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ORIGINAL ARTICLE

Vitamin D and Hypoxia-Inducible Factor (HIF-1α) Serum Levels as Markers for Progression of Nephropathy in Type 2 Diabetic Patients

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SUMMARY

Background: **Vitamin D is a locally acting hormone, which plays a major role in skeletal health. Previous studies reported an important role of vitamin D in modulation of inflammatory response. We aimed to investigate the role of vitamin D deficiency and hypoxia-inducible factor (HIF-1α) as markers for the progression of diabetic nephropathy in Saudi patients with type 2 diabetes mellitus (T2DM).**
Methods: **We included 174 Saudi patients with T2DM in addition to 60 healthy control subjects. Patients were classified according to urinary Albumin to Creatinine Ratio (ACR) into three groups: Group AI: ACR < 30 µg/mg, Group AII: ACR levels of 30 - 300 µg/mg and Group AIII: ACR > 300 µg/mg. We estimated fasting blood glucose, HbA1c, lipid profile, serum creatinine, hemoglobin concentration (Hb), estimated glomerular filtration rate (eGFR), urine albumin/creatinine ratio, serum 25 hydroxyvitamin D, calcium, parathyroid hormone (PTH), tumor necrosis factor (TNF-α), C- reactive protein (CRP), and hypoxia-inducible factor (HIF-1α).**
Results: **There was a significant difference among studied groups regarding serum levels of vitamin D, calcium, PTH, TNF-α, CRP, and HIF-1α levels. The level of vitamin D was lower in diabetic patients in comparison to the controls and was significantly related to the severity of renal nephropathy as indicated by the level of albumin in urine. Moreover, vitamin D levels showed significant negative correlation with the inflammatory markers: TNF-α, CRP, and HIF-1α levels.**
Conclusions: **Vitamin D deficiency and elevated HIF-1α serum levels showed a significant correlation to progression of nephropathy in Saudi patients with T2DM.**
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INTRODUCTION

Diabetes Mellitus (DM) is an increasing global health challenge [1]. Type 2 DM (T2DM) is the leading cause of end stage chronic kidney disease (CKD) as 30% to 40% of diabetic patients develop to nephropathy [2]. Diabetic nephropathy is specified by hypertrophic changes in glomerular and tubular epithelium in addition to increased thickening of basement membranes

which subsequently lead to glomerular sclerosis and interstitial tubular fibrosis. Risk factors of developing nephropathy in diabetic patients include: candidate gene polymorphisms, uncontrolled diabetes, and patients with obesity and hypertension [3]. Diagnosis of nephropathy in diabetic patients depends mainly on detection of albumin in urine, decreased glomerular filtration rate (GFR), and deterioration in kidney functions tests [4,5]. The pathophysiological changes of diabetic nephropathy are most likely related to the metabolic and hemodynamic abnormalities. Recently, many studies have proposed an important role of chronic inflammation in the initiation and progression of nephropathy in diabetic patients. However, the exact mechanisms and markers implicated in the pathogenesis of diabetic nephropathy are complex and involve multiple pathways [6,7]. Pathological markers predicting early nephropathy needs more exploration. Early detection and rapid intervention in cases with diabetic nephropathy may slow the rate of disease progression [8].

In humans, vitamin D obtained from diet or that is released from the skin under the effect of ultraviolet rays is first converted in the liver to 25-hydroxyvitamin D, which is the predominant circulating metabolite. 25-hydroxyvitamin D is commonly used as an indicator of vitamin D status in the body. Subsequently, 25-hydroxyvitamin D is further hydroxylated in the kidney by 1α-hydroxylase enzyme to calcitriol, which is the biologically active form of vitamin D.

Due to aging, dietary changes, reduced outdoor activities, low sun exposure, and other causes, vitamin D deficiency or insufficiency is quite common worldwide. Vitamin D and its active form calcitriol are well known steroids involved in calcium homeostasis. Recent studies suggest that vitamin D may play an important role in different physiological pathways such as modulation of inflammatory processes [9].

Vitamin D has ameliorative effects against inflammatory processes by anti-prostaglandins and inhibition of p38 stress kinase signaling. It can inhibit the progression of diabetic nephropathy by inhibiting the activation of some signaling pathways, such as nuclear factor κB (NF-κB) signaling, with the consequent production of pro-inflammatory cytokines [10,11]. Vitamin D can inhibit the renin-angiotensin system (RAS) through down-regulation of renin expression and, hence, can play a protective role in diabetic nephropathy [12,13]. Moreover, adding vitamin D to the protocol of treatment was reported to improve kidney functions in patients with chronic kidney disease [14].

