Relationship of vitamin D level and microalbuminuria in type 2 diabetic patients

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Background Great prevalence of patients with type 2 diabetes mellitus (T2DM) has been witnessed in the last decades worldwide. The global prevalence has been estimated to reach 6.4% among adults at the beginning of the current decade. Diabetic nephropathy is considered the most common complication that affects the kidney and plays as a leading cause of end-stage kidney disease. The presence of proteinuria in diabetic patients is a sign of risk. Vitamin D is a multitrait steroid hormone involved in a wide spectrum of cell regulatory and metabolic functions. Multiple diseases were observed to be frequently associated with low levels of vitamin D.

Objective To estimate the level of vitamin D in T2DM patients, to assess its relation to microalbuminuria, and to estimate the effect of vitamin D replacement on these patients.

Patients and methods A case–control study was conducted on 50 T2DM patients having microalbuminuria (group 1), 50 T2DM patients with no microalbuminuria (group 2), and 50 apparently healthy persons (group 3) (the control group), Routine tests such as complete blood count, fasting blood glucose, 2 h postprandial blood glucose, glycosylated hemoglobin', liver enzyme tests [alanine aminotransferase (ALT), aspartate aminotransferase (AST)], kidney function tests (urea, creatinine), lipid profiles [cholesterol, Triglyceride (TG)], serum albumin, calcium, phosphorus, intact parathyroid hormone (PTH), 25-hydroxycholecalciferol in the blood, and urinary albumin/creatinine ratio were done.

Results The study showed highly statistically significant decrease of vitamin D level in group 1 (diabetes with microalbuminuria) compared with group 2 (diabetes without microalbuminuria) and group 3 (control) ($P<0.001$). A statistically significant negative correlation was found between microalbuminuria and vitamin D ($r=-0.946$). It also showed a highly statistically significant decrease of microalbuminuria after treatment with vitamin D compared with before treatment .There was a highly statistically significant decrease of glycosylated hemoglobin after vitamin D replacement.

Conclusion This study demonstrated that vitamin D was significantly deficient in diabetic patients in comparison with the control group and it is significantly deficient in T2DM with microalbuminuria compared with T2DM without microalbuminuria. It showed that microalbuminuria was significantly improved after treatment with vitamin D but not to nonmicroalbminuric level. These findings indicated that there is a potential role of vitamin D in diabetic nephropathy pathogenesis.

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Introduction

Type 2 diabetes mellitus (T2DM) is a public health problem that threatens the economies of all nations, particularly the developing countries. The total number of diabetic patients are at least 285 million people worldwide and the number is expected to reach 438 million by 2030. Egypt is expected to be among the top 10 countries that has the highest prevalence rates of diabetes in the world by 2025 [1].

Diabetes comes among the 11th most important risk factors of early death in Egypt; it is the most important cause of life lost in all ages [2]. Furthermore, diabetic nephropathy (DN) is the number one diabetic complication that stands behind end-stage kidney disease [3]. DN is a common syndrome with the following characteristics:

(1) Urinary albumin/creatinine ratio (UACR) of more than 30 mg/mmol.
(2) Persistent albuminuria (>300 mg/day or >200 μg/min) on two occasions at least, of 3–6 months apart.
(3) Growing decline in the glomerular filtration rate (GFR).
(4) High arterial blood pressure.

Albuminuria is considered a predicting factor of advanced stages of chronic kidney diseases (CKDs), end-stage renal disease, cardiac/vascular disorders, and mortality [4]. Detection of proteinuria in T2DM

patients is a prominent marker of risk; as the amount of proteinuria is equivalent to the degree of risk of having lower GFR [5]. A stronger relation is found between renal protection and the degree of albuminuria reduction than that of low blood pressure; a finding which indicates that albuminuria may be a targeted preventive therapeutic agent of renal and cardiovascular disorders [6]. Vitamin D is a multieffect steroid hormone that controls a broad range of cell regulatory and metabolic functions [7]. Preserving calcium and homeostasis of phosphorus are two of the most well-known endocrinal functions of vitamin D, although, various studies during the past decades have revealed a wide spectrum of events for vitamin D that extends beyond its regulation of calcium and phosphorus metabolism [8]. Animal and cell-culture investigations have shown that vitamin D supplementation muffled transcription of rennin, reduced serum angiotensin II levels, averted loss of podocytes and glomerulosclerosis, and reduced albumin excretion in urine [9]. Human low levels of vitamin D are usually detected in patients with hypertension [10], cases of insulin resistance, metabolic syndrome, and diabetic patients, all known risk factors for the development of albuminuria [11]. On the other hand, low vitamin D levels were found to have a potential role in worsening renal injuries in patients with diabetes [12]. Many studies were conducted to know the relationship between inflammatory markers in CKD with the level of 1,25 (OH)D. They found that there is a relationship between CKD and the level of 1,25(OH)D [13]. The decrease in vitamin D metabolites is related to the increase in inflammation in CKD patients. The active metabolites of vitamin D directly influence the endothelial function and are inversely related to the degree of arterial calcification in CKD patients [14].

