ORIGINAL ARTICLE

Vitamin D deficiency increases risk of nephropathy and cardiovascular diseases in Type 2 diabetes mellitus patients

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Background: Vitamin D (VD) deficiency is associated with insulin function and secretion. It is linked with diabetes mellitus (DM) progression, and complications were also recorded. Therefore, the current study aimed to investigate serum VD level in Type 2 DM (T2DM) patients and its association with diabetic nephropathy and cardiovascular diseases (CVD). **Materials and Methods:** In this cross-sectional study, 205 patients with Type 2 diabetes age ranged from 39 to 75 years old were enrolled. Serum VD, high-sensitivity C-reactive protein (hs-CRP), and hemoglobin A1c (HbA1c) were measured. In addition, urinary albumin:creatinine ratio (ACR) was estimated. **Results:** Patients with Type 2 diabetes had a 78.5% VD level <30 ng/m. ACR and hs-CRP levels were significantly increased in patients with diabetes with VD <30 ng/m (P = 0.011 and P = 0.008, respectively). Female had significantly lower VD level than male P < 0.001. Patients exposed to sunlight had significantly higher VD level and lower hs-CRP levels compared with less-exposed, P value (0.001 and <0.001), respectively. Exercise significantly increased VD and decreased ACR levels in DM patients, P value (0.046 and 0.002), respectively. VD was positively associated with age (r = 0.355 P = 0.040) and negatively correlate with BMI (r = −0.502 P = 0.009), duration of disease (r = −0.498 P = 0.003), ACR (r = −0.384 P = 0.015), and HbA1c (r = −0.327 P = 0.032). **Conclusion:** The evidence from this study suggest that patients with Type 2 diabetes with VD deficiency are at higher risk for developing CVD and nephropathy.

Key words: Albumin:creatinine ratio, cardiovascular diseases, high-sensitivity C-reactive protein, nephropathy, Sudan, type 2 diabetes mellitus, Vitamin D

How to cite this article: Aljack HA, Abdalla MK, Idris OF, Ismail AM. Vitamin D deficiency increases risk of nephropathy and cardiovascular diseases in Type 2 diabetes mellitus patients. J Res Med Sci 2019;24:47.

INTRODUCTION

Vitamin D (VD) is being recognized recently as antiproliferative, stimulating cell differentiation, immunomodulatory, and anti-inflammation activity.[1] Therefore, it has a role in human health including cancer, infectious, respiratory, autoimmune, and cardiovascular diseases (CVDs).[2] Recently, VD has been linked with the development of Type 2 diabetes mellitus (T2DM), through its direct effect on pancreatic β-cell function, insulin secretion, and action.[3]

VD deficiency is highly prevalent worldwide,[4-8] about 1 billion in the world[9] and up to 50% of the adult population in developing countries lack VD.[10] In addition, the prevalence rate of hypovitaminosis D in the United States adults was 41.6%.[4] Many factors increase the deficiency of VD including less sunlight exposure, darkness skin, winter, elderly, use of clothes covering most of the body, female gender, and obesity.[11,12] Several previous studies proved that VD deficiency is highly prevalent in Type 1 and T2DM.[5] Few studies in Sudan reported an association between T2DM and VD deficiency. Furthermore, VD deficient is common in T2DM female than male.[13,14]

Access this article online

Quick Response Code:

Website: www.jmsjournal.net

DOI: 10.4103/jrms.JRMS_303_18

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Received: 26-05-2018; **Revised:** 13-11-2018; **Accepted:** 17-02-2019

VD prevents the endothelial damage of the kidney which leads to microalbuminuria (MAU), by its role in negative regulation of renin–angiotensin–aldosterone system.[15] One study suggested that patients with Type 2 diabetes and chronic renal disease have an exceptionally high rate of severe VD deficiency.[16] A number of observations have reported a correlation between MAU and VD deficiency in T2DM,[15] and the prevalence of albuminuria was enhanced with decreased levels of VD.[17] Moreover, VD deficiency independently associated with prevalent of CVD in patients with Type 2 diabetes with mild kidney dysfunction.[18]

Early detection of nephropathy and CVD risks provides the way for early intervention to reduce T2DM complications. Therefore, the present study aimed to investigate whether VD has diagnostic and predictive risk factors for diabetic nephropathy and CVD in T2DM patients.