HIF-1α is a protein complex which plays a crucial role in body's response to decreased oxygen levels. Recent studies support the implication of HIF-1α as an important inflammatory cytokine in renal sclerosis associated with diabetes mellitus [15]. Additionally, inflammatory markers such as TNF-α and CRP have nowadays become widely available in the setting of the clinical practice. These inflammatory markers can be used as markers of early diabetic nephropathy as has been shown in recent studies [16].

The current study was conducted to elicit the relationship of vitamin D and inflammatory and hypoxic biomarkers with aggressiveness of nephropathy in type 2 diabetic Saudi patients.

MATERIALS AND METHODS

The current case-control study comprised 174 patients suffering from type 2 diabetes mellitus for more than 5 years. Patients were recruited from Taif region Saudi Arabia, during the period from June 2020 to October 2020. Patients were diagnosed with type 2 diabetes according to the American Diabetes Association 2010 [17]. The study was carried out at the College of Applied Medical Sciences, Taif University.

The study included 103 males and 71 females suffering from type 2 diabetes, with ages ranging from 30 to 60 years. A control group of healthy, age- and gender-matched individuals was included. The control group comprised 35 males and 25 females; all control individuals were subjected to clinical examination and evaluation for microalbuminuria by commercially available strip test to exclude microalbuminuria. The anthropometric measures and medical histories were evaluated and recorded.

Patients were excluded from the study if suffering from hypertension, or other renal, hepatic, cardiac, neoplastic, rheumatic, infectious diseases, or endocrine diseases other than diabetes. Patients who use medication that could affect the metabolism of glucose other than diabetes treatment such as corticosteroids or thyroxin were excluded from the study.

Patients were stratified into 3 groups based on the albumin-to-creatinine ratio (ACR), as follow: Group AI: normal or mildly increased albumin in urine < 30 µg/mg; Group AII: moderately increased 30 - 300 µg/mg; and Group AIII: severely increased > 300 µg/mg) [18].

Written informed consent was obtained from all participants in addition to the approval of the Research Ethics Committee, Taif University.

Sampling

Blood and urine samples were collected in the morning from all participants after fasting for 12 hours. Blood samples were collected in plain and EDTA tubes. The sera were separated after centrifugation then stored at -20°C until further analysis. Estimation of the glycated hemoglobin (HbA1c) was performed from the EDTA blood samples.

Analysis of blood glucose, lipid profile, calcium, and creatinine

Fasting blood glucose levels and lipid profile, total cholesterol (TC), high dentistry lipoprotein- cholesterol (HDL-C), and triacylglycerol (TG), were analyzed using enzymatic colorimetric techniques according to

Trinder [19], Allain et al. [20], Lopes-Virella et al. [21], and Glick et al. [22], respectively, utilizing kits purchased from ELITech Group (Puteaux, France). LDL-cholesterol was estimated based on Friedewald et al. [23]; LDL-C (mg/dL) = total cholesterol - (HDL-C + TG/5). Glycated hemoglobin (HbA1c) was analyzed using the automated glycosylated hemoglobin analyzer (Bio-Rad, USA). Serum creatinine (Scr) was evaluated by utilizing the automated chemistry analyzer (Beckman, USA). Estimated glomerular filtration (eGFR) results were calculated by an equation developed by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI**)** formula [24].

The urine ACR (µg albumin/mg creatinine) was evaluated immediately after the colorimetric estimation of urine creatinine by the Randox Jaffé creatinine assay kit and the enzyme-linked immunosorbent assay (ELISA, Orgentec Diagnostika GmbH, Germany) determination of urinary albumin [25].

Estimation of serum vitamin D, calcium, and parathyroid hormone (PTH)

ELISA assay kits were utilized for the estimation of serum 25-hydroxyvitamin D (Abcam Human vitamin D ELISA Kit, USA (Cat No. ab213966)). The detection range was 0.5 - 1,010 ng/mL with a sensitivity of 1.98 ng/mL. The PTH serum levels were estimated by the Abcam Human PTH ELISA kit, USA (ab 230931), based on the company guidelines with a detection range of 4.69 - 300 pg/mL and sensitivity of 0.761 pg/mL. Serum calcium was determined calorimetrically with a kit from Abcam, USA (Cat No. ab102505).