This study aimed to estimate the level of vitamin D in T2DM patients, to assess its relation to microalbuminuria and to study the effect of vitamin D replacement in these patients.

Patients and methods

This was a case–control study conducted during the period from October 2014 till July 2016 which included 100 T2DM patients in addition to 50 matched healthy persons serving as the control group. All these patients were selected from the nephrology outpatient clinics and Internal Medicine Department in Al-Zahraa University Hospital and Student Hospital of Cairo University. Written informed consent was obtained from all patients according to its ethical committee.

Patients included were diagnosed based on the standard diagnostic criteria for diabetic non-pregnant adults according to the American Diabetes Association [15]:

(1) A1C of at least 6.5%. The test was done in a laboratory using the National Glycolhemoglobin Standardization Program certified to the Diabetes Control and Complications Trial assay.
(2) FPG of at least 126 mg/dl (7 mmol/l) (having no caloric intake for 8 h at least).
(3) Two-hour plasma glucose of at least 200 mg/dl (11.1 mmol/l) in an OGTT. The test was done according to the guidelines of WHO, a glucose load of the 75 g anhydrous glucose dissolved in water.
(4) In cases of hyperglycemia or hyperglycemic crisis, a random plasma glucose of at least 200 mg/dl (11.1 mmol/l).
(5) In patients having no unequivocal symptomatic hyperglycemia, these criteria were assured by repeating the test.

These patients were divided into:

Group 1: included '50 patients with T2DM with microalbuminuria'.
Group 2: included '50 patients with T2DM without microalbuminuria'.
Group 3: included '50 healthy persons as controls'.

The exclusion criteria were as follows: type 1 DM, primary glomerulonephritis or secondary nephritis, pregnant women and breastfeeding, poorly controlled hypertension 'systolic blood pressure of more than 160 mmHg and/or diastolic blood pressure of more than100 mmHg', renal failure, liver disease, congestive heart failure, autoimmune diseases, primary hyperparathyroidism, malignancy, alcoholism, malabsorption, UACR of more than 30 mg/mmol, and serum albumin of less than 3.0 g/dl.

All included patients and controls were subjected to the following procedures:

Informed consent, complete personal history, complete physical examination, and laboratory investigations in which 5 ml of fasting venous blood samples was collected from each patient included in the study and divided into aliquots: aliquot (a) 2 ml of blood was added to a tube containing EDTA for complete blood count determination which was performed on coulter counter T890 (Coulter Counter, Harpenden, UK) and glycosylated hemoglobin (HbA1c)

determination by cation exchange resin. Aliquot (b) 3 ml of blood was left to clot and centrifuged at 3000*g* for 5 min and the separated serum was stored at −20°C until analysis of liver function tests: urea, calcium, and phosphorous, which were carried out on Dimension RxL Max analyzer (Siemens Healthcare GmbH – Henkestr, Erlangen, Germany) by colorimetric techniques. Serum parathyroid hormone (PTH) was assessed by sandwich ELISA kit supplied from BioVendor (Laboratorni Medicina, Karasek, Brno, Czech Republic) [16].

An early morning mid-stream urine sample was taken. Prior to use, the cloudy samples were exposed to centrifugation and the clear floating segment was stored at −20°C to be analyzed. Concentrations of albumin were assessed in urine using a Minineph microalbumin kit by nephlometry method on Minineph nephelometer (AD200) (The Binding Site, Birmingham, UK) [17]. Albumin concentrations of the samples were compared against its creatinine concentrations (according to Jaffe reaction) on a Dimension RxL Max analyzer, and the albumin/creatinine ratio was also calculated [18].