MATERIALS AND METHODS

This is a cross-sectional study conducted on randomly selected patients with Type 2 diabetes attending Military Hospital in Khartoum State (hospital for referring to it from all States of Sudan). After informed consent, blood samples were collected from 205 clinically diagnosed patients and consist of 94 males and 111 females. Patients with diabetes with known inflammatory, cardiovascular, liver, renal diseases, and/or with VD supplement were excluded from the study. The diagnoses were based on clinical history records. Before the demographic determination and classification instructed questionnaire was used, we asked the patients about age, sex, education level, lifestyle (based on the economic status), sun exposure, and family history. Moreover, glycemic control, BMI, and VD status were classified based on the reference range of each. Hemoglobin A1c (HbA1c) ≤8% considered as controlled and >8% as uncontrolled. Whereas, BMI 18.5–25 kg/m^2 defined as normal weight, >25–30 kg/m^2 overweight, and >30 kg/m^2 obese. Meanwhile, VD level ≤30 ng/ml considered as deficient and >30 ng/ml as sufficient.

Ethical consideration

The study was approval by the Local Ethical Committee of Al-Neelain University (issued on May 5, 2015) and Military Hospital Managers and carried out in accordance to International Ethical Guideline. Written informed consent was obtained from all participants.

Measurement of body mass index

Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared (kg/m^2).

Measurement of Vitamin D

Serum VD level was measured using the competitive inhibition enzyme-linked immunosorbent assay or ELISA (EUROIMMUN AG, Germany). Brief according to manufacture's protocol, 200 µl sample was added into biotin-coated monoclonal anti-VD antibodies, followed by competitive binding of 100 µl VD-labeled. Unbound VD were washed. Streptavidin-peroxidase (100 µl) was added to detect bound biotin labeled 25-OH VD, and then, peroxidase substrate tetramethylbenzidine (100 µl) was added to promote a color reaction. The color intensity was inversely proportional to the 25-OH VD concentration in the sample. Samples results were calculated using standard curve. The detection limit is 1.6 ng/mL (sensitivity) and specifically detects 25-VD and VD_3.

Measurement of high-sensitivity C-reactive protein

Serum levels of high-sensitivity C-reactive protein (hs-CRP) in patients were measured using the particle-enhanced immunoturbidimetric assay method Cobas C-311®. Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies, and then, the precipitate was determined turbidimetrically.

Measurement of albumin:creatinine ratio

According to the manufacturer, urine albumin and creatinine were analyzed by Cobas C-311® fully automated analyzer. Anti-albumin antibodies react with antigen in the sample to form antigen-antibody complexes, following agglutination, and then, complexes were measured turbidimetrically. Moreover, creatinine reacts with picrate in alkaline solution to form a yellow-red adduct. The rate of the dye formation is directly proportional to the creatinine concentration in the specimen and is measured photometrically.

Measurement of hemoglobin A1c

The analysis of HbA1c was done in fully automated closed system – Roche Cobas C-311®. Total hemoglobin and HbA1c concentrations are determined after hemolysis of the anticoagulated whole blood specimen. The total hemoglobin was measured using spectrophotometer. HbA1c was determined by immune turbidimetrically. The ratio of both concentrations was yield the final percentage of HbA1c results. The anticoagulated whole blood specimen was hemolyzed with HbA1c hemolysis reagent. The released Hb was proteolytically degraded by pepsin, to make the β-N-terminal structures more accessible for the immunoassay. In addition, the heme portions are oxidized for the Hb assay. Total Hb was determined in the hemolysate using a cyanide-free colorimetric method based on the formation of a brownish-green chromophore (alkaline hematin D-575) in alkaline detergent solution. The color intensity was proportional to the Hb concentration. HbA1c is measured using monoclonal antibodies attached to latex particles. The antibodies bind the β-N-terminal fragments of HbA1c. Remaining free antibodies were agglutinated with a synthetic polymer carrying multiple copies of the

β-N-terminal structure of HbA1c. The change in turbidity was inversely related to the amount of bound glycopeptides and is measured turbidimetrically at 550 nm. The test sensitivity is 3%. Acetylated Hb, carbamylated Hb, and labile HbA1c do not affect the assay result. Glycated HbF is not detected.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, USA) was used for the data analysis. Data are presented as frequencies, percentage, and means ± standard deviation. The Student's *t*-test was used to compare mean levels of study parameters between groups. Categorical variables were compared using Chi-square and multiple regression tests. Pearson's correlation coefficient test was employed to evaluate the relationship between continuous variables. $P \leq 0.05$ was used as the statistical significance.