Assay of tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP)

The serum TNF-α levels were assayed using the Abcam Human TNF-α ELISA Kit, UK (ab181421). The detection range was 15.6 - 1,000 pg/mL, and the sensitivity was 4.4 pg/mL. CRP was evaluated by the immunoturbidimetric method (CRP II Latex X2, Denka Seiken Co. Ltd., Tokyo, Japan), utilizing the autoanalyzer (Toshiba, Tokyo, Japan). The measurement range of this assay was 0.01 - 32 mg/dL.

Estimation of hypoxic marker - hypoxia-inducible factor-1α (HIF-1 α)

The serum levels of HIF-1α were determined by a commercial ELISA kit (product number: KA1247, Abnova, Taiwan, China).

Statistical analysis

Statistical Package for Social Sciences (SPSS) for Windows version 20.0 (IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA) was used for data analysis. Data were presented as mean ± standard deviation (SD) and one-way analysis of variance (ANOVA) followed by Tukey's Honest Significant Difference (HSD) post-hoc test were used for multiple comparisons between groups. Pearson's correlation coefficient was used to assess the association between vitamin D and other studied parameters. p-values were considered statistically significant at < 0.05.

RESULTS

Demographic and biochemical basal characteristics of studied subjects

The studied groups were matched for gender distribution; there was no significant difference observed between them. The mean age for both patient groups AII and AIII was high in comparison to the control group, and the results showed a statistically significant difference ($p < 0.01$). Also, the mean age of patients of group AIII was significantly higher than that of diabetic patients of group AI ($p < 0.01$). Higher blood pressure levels were recorded with a significant difference between the studied groups (group AIII of patients showed higher levels than both the control and AI diabetic patients groups; the AII group also showed higher levels of blood pressure with significant difference than both control and AI groups).

Biochemical basal characteristics, blood glucose, Hb, HbA1c, TC, TG, HDL, and LDL showed a significant difference between the four studied groups ($p < 0.0001$, ANOVA test). However, eGFR was only significantly different in the AIII group in comparison to control ($p < 0.01$). Diabetic patients with microalbuminuria (group AII) showed a significant difference for all biochemical parameters when compared to control and group AI diabetic patients ($p < 0.01$, $p < 0.05$ for TC, Tukey's HSD post-hoc test). Diabetic patients with macroalbuminuria (group AIII) showed a significant difference when compared to control, AI, and AII diabetic patients, ($p < 0.01$, Tukey's HSD post-hoc test). ACR followed the same results except it showed an insignificant difference between the control and AI diabetic patients groups (Table 1).

TNF-α and CRP levels of the studied groups

The results of the studied inflammatory markers are summarized in Table 2. There was a significant rise of TNF-α and CRP levels in the diabetic patients group AIII as compared to diabetic patients AII or AI in addition to the controls ($p < 0.01$, Tukey's HSD post-hoc test). A similar rise was observed in AII diabetic patients versus AI patients and controls.

Vitamin D and related biochemical parameters in the studied groups

As shown in Table 3, there was a significant difference among studied groups as regards vitamin D, calcium, and PTH levels ($p < 0.0001$, ANOVA test). Moreover, vitamin D in AIII diabetic patients group showed significant difference versus T2DM AII or AI groups ($p < 0.01$, Tukey's HSD Post-hoc test). However, calcium and PTH levels in AI diabetic patients revealed an insignificant difference in its level when compared to con-

Table 1. Demographic and biochemical characteristics of studied subjects.