Serum vitamin D 25(OH)D was determined using the electrochemiluminescence binding assay on Cobas e411 immunoassay analyzer (Roche Diagnostics GmbH, Mannheim, Germany); vitamin D deficiency was recognized as 25(OH)D of up to 20 ng/ml. Vitamin D insufficiency was also determined as 21–29 ng/ml. The recommended preferred level for vitamin D is at least 30 ng/ml [19].

Patients with vitamin D deficiency were subjected to replacement therapy for 6 months with cholecalciferol 4000 IU daily by oral intake and during this period there was no change in their hypoglycemic treatment. After the treatment, both baseline clinical and laboratory markers were repeated to assess the relationship of vitamin D and microalbuminuria and the effect of its replacement on microalbuminuria.

Statistical analysis

Statistical program for the social sciences (SPSS) version 15.0 (SPSS Inc., 233 South Wacker Drive, Chicago, IL, USA) was used to analyze the collected data. Quantitative data were expressed in mean±SD. Qualitative data were expressed in frequency and percentage.

Probability (*P*-value):

(1) *P*-value of less than 0.05 was considered 'statistically significant.'
(2) *P*-value of less than 0.001 was considered as 'highly statistically significant.'
(3) *P*-value of more than 0.05 was considered 'statistically insignificant.'

Results

A case–control study was performed during the period from 'October 2014 to July 2016' which included 100 patients with T2DM fulfilling the inclusion and exclusion criteria and 50 apparently healthy persons as the control group.

These patients were divided into the following.

Group 1: '50 patients with T2DM with microalbuminuria.'
Group 2: '50 patients with T2DM without microalbuminuria.'
Group 3: '50 apparently healthy individuals as the control group.'

The demographic, clinical, and laboratory data of the studied population is shown in Table 1

Our results showed a highly statistically significant increase of BMI in group 1 (diabetic with microalbuminuria) compared with group 2 (diabetes without microalbuminuria) and the control group ($P<0.000$) (Table 2).

Our results have shown a highly statistically significant increase of HbA1c in group 1 (diabetic with microalbuminuria) compared with group 2 (diabetic without microalbuminuria) and group 3 (control) ($P<0.001$) (Table 3).

Our results have shown a highly statistically significant low level of vitamin D in group 1 (diabetes with microalbuminuria) compared with group 2 (diabetes without microalbuminuria) and group 3 (control) (Table 4).

Our results have shown a statistically significant negative correlation ($r=-0.946$) between vitamin D level and microalbuminuria ($P<0.001$) (Table 5 and Fig. 1).

We found that 71 patients had low vitamin D level (of the patients, 24 patients were diabetic without microalbuminuria and 47 patients were diabetic with microalbuminuria) and they were subjected to its replacement for 6 months with Cholecalciferol 4000 IU daily by oral intake; all baseline clinical and laboratory markers were repeated. There was no change in antihyperglycemic medication during this period.

Table 1 Demographic, clinical, and laboratory data of the studied population

	Group 1 diabetic with microalbuminuria	Group 2 diabetic without microalbuminuria	Group 3 controlled
Age (years) (mean±SD)	50.58±3.89	48.02±4.64	47.7±6.01
Sex (%)			
Male	68	54	56
Female	32	46	44
BMI (mean±SD)	30.4±1.2	28.7±0.08	28.7±1.2
Glycosylated hemoglobin	8±0.04	7.1±0.08	5.4±0.04
Urea (mg/dl)	29.1±5.9	25.1±5.6	22.1±5.6
ALT (μ/l)	13.72±3.91	13.3±2.56	13.64±3.47
AST (μ/l)	13.11±3.47	13.21±2.76	12.86±2.86
Serum albumin (g/dl)	4.04±0.064	4.02±0.063	4.1±0.02
Calcium (mg/dl)	9±0.08	8.8±0.2	9.2±0.04
PO_4 (mg/dl)	4.4±0.08	4.6±0.06	4.2±0
PTH (pg/ml)	35.2±8.42	27.08±4.9	23.4±4.9
ALP (μ/l)	57.4±9.9	55.2±7.9	50.6±6.58
Vitamin D (ng/ml)	20.4±4	30.7±5.3	38.1±3.7
UACR (mg/mmol)	3.3±.05	1.9±0.05	1.7±0.05

ALT, alanine aminotransferase; AST, aspartate aminotransferase; PTH, parathyroid hormone; UACR, urinary albumin/creatinine ratio.

