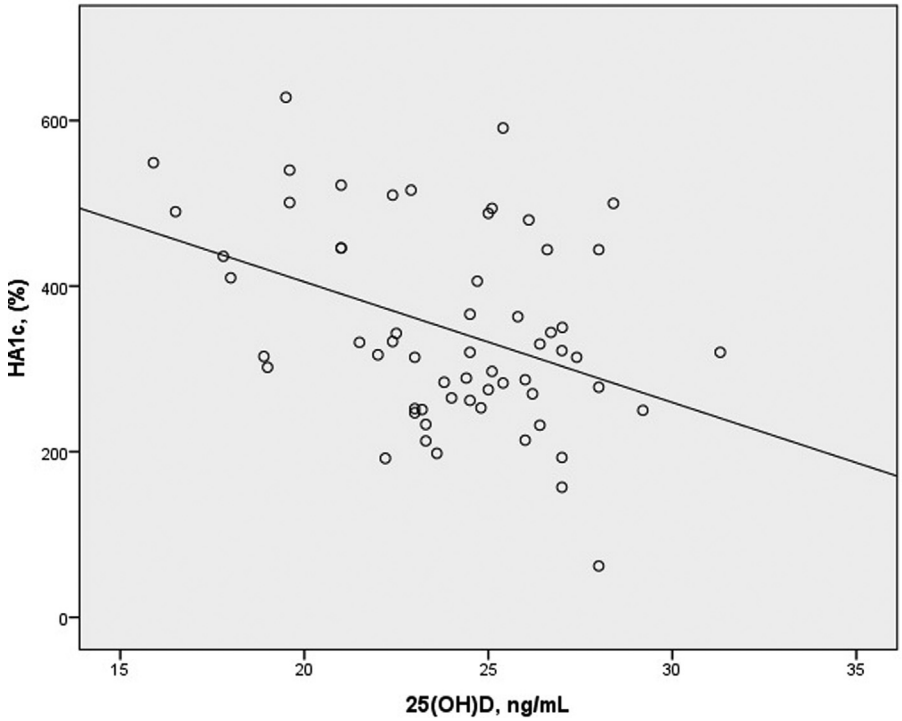
Figure 2



Comparison of 25(OH)D, ng/ml data of the studied cases according to chronic kidney disease stages. 25(OH)D, 25-hydroxyvitamin D; HbA1c, glycated hemoglobin.

Despite about 80% of patients included in this study were rural (expected to be exposed sufficiently to sunlight), most of them are either vitamin D deficient (46%) or insufficient (20%), which may refer to the underestimated problem of wide prevalence hypovitaminosis D among Egyptians. On the other hand, our study participants cannot represent a complete representative sample of Egyptians because

Figure 3



Correlation between 25-hydroxyvitamin D and HbA1c levels in patients with type 2 diabetes at all stages of chronic kidney diseases showing inverse relationship. HbA1c, glycated hemoglobin.

all of them are diabetics and also have different stages of CKD. Among type 2 diabetic patients with normal renal function, the relationship between serum 25 (OH)D and glycemic control yielded inconsistent results. In a prospective study of more than 5000 healthy Danish individuals aged 30–65 years, hypovitaminosis D was associated with worsened insulin resistance and subsequent hyperglycemia [12]. Another supporting large-scale AusDiab study show an inverse relationship between serum 25(OH)D with metabolic parameters; for example, fasting glucose, insulin resistance, triglyceride level, and waist circumference were reported, observing an increased 5-year risk of new-onset metabolic syndrome among healthy adults (Gagnon *et al.*, 2012). Based on a meta-analysis of 11 prospective studies, individuals with higher serum 25(OH) levels (>80 vs. <48.8 nmol/l) were at 41% diminished risk of developing type 2 diabetes [13]. Conversely, several studies reported discordant results, whereby hypovitaminosis D was not an independent predictor of incident or worsening type 2 diabetes in healthy adults, as its effect was predominantly modulated by BMI [14]. Trials on the effects of cholecalciferol supplementation on glucose homeostasis in type 2 diabetes with CKD patients were underexplored. In the Nurses' Health Study, involving nondiabetic middle-aged women, the relative risk of type 2 diabetes was lowered by 17–33% with at least 400 IU cholecalciferol and 1200 mg calcium intake after 20 years of follow-up [15]. This was consistent with a post-hoc analysis that observed favorable impacts on glycemic traits after a 3-year therapy with 700 IU cholecalciferol and 500 mg calcium citrate among the elderly Caucasians with impaired fasting glucose. In healthy middle-aged Japanese, increased vitamin D and calcium intake was protective against type 2 diabetes, with a 40% relative risk reduction after 5 years [16]. Conversely, in the 7-year follow-up of the Women's Health Initiative Study, 400 IU cholecalciferol and 1000 mg elemental calcium did not diminish the incident type 2 diabetes risk among postmenopausal women [17]. The discrepancy between these intervention studies could be attributed to the differences in participants' characteristics, the severity of the underlying metabolic disorders, vitamin D status, and supplementation regime. The importance of identification of the susceptible group, who will most likely benefit from vitamin D repletion therapy, is highlighted in a recent meta-analysis involving 1797 patients with type 2 diabetes, which reported a favorable improvement in fasting glucose post-vitamin D supplementation only among those with poorly controlled glycemia (HbA1c ≥8%) [18]. Of note, these trials were not carried out in CKD cohorts, who were phenotypically distinct from the non-CKD population and therefore required a different treatment approach. This highlights the need for

similar studies on patients with diabetes and concomitant different stages of CKD.

Limitations of the study

The present study had four limitations:

(1) The study was prospective and not retrospective and it wasn't one of the limitations.
(2) The effect of vitamin D on glucose metabolism was not studied directly.
(3) The relatively small number of patients in CKD stage 1.
(4) The possible impact of antidiabetic agents was not taken into consideration.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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