	Control group n = 60	Diabetic patients n = 174			p-value
		Group AI Normoalbuminuria n = 60	Group AII Microalbuminuria n = 56	Group AIII Macroalbuminuria n = 58	
Gender					**0.46 [X]**
male	**35 (58.4%)**	**31 (51.66%)**	**37 (66%)**	**35 (60.35%)**	
female	**25 (41.6%)**	**29 (48.33%)**	**19 (34%)**	**23 (39.65%)**	
Age (years)	**49 ± 12.5**	**51 ± 11.4**	**55 ± 10.2 ****	**59 ± 9.7 ****	**0.0001 ***
Systolic BP	**121.6 ± 22.4**	**133.2 ± 18.6 *****	**139.2 ± 21.5 ****	**144.3 ± 25.3 ** [a1]**	**0.0001 ***
Diastolic BP	**75.3 ± 9.4**	**80.1 ± 8.7 *****	**83.7 ± 9.9 ****	**86.4 ± 10.3 ** [a]**	
Blood glucose (mg/dL)	**91.33 ± 19.50**	**162.038 ± 23.38 ****	**186.44 ± 27.20 **[a]**	**235.72 ± 47.51 **[ab]**	**< 0.0001 ***
Hb (g/dL)	**13.5 ± 2.1**	**12.8 ± 1.6**	**11.9 ± 1.7 ** [a1]**	**11.1 ± 1.9 ** [a]**	**< 0.0001 ***
HbA1c %	**5.19 ± 1.09**	**7.55 ± 1.10 ****	**8.94 ± 0.96 **[a]**	**9.79 ± 1.11 **[ab]**	**< 0.0001 ***
eGFR (mL/min/1.73m^2)	**106.3 ± 13.6**	**102.7 ± 12.8**	**101.2 ± 15.2**	**97.8 ± 16.1 ****	**0.015 ***
Triglycerides (mg/dL)	**128.40 ± 11.34**	**197.25 ± 25.17 ****	**226.08 ± 15.20 **[a]**	**255.14 ± 18.95 **[ab]**	**< 0.0001 ***
Total cholesterol (mg/dL)	**147.69 ± 14.04**	**232.46 ± 9.17 ****	**250.14 ± 9.31 **[a1]**	**296.82 ± 59.73 **[ab]**	**< 0.0001 ***
HDL (mg/dL)	**65.03 ± 7.49**	**53.50 ± 9.21 ****	**47.38 ± 7.23 **[a]**	**40-92 ± 6.69 **[ab]**	**< 0.0001 ***
LDL (mg/dL)	**57.69 ± 7.69**	**144.21 ± 9.41 ****	**187.20 ± 13.75 **[a]**	**244.78 ± 8.36 **[ab]**	**< 0.0001 ***
ACR *(µg albumin/mg creatinine)*	**16.07 ± 3.48**	**17.86 ± 3.59**	**78.85 ± 12.89 ** [a]**	**445.76 ± 95.59 **[ab]**	**< 0.0001 ***

[¥] Hb - Hemoglobin, HbA1c - glycated hemoglobin, HDL - high dentistry cholesterol, LDL - low density cholesterol, AC - urinary albumin excretion (µg albumin/mg creatinine).
*** Significant p < 0.0001 between four studied groups by ANOVA.**
[X] Chi squared test used for calculation of significance.
**** Significant difference p < 0.01 from control group by Tukey's HSD post-hoc test.**
***** Significant difference p < 0.05 from control group by Tukey's HSD post-hoc test.**
[a] Significant p < 0.01 compared to AI diabetic patients group by Tukey's HSD post-hoc test.
[a1] Significant p < 0.05 compared to AI diabetic patients group by Tukey's HSD post-hoc test.
[b] Significant p < 0.01 compared to AII diabetic patients by Tukey's HSD post-hoc test.

Table 2. Inflammatory markers in the studied groups.

	Control group n = 60	Diabetic patients n = 174			p-value
		Group I n = 60	Group II n = 56	Group III n = 58	
TNF-α (pg/mL)	**3.89 ± 1.38**	**32.45 ± 6.27 ****	**60.61 ± 7.12 **[a]**	**91.50 ± 7.33 **[ab]**	**< 0.0001 ***
CRP (mg/dL)	**1.96 ± 0.99**	**8.16 ± 1.38 ****	**25.74 ± 7.11 **[a]**	**50.06 ± 11.09 **[ab]**	**< 0.0001 ***

[¥] TNF-α - tumor necrosis factor-α, CRP - C-reactive protein.
*** Significant p< 0.0001 between four studied groups by ANOVA.**
**** Significant difference from control group by Tukey's HSD post-hoc test.**
[a] Significant p < 0.01 compared to normoalbuminuria diabetic patients group by Tukey's HSD post-hoc test.
[b] Significant p < 0.01 compared to microalbuminuria diabetic patients by Tukey's HSD post-hoc test.

trol. In addition, PTH levels in AII/AIII diabetic patients showed insignificant statistical difference ($p > 0.05$, Tukey HSD post-hoc test). PTH levels exhibited a lower statistically significant rise in the AII patients group when compared to control ($p < 0.05$, Tukey's HSD post-hoc test).