Table 2 BMI of the studied groups

	Group 1 diabetes with albuminuria (*N*=50) (mean ±SD)	Group 2 diabetes without albuminuria (*N*=50) (mean ±SD)	Group 3 control (*N*=50) (mean ±SD)	*P* value
BMI	30.4±1.2	28.7±0.8	28.7±1.2	*P*<0.000

Table 3 Level of glycosylated hemoglobin of the studied groups before treatment

	Group 1 diabetes with albuminuria (*N*=50) (mean±SD)	Group 2 diabetes without albuminuria (*N*=50) (mean±SD)	Group 3 control (*N*=50) (mean ±SD)	*P* value
Glycosylated hemoglobin	8±0.4	7.1±0.8	5.4±0.4	*P*<0.001

Those patients were divided into two groups:

Group A: 47 patients that were diabetics with microalbuminuria.
Group B: 24 patients that were diabetics without microaluminuria.

Our results have shown a highly statistically significant increase in the level of vitamin D after its administration in group A (*P*<0.001) (Table 6).

Our results have shown a highly statistically significant increase in the level of vitamin D after its administration in group B (*P*<0.001) (Table 7).

Our results have shown a highly statistically significant decrease of HbA1c after treatment with vitamin D in group A (*P*<0.001) (Table 8).

Table 4 Vitamin D level of the studied groups before its replacement

	Group 1 diabetes with albuminuria (*N*=50) (mean ±SD)	Group 2 diabetes without albuminuria (*N*=50) (mean ±SD)	Group 3 control (*N*=50) (mean ±SD)	*P* value
Vitamin D	20.4±4	30.7±5.3	38.1±3.7	*P*<0.001

Table 5 Pearson's correlation coefficient between albuminuria and vitamin D level

	Vitamin D level	*P* value
Microalbuminuria	*r*=0.946	<0.001

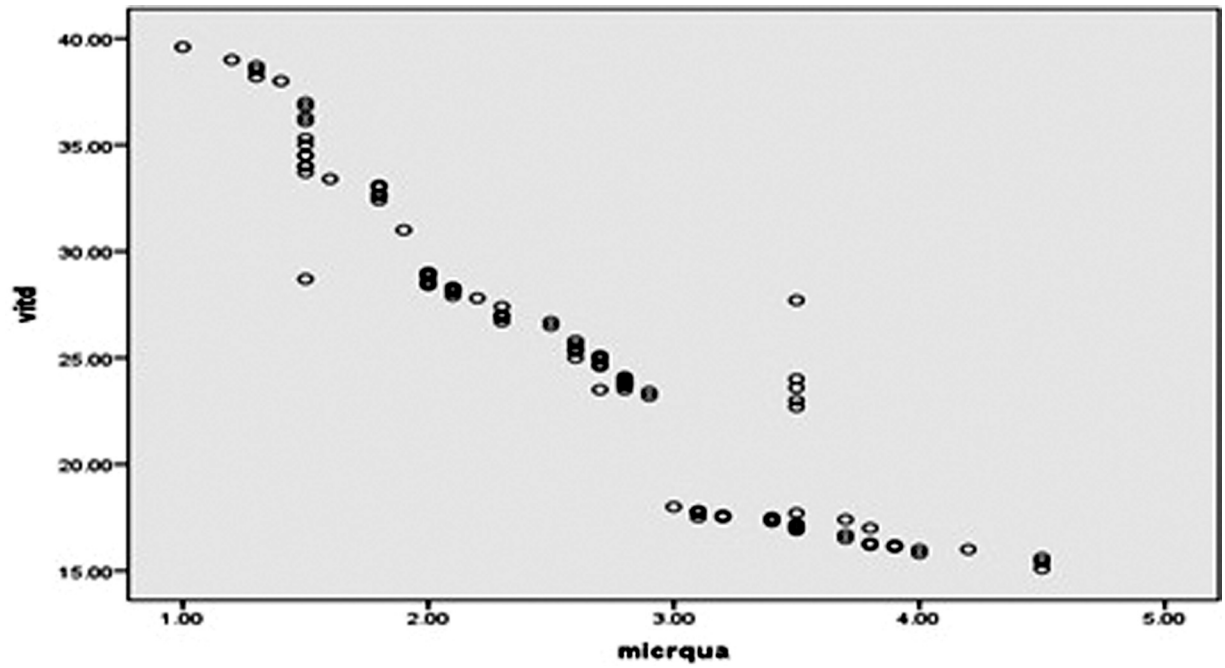
Our results have shown a highly statistically significant decrease of HbA1c after treatment with vitamin D in group B (*P*<0.001) (Table 9).