RESULTS

Demographic and baseline characteristics of study population

Patients demographic analyses showed that, in total, 205 patients were recruited, 111 (54%) were female and 94 (54%) were male. Of 205 patients with Type 2 diabetes, 161 (78.5%) had VD deficient and 44 (21.5%) had VD sufficient. High frequency of BMI status was reported in overweight 94 (46%) followed by obese 64 (31%) and normal weight 47 (23%). Of all patients, 141 (68.8%) were diabetic uncontrolled. Whereas 176 (87.1%) were sun exposure <5 hr. Likewise, patients had not physical exercise 101 (52.1%). Demographic and baseline characteristics are summarized in Table 1.

Nonparametric association of study variables in groups classified according to Vitamin D status

The results of the study revealed that gender, BMI, and sun exposure were significantly associated with VD deficient ($P < 0.001$, $P = 0.004$, and $P = 0.006$, respectively). Furthermore, they increased the risk of VD deficient (gender – odd ratio [OR]: 3.89 with confidence interval [CI]: [1.89–7.98]; BMI – OR: 5.97 with CI: [1.79–19.79]; and sun exposure – OR: 3.5 with CI: [1.45–8.39]). The results of the association were shown in Table 2.

Comparison of study parameters in groups classified based on Vitamin D status, gender, sun exposure, physical exercise, and glycemic control

The mean values of hs-CRP and albumin: creatinine ratio (ACR) levels were significantly higher in patients with diabetes with VD deficient than in those with VD sufficient ($P = 0.008$ and $P = 0.011$, respectively). However, the mean value of HbA1c level revealed insignificant difference.

Table 1: Demographic and baseline characteristics of Type 2 diabetes mellitus patients

Characteristic	Frequency (%)
Gender	
Male	94 (46)
Female	111 (54)
Age (years)	
<55	96 (47)
>55	109 (53)
BMI	
Normal weight	47 (23)
Overweight	94 (46)
Obese	64 (31)
Diabetic control	
Controlled	64 (31.2)
Uncontrolled	141 (68.8)
Education status	
Low	36 (18)
Moderate	132 (65)
High	35 (17)
Lifestyle	
Low	69 (35.0)
Moderate	113 (57.40)
Good	15 (7.6)
Physical exercise	
Yes	93 (47.9)
No	101 (52.1)
Sun exposure	
<5 h	176 (87.1)
>5 h	26 (12.9)
Family history of DM	
First degree	108 (36.5)
Second degree	137 (46.3)
No	51 (17.2)
Family history of CVD	
Yes	26 (14.1)
No	159 (85.9)
Cholesterol-lowering agent	
Yes	84 (47.2)
No	94 (52.8)
VD status	
VD deficient	161 (78.5)
VD sufficient	44 (21.5)

The data reported as frequency and percentage. BMI=Body mass index; VD=Vitamin D, CVD=Cardiovascular diseases; DM=Diabetes mellitus

The mean level of VD (20.0 ± 8.90 ng/ml) was significantly reduced in females compared to that in males (30.2 ± 12.2 ng/ml) with $P = 0.000$, whereas hs-CRP (5.91 ± 2.61 mg/l) was considerably elevated (3.54 ± 2.55 mg/l) with $P = 0.005$.

The mean VD level (23.6 ± 11.2 ng/ml) was significantly lower in group exposed to sun <5 h compared to sun exposed >5 h (32.8 ± 12.3 ng/ml) with $P = 0.001$; in contrast, hs-CRP was significantly elevated (5.15 ± 6.48 mg/l) than (2.81 ± 1.91 mg/l) with $P = 0.000$.

Patients on physical exercise significantly had higher VD and lower ACR levels, with $P = 0.046$ and $P = 0.002$, respectively. On the other hand, uncontrolled patients with diabetes had significantly higher ACR with $P = 0.019$; the results were presented in Table 3.