Table 3. Vitamin D and related biochemical parameters in the studied groups.

	Control group n = 60	Diabetic patients n = 174			p-value
		Group I n = 60	Group II n = 56	Group III n = 58	
Vitamin D (ng/mL)	35.31 ± 3.82	26.04 ± 8.32 **	22.29 ± 7.50 **[a]	15.77 ± 4.74 **[ab]	< 0.0001 *
Calcium (mg/dL)	9.63 ± 0.57	9.76 ± 0.53	8.72 ± 1.35 **[a]	7.44 ± 1.19 **[ab]	< 0.0001 *
PTH (pg/mL)	34.38 ± 5.17	35.91 ± 5.78	40.11 ± 11.04 **[a1]	43.76 ± 11.47 **[a]	< 0.0001 *

[¥] PTH - parathormone hormone.
* Significant p < 0.0001 between four studied groups by ANOVA.
** Significant difference from control group by Tukey's HSD post-hoc test.
[a] Significant p < 0.01 compared to normoalbuminuria diabetic patients group by Tukey's HSD post-hoc test.
[a1] Significant p < 0.05 compared to normoalbuminuria diabetic patients group by Tukey's HSD post-hoc test.
[b] Significant p < 0.01 compared to microalbuminuria diabetic patients by Tukey's HSD post-hoc test.

Table 4. Hypoxia marker in the studied groups.

	Control group n = 60	Diabetic patients n = 174			p-value
		Group I n = 60	Group II n = 56	Group III n = 58	
HIF-1α (pg/mL)	17.65 ± 3.85	24.86 ± 4.57 **	30.73 ± 6.88 **[a]	34.77 ± 6.43**[ab]	< 0.0001 *
Hb (g/dL)	13.5 ± 2.1	12.8 ± 1.6	11.9 ± 1.9	11.1 ± 1.6	

[¥] HIF-1α - hypoxia-inducible factor-1α.
* Significant p < 0.0001 between four studied groups by ANOVA.
** Significant difference to control group by Tukey's HSD post-hoc test.
[a] Significant p < 0.01 compared to normoalbuminuria diabetic patients group by Tukey's HSD post-hoc test.
[b] Significant p < 0.01 compared to microalbuminuria diabetic patients by Tukey's HSD post-hoc test.

Table 5. Pearson's correlation coefficients for serum vitamin D (ng/mL) levels and inflammatory and hypoxia markers in studied groups.

Biochemical parameters	Vitamin D (ng/mL)	
	r	p
TNF-α (pg/mL)	-0.72635	< 0.0001
CRP (mg/dL)	-0.6423	< 0.0001
HIF-1α (pg/mL)	-0.58438	< 0.0001

TNF-α - tumor necrosis factor, CRP - C-reactive protein, HIF-1α - hypoxia-inducible factor-1α.

Hypoxia-inducible factor in the studied groups

HIF-1α levels were significantly different between the studied groups ($p < 0.0001$, ANOVA test) and displayed a significant rise in the AIII diabetic patients group as compared to other groups ($p < 0.01$, Tukey's HSD post-hoc test). In addition, a similar significant rise was observed in the AII diabetic patients group versus both AI patients and control ($p < 0.01$, Tukey's HSD post-hoc test) (Table 4).

Correlations between vitamin D levels with various inflammatory and hypoxia markers in studied groups

Vitamin D levels were significantly negatively correlated with TNF-α, CRP, and HIF-1 α levels ($p < 0.0001$ for all parameters) as shown in Table 5 and Figures 1, 2, and 3.

Table 6. Pearson's correlation coefficients of serum vitamin D (ng/mL) levels and urinary albumin-to-creatinine ratio (µg albumin/mg creatinine) in studied groups.

Biochemical parameters	Vitamin D (ng/mL)	
	r	p
ACR (µg albumin/mg creatinine)	-0.54722	< 0.0001

ACR - urinary albumin-to-creatinine ratio (µg albumin/mg creatinine).

Table 7. Logistic regression analysis of albuminuria risk factors in T2DM.