Our results have shown a highly statistically significant decrease in the level of microalbuminuria after treatment with vitamin D in group A (*P*<0.001) (Table 10).

Discussion

Vitamin D is a group of fat-soluble prohormones. Vitamin D has a fundamental role in bone metabolism and is involved in various immune-modulating and anti-inflammatory mechanisms. Approximately 30–50% of the general population are reported to have low vitamin D levels or suffer from insufficiency or deficient vitamin D which is a global health problem [20]. Many studies observed the relationships between low levels of vitamin D and

Figure 1



The correlation between vitamin D level and microalbuminuria.

Table 6 Vitamin D level before and after its administration in group A

	Before treatment (*N*=47) (mean±SD)	After treatment (*N*=47) (mean±SD)	*P* value
Vitamin D	20.1±3.9	22.65±4	<0.0001

Table 7 Vitamin D level before and after its administration in group B

	Before treatment (*N*=24) (mean±SD)	After treatment (*N*=24) (mean±SD)	*P* value
Vitamin D	27.96±0.82	31.1±1.3	<0.0001

Table 8 Level of glycosylated hemoglobin before and after treatment with vitamin D in group A

	Before treatment (*N*=47) (mean ±SD)	After treatment (*N*=47) (mean ±SD)	*P* value
Glycosylated hemoglobin	7.9±0.5	7.2±0.37	<0.0001

Table 9 Level of glycosylated hemoglobin before and after treatment with vitamin D in group B

	Before treatment (*N*=24) (mean ±SD)	After treatment (*N*=24) (mean ±SD)	*P* value
Glycosylated hemoglobin	7.47±0.4	7.0±0.30	<0.0001

Table 10 Level of microalbuminuria before and after treatment with vitamin D in group A

	Before treatment (*N*=47) (mean ±SD)	After treatment (*N*=47) (mean ±SD)	*P* value
Microalbuminurea	3.2±0.58	2.4±0.35	<0.0001

many disease states such as 'hypertension, cancer, cardiovascular disease, and chronic CKD' [21]. Although the relationship between vitamin D and insulin secretion, insulin resistance, and β-cell dysfunction are pointed out in diabetes mellitus patients, evidence regarding vitamin D levels and DM is contradictory, and well-controlled studies are needed [13]. Various studies have reported the given effects of vitamin D on the 'renin–angiotensin complex, inflammation, and mineral bone disease,' which may be correlated with the etiology and prognosis of CKD. Progressive evidences denote vitamin D deficiency as a risk factor for DM and CKD; however, whether vitamin D deficiency also predisposes to death from DM and CKD remains to be confirmed.

Vitamin D is found in two major compounds, VD3 (cholecalciferol) and D2 (Ergocalciferol), depending on their sub-chain structure. However, 1,25(OH)2 D3 is the active form of vitamin D, and so it should not be used in the evaluation of serum levels, as it has a short half-life [22].

T2DM represents a global pandemic disorder with estimates of nearly 382 000 000 diabetic persons in

2013 and an expected rise to 592 000 000 by 2035 with a parallel expected increase in its complications, one of which is nephropathy [23]. DN is considered one of the most common microvascular complications of DM detected in ~44% of end-stage renal disease patients who are subjected to hemodialysis.

This study aimed to estimate the level of vitamin D in T2DM patients, to assess its relation to microalbuminuria and to investigate the effect of its administration on these patients.

One hundred patients with T2DM participated in the current study, who were divided into two equals groups. The first group included 50 patients with T2DM without microalbuminuria and the second group included 50 patients with T2DM with microalbuminuria, in addition to 50 apparently healthy individuals who served as the control group.

This study showed a highly statistically significant increase of BMI in group 1 (diabetic with microalbuminuria) compared with group 2 (diabetic without microalbuminuria) and the control group ($P<0.001$).

In accordance to this study, a study by Roett *et al.* [24] showed a strong and independent association between BMI and a potential risk of being diagnosed as T2DM patient. Such association between BMI category and the risk of T2DM is stronger in individuals with a higher BMI compared with other people with a lower BMI.

The current study showed a highly statistically significant increase in HbA1c in DM patients with microalbuminuria when compared with DM patients with no microalbuminuria.

Congruent with the current study, Sheikh *et al.* [25] reported a prompt incipience of microalbuminuria in their study population, a finding which could be because of the poor glycemic control (HbA1c>7%) or genetic factors. Microalbuminuria and HbAlc tests should be done in both new and confirmed diagnoses of T2MD patients as a good early marker of kidney dysfunction and bad glycemic control.