Table 2: Nonparametric association of study variables in groups classified according to Vitamin D status

Parameter	Frequency (%)		OR (CI)	*P*
	VD deficient	VD sufficient		
Gender				
Male	62 (38.70)	32 (71.10)	3.89 (1.89-7.98)	<0.001
Female	98 (61.30)	13 (28.90)		
Age (years)				
<55	79 (49.01)	15 (34.09)	1.86 (0.92-3.73)	0.054
>55	82 (50.99)	29 (65.91)		
BMI				
Normal weight	30 (19.00)	19 (40.40)		
Overweight	72 (45.50)	20 (42.60)	5.97 (1.79-19.79)	0.004
Obese	56 (40.50)	8 (17.00)	2.24 (0.76-6.59)	0.143
Diabetic control				
Controlled	50 (31.00)	16 (36.36)	0.78 (0.39-1.58)	0.310
Uncontrolled	111 (69.00)	28 (63.64)		
Physical exercise				
Yes	71 (44.65%)	26 (56.52%)	0.62 (0.32-1.20)	0.105
No	88 (55.35%)	20 (43.48%)		
Sun exposure (h/day)				
<5	147 (91.30)	33 (75.00)	3.5 (1.45-8.39)	0.006
>5	14 (8.70)	11 (25.00)		

Categorical variables reported as frequencies and percentage. Chi-square and multiple regression tests have been done to compare between variables. BMI=Body mass index; OR=Odd ratio; CI=Confidence interval, VD=Vitamin D

Correlation between Vitamin D level and variables indicative cardiovascular diseases, nephropathy, and glycemic control

The relationship between VD and other studied variables and parameters was determined. A significant positive correlation was found between VD and age ($r = 0.355$; $P = 0.040$). In addition, VD was negatively correlate with BMI ($r = -0.502$; $P = 0.009$), duration of disease ($r = -0.498$; $P = 0.003$), ACR ($r = -0.384$; $P = 0.015$), and HbA1c ($r = -0.327$; $P = 0.032$). On the other hand, no correlations were observed between VD and other variables. The results were presented in Figure 1.

DISCUSSION

Latest studies in Sudan revealed a higher prevalence of VD deficiency among T2DM patients, which attributed to nutritional, skin color, sun exposure times, and uses of sun blockers. This finding signifies the need to determine a possible relationship between VD, ACR, hs-CRP levels, and study variables. Therefore, monitoring of VD was valuable for the diagnosis, supplementation regimen if necessary and thus prevention of T2DM complication.

The results of characteristics demonstrated that T2DM is more common in females, overweight, and most patients