Parameters	Sig.	Odds ratio	95% CI	
			Lower	Upper
Age (years)	0.002 *	1.221	1.088	1.370
Gender	0.449	0.750	0.356	1.580
Hypertension	0.270	1.505	0.728	3.111
Glucose (mg/dL)	< 0.001 **	1.081	1.050	1.112
HbA1c (mg/dL)	0.476	0.795	0.377	1.675
TG (mg/dL)	0.286	1.595	0.772	3.298
TC (mg/dL)	0.490	0.819	0.389	1.725
LDL (mg/dL)	0.199	2.772	0.333	7.291
HDL (mg/dL)	< 0.001 **	0.877	0.826	0.931
TNFα (pg/mL)	0.299	7.794	0.935	20.497
CRP (mg/L)	< 0.001 **	1.642	1.276	2.114
Vit D (ng/mL)	< 0.001 **	0.830	0.764	0.902
Calcium total (mg/dL)	0.295	1.643	0.795	3.397
PTH (ng/L)	0.505	0.843	0.400	1.777
HIF1 (pg/mL)	0.002 *	1.094	1.034	1.158

p-value > 0.05 NS, * p-value < 0.05 S, ** p-value < 0.001 HS.

Correlations between vitamin D levels and ACR in studied groups

Table 6 and Figure 5 show that vitamin D levels were significantly negatively correlated with ACR ($p < 0.0001$).

Logistic regression analysis of albuminuria risk factors in type 2 diabetes mellitus

Table 7 shows that increased age, glucose, CRP, HIF-1, low vitamin D, and HDL levels are significant predictor risk factors for albuminuria in T2DM. While gender, hypertension, HbA1c, TG, TC, LDL, TNFα, calcium total, and PTH are insignificant.

DISCUSSION

Diabetes mellitus (DM) is a highly prevalent disease with a significant socioeconomic load globally. In most countries, the prevention and treatment of DM has been the main priority for health systems. Several reports showed that the prevalence of DM and the metabolic syndromes were linked to the low levels of vitamin D [26,27].

We studied the serum levels of vitamin D, calcium, parathyroid hormone, and lipid profile in diabetic patients. Our results revealed that vitamin D levels were reduced in our diabetic patients compared to the controls. The level of vitamin D deficiency was significantly related to the severity of renal nephropathy as indicated by the level of albuminuria. Bayani et al. previously reported that in diabetic patients, vitamin D was significantly lower than in healthy people [28].

Our results showed a significant decrease in serum calcium and an increase in parathyroid hormone in all studied patients compared to the controls. In our diabetic groups, the serum level of total cholesterol, triglycerides, and LDL- cholesterol were significantly increased,

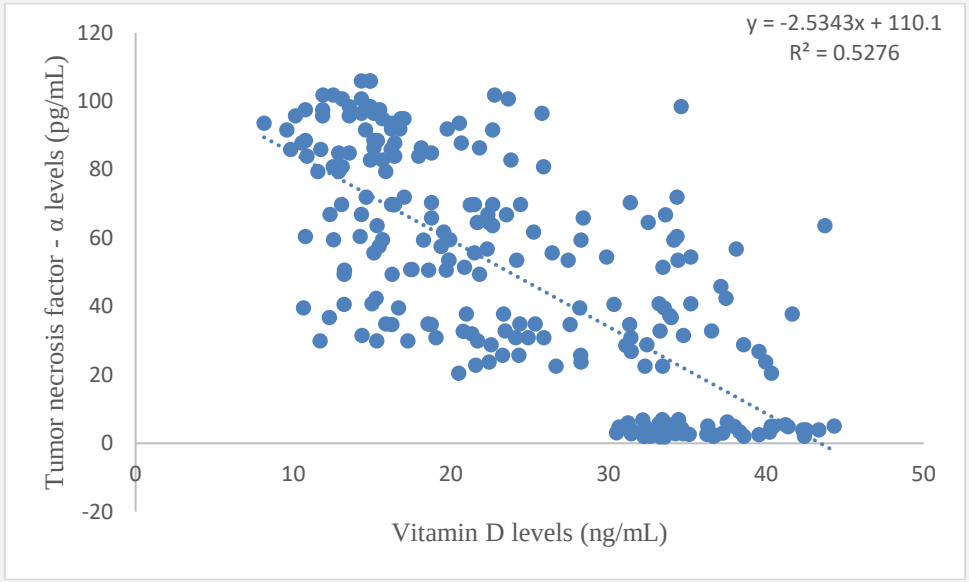

Figure 1. Correlation coefficient between serum vitamin D levels (ng/mL) and tumor necrosis factor-α levels (pg/mL) in studied group (r = -0.72635, p < 0.0001).