Similar to the current study, a study in Pakistan by Anwarulla *et al.* [26] t showed a statistically significant correlation between the prevalence of microalbuminuria and HbA1c levels

This study showed a highly statistically significant low level of vitamin D in diabetic patients with microalbuminuria when compared with diabetic patients without microalbuminuria and control group.

In agreement with the current study, the largest cross-sectional study to date from National Health and Nutrition Examination Survey data by Scragg *et al.* [27] serum 25(OH)D concentration was inversely associated with diabetes.

Vitamin D receptors exist in pancreatic beta cells which regulate insulin secretion. Consequently, vitamin D deficiency may have a potential role in impaired insulin secretion in T2DM patients. In addition, the stimulation between vitamin D and the expression of the insulin receptor may explain that its deficiency may have a relation with insulin resistance [28].

This study showed a strong negative correlation between vitamin D level and microalbuminurea.

In agreement with the current study, the study done by De Boer *et al.* [29] showed a relationship between the increase in albuminuria and decrease in plasma 25 (OH)D.

The direct cellular effects of vitamin D deficiency, including podocyte loss and increasing glomerulosclerosis, may be contributing to the increasing risk of albuminuria [30]. It has been demonstrated that the major factors involved in the development of glomerulosclerosis and interstitial fibrosis of DN (e.g. TGF-β and angiotensin II) could negatively regulate the receptor-mediated endocytosis participating in enhanced vitamin D binding protein excretion [31].

Regarding 24 patients who were diabetic without microalbuminuria with a low level of vitamin D, chronic hyperglycemia even without overt albuminuria may be potentially involved in the low rate of vitamin D3 hydroxylation in the kidney, which favors a decrease in the synthesis of this vitamin [32].

We found that three patients who were diabetic with microalbuminuria and normal vitamin D level were all men with normal BMI. There are a lot of factors that impact the vitamin D status as the synthesis in the skin and absorption of vitamin D, such as sunlight exposure and sea food intake that may have effects on vitamin D status in such categories of patients. This denotes that microalbuminuria may be related to other factors rather than vitamin D deficiency.

This study showed a highly statistically significant decrease of microalbuminurea after administration with vitamin D ($P<0.001$). In line with the present study, a previous study by De Zeeuw *et al.* [33] has shown that paricalcitol diminished residual albuminuria in patients with DN. And Momeni *et al.* [12] study showed that the administration of vitamin D in T2DM patients with vitamin D deficiency or insufficiency leads to normalization of serum vitamin D level and decreased proteinuria.Thus, we concluded that correction of vitamin D deficiency may be an effective and safe modality of the treatment for DN.

In contrast to the current study, a study done by Ahmadi *et al.* [34] has shown that the level of 25 (OH)D in diabetic patients receiving vitamin D is significantly increased, but there was no significant decrease in proteinuria or a change in GFR after 3 months of treatment.

Also, meta-analysis by Derakhshanian *et al.* [35] showed the higher risk for nephropathy in vitamin D-deficient patients with diabetes. Pooling the results of available clinical trials after vitamin D supplementation did not support the causality in this association.

The current study showed highly statistically significant decrease of HbA1c after treatment with vitamin D.

In agreement with this study, a study by Bonakdaran *et al.* [36] showed a significant reduction in HbA1c in diabetic patients after administration with vitamin D.

Contrary to the current study, a study by Momeni *et al.* [12] did not show any improvement of glycemic control indices in patients receiving vitamin D

Conclusion

Our study showed that T2DM patients had a highly significant decrease of vitamin D level when compared with healthy individuals and when compared with the microalbuminuric T2DM patients' group with the nonmicroalbuminuric T2DM patients' group . Also, there are negative correlations between vitamin D level and HbA1c, albuminureia and BMI. These findings indicate that the vitamin D deficiency may be a risk factor T2DM or may be involved in the pathogenesis of DN which will pave the way for novel prophylactic or therapeutic interventions since DN is a significant progress to CKD contributor to mortality in T2DM patients. Finally, we recommend repeating this study on healthy individuals who have a family history of T2DM and follow up of vitamin D level and incidence of T2DM aiming to know if vitamin D replacement decreases the incidence of T2DM. We recommend also repeating this study on a large scale to detect the relation of vitamin D and microalbuminuria which remains a controversy.

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Conflicts of interest

There are no conflicts of interest.

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