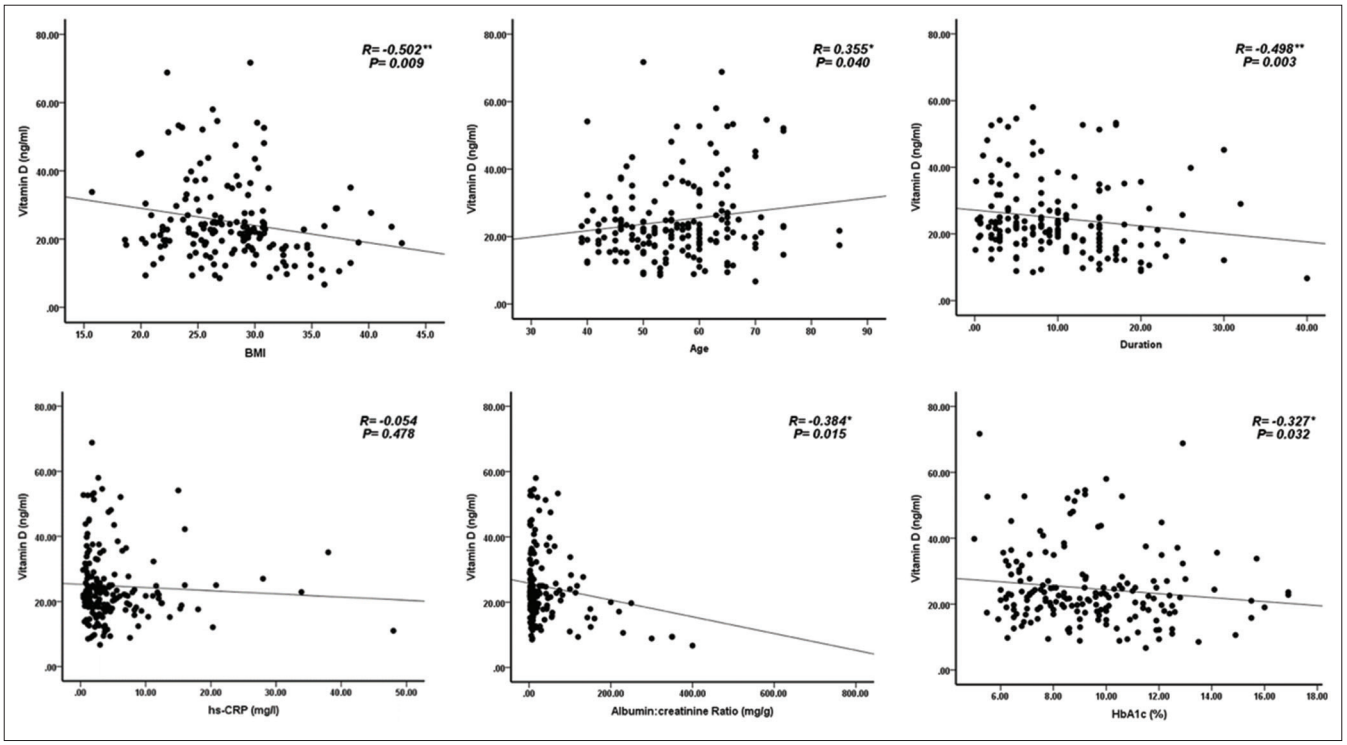

Figure 1: Pearson's correlation coefficient test has been used to correlate between Vitamin D and variables indicative cardiovascular diseases, nephropathy, and glycemic control. (r)=Pearson correlation; (−)=Negative correlation; BMI = Body Mass Index; hs-CRP = high-sensitivity C-reactive protein; HbA1c = Glycated hemoglobin

Table 3: Study parameters in patients group classified according to Vitamin D status, gender, sun exposure, physical exercise, and glycemic control

Study parameters in case group classified according to VD status			
Parameter	**VD deficient (mean±SD)**	**VD sufficient (mean±SD)**	***P***
HbA1c (%)	9.46±2.43	8.82±2.63	0.159
hs-CRP (mg/L)	4.24±4.11	2.72±2.61	0.008
Albumin:creatinine ratio (mg/g)	47.8±117	19.9±22.7	0.011
Patients classified according to gender			
Parameter	**Male (mean±SD)**	**Female (mean±SD)**	***P***
Vitamin D (ng/ml)	30.2±12.2	20.0±8.90	<0.001
hs-CRP (mg/L)	3.54±2.55	5.91±2.61	0.005
Albumin:creatinine ratio (mg/g)	30.6±8.60	42.9±7.21	0.271
Patients classified according to sun exposure			
Parameter	**Sun exposure <5 h/day (mean±SD)**	**Sun exposure > 5 h/day (mean±SD**	***P***
Vitamin D (ng/ml)	23.6±11.2	32.8±12.3	0.001
hs-CRP (mg/L)	5.15±6.48	2.81±1.91	<0.001
Albumin:creatinine ratio (mg/g)	38.6±6.33	24.0±5.79	0.092
Patients classified according to physical exercise			
Parameter	**Patients with physical exercise (mean±SD)**	**Patients without physical exercise (mean±SD)**	***P***
Vitamin D (ng/ml)	27.0±12.4	23.3±11.0	0.046
hs-CRP (mg/L)	4.29±5.35	5.20±6.91	0.309
Albumin:creatinine ratio (mg/g)	21.1±41.5	48.6±77.4	0.002
Patients classified according to glycemic control			
Parameter	**Controlled DM (mean±SD)**	**Uncontrolled DM (mean±SD)**	***P***
Vitamin D (ng/ml)	25.7±11.4	24.3±11.9	0.462
hs-CRP (mg/L)	4.06±2.63	5.12±2.65	0.267
Albumin:creatinine ratio (mg/g)	20.6±6.59	43.8±7.21	0.019

Two tails Student's *t*-test has been employed to compare between variables. The results expressed as mean±SD, and *P*<0.05 was statistically considered significant. VD=Vitamin D; HbA1c=Glycated hemoglobin; hs-CRP=High-sensitivity C-reactive protein; SD=Standard deviation; DM=Diabetes mellitus

were poorly glycemic control, physically inactive, and less sun exposure. Meanwhile, the current study reported a high prevalence of VD deficiency (78.5%) in our population. These findings have been documented in many previous studies that obese and females are more vulnerable to VD deficiency; these findings were reported in nondiabetic and patients with diabetes.[19,20] Moreover, supported with another study that female gender is an independent predictor of VD deficiency.[21] Therefore, speculated to many factors could lead to VD deficiency, such as nutritional, physical activity, and spend more indoor times despite dark skin color. Furthermore, supporting with previous observations,[22,23] the results of the current study demonstrated that, females had increased hs-CRP than males.