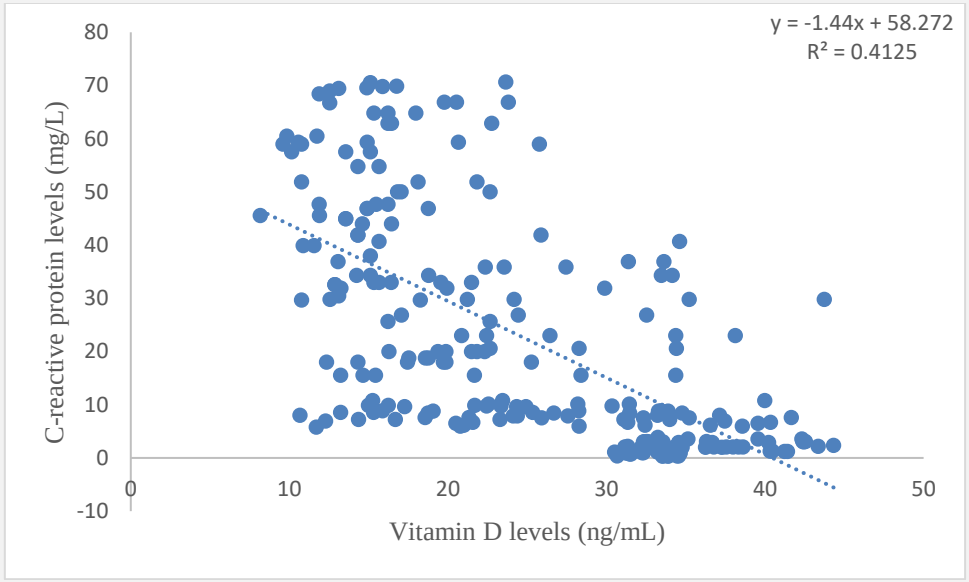

Figure 2. Correlation coefficient between serum vitamin D levels (ng/mL) and C reactive protein levels (mg/dL) in studied group (r = -0.6423, p < 0.0001).

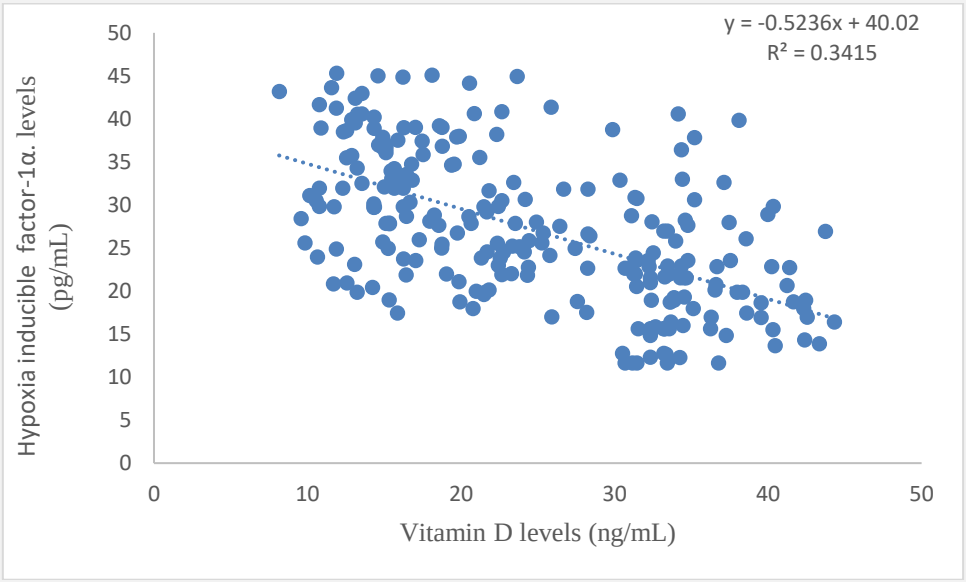

Figure 3. Correlation coefficient between serum vitamin D levels (ng/mL) and hypoxia-inducible factor-1α levels (pg/mL) in studied group (r = -0.58438, p < 0.0001).