In addition, exposure to sunlight significantly increased VD and decreased hs-CRP levels. Likewise, physical exercise increases the VD level. Our study confirmed by previous observations, suggesting that low level of sun exposure and physical inactivity affected VD status.[24] Furthermore, physical exercise may increase serum level of VD by increasing skin exposure to sunlight,[24] increasing lipolysis, and enhancing mobilization of deposited VD from the fat compartments.[25]

Concurrent with previous findings, the present study demonstrated that patients with diabetes with VD deficient had higher levels of hs-CRP than those with VD sufficient. The previous study has shown that VD deficiency may contribute to systemic inflammation,[26] due to its role to inhibit production of inflammatory markers such as interferon-gamma, interleukin 2 (IL-2), and IL-5 by Th-1 lymphocytes, and it also inhibits synthesis of IL-6 by monocytes, which is the primary stimulant of hs-CRP production.[10] Moreover, VD deficiency associated with inflammation-linked vascular endothelial dysfunction that leads to macrovascular and microvascular complications.[1]

The study provides evidence that mean ACR was significantly higher in diabetic with VD deficient than those with VD sufficient. Moreover, the negative association between ACR and VD was demonstrated. These findings incompatible with previous report that patients with diabetes had increased level of ACR; on the other hand, VD supplementation diminished ACR.[9] Since ACR considered as an early predictor marker for nephropathy, and VD has a role in proteinuria homeostasis.[27] Therefore, suggesting that, VD deficiency associated with nephropathy in T2DM patients. Furthermore, these findings have been reinforced by the negative association between VD level and duration of DM. In addition to that, physical exercise significantly decreased ACR level. Previous reports revealed similar finding that physical activity is associated with lower

albumin excretion in the diabetic population despite unknown mechanism of physical exercise on ACR; it may be due to its effects on the vascular endothelium that mediated by nitric oxide which acts as a vasorelaxant.[28]

The present study focused to compare mean VD level of uncontrolled diabetic patients with controlled. In spite of an insignificant difference was found, yet, it agreed with previous a study that demonstrated insignificant differences in mean HbA1c between groups of VD status.[26] Likewise, Pearson's regression analyses revealed that VD level negatively correlated with HbA1c. Since the role of VD in pancreatic β-cell functions, which acts to enhance insulin secretion and conversion from proinsulin to insulin, VD might facilitate insulin action by simulating the insulin receptors expression and regulation of the calcium pool.[29] Therefore, VD must be considered in blood glucose homeostasis and thus diabetic complications.[30]

In fact, that VD level was negatively related with BMI,[31] duration of disease,[32] and glycemic control.[2,32] In other studies, the significant association between hypovitaminosis D and albuminuria was reported, and the possible effect of low VD level on nephropathy progression can be determined.[33] In addition, drug trial study proved that the administration of VD in patients with Type 2 diabetes with hypovitaminosis D leads to normalization of serum VD level and decrease proteinuria.[34]

The limitations of this study the number of sample size. Other limitations of this study were measuring some laboratory parameters related to VD regulation such as parathyroid hormone, phosphorus, and calcium levels. The major limitation of this study was that patients selected depending on their previous clinical data, no preinvestigations have done to overcome drawback confounding factors.

CONCLUSION

The data of the present study suggest that the frequency of VD deficiency is higher in our population. Type 2 diabetic with VD <30 ng/ml had higher levels of ACR and hs-CRP. Moreover, VD level was inversely associated with BMI, duration of disease, ACR, and HbA1c. Therefore, VD deficiency might be a risk factor for developing CVD and nephropathy in patients with Type 2 diabetes. Therefore, monitoring and VD supplementation regimens are recommended.

Acknowledgment

The authors gratefully acknowledge Dr. Nazik Mahmoud (Specialist of Diabetes and Endocrinology) at diabetic center of military hospital for her valuable assistance in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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