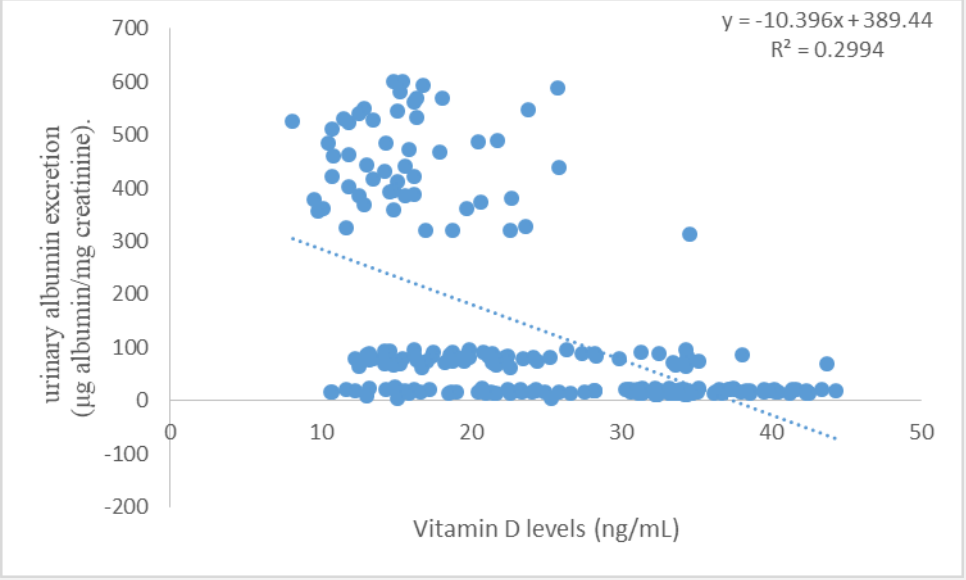

Figure 4. Correlation coefficient between serum vitamin D levels (ng/mL) and urinary albumin excretion (µg albumin/mg creatinine) in studied group (r = -0.54722, p < 0.0001).

and HDL-cholesterol was significantly reduced. In agreement with our findings, previous studies demonstrated that dyslipidemia complicated with diabetes has been involved in the development of diabetic nephropathy because dyslipidemia facilitates glomerulosclerosis in diabetic patients. In this context, Rutledge et al. and Hirano et al. documented the significant role of dyslipidemia in the development and progression of diabetic nephropathy. Impairment of lipoprotein metabolism, such as high levels of very low-density lipoprotein (VLDL) and LDL-cholesterol and a decline in HDL-cholesterol, is observed in patients with diabetes [29, 30].

Our findings about the association of parathyroid hormone and albuminuria are consistent with the results reported by Wang et al., who found that serum level of PTH was increased in parallel with albuminuria, however, inversely correlated with eGFR 31.

The significant association between vitamin D and albuminuria reported in the current study are in agreement with the results of some previous studies [33,34] but not with others [31,34-36].

In contrast to our findings, Guessous et al. reported that serum vitamin D levels were similar in both CKD patients and non-CKD subjects in Swiss population [36]. They did not report any significant correlation of serum vitamin D levels with CKD, albuminuria, or declined kidney functions in a follow-up research for 5.5 years [36]. On the other hand, an observational study included 1,193 participants with type 1 diabetes over a period of 16 years found that higher risk of albuminuria was associated with vitamin D deficiency compared with sufficient vitamin D status [37]. Moreover, in agreement to our findings, a cross sectional study by Sunkar et al. including 150 advanced CKD patients showed an association of CKD with hypocalcemia, hypovitaminosis D, and anemia [38].

In the current study, we found that the serum vitamin D levels were significantly negatively correlated with TNF-α, CRP, and HIF-1 α levels. Several reports considered renal inflammation as the fundamental pathological process in the pathogenesis of diabetic kidney diseases (DKD) [39,40]. Also, vitamin D reduces the release of cytokines and lymphocyte production, diminishing the inflammatory mediators [41]. Timms et al. reported that reduction in the serum vitamin D is participating in the microangiopathy by affecting the formation of CRP and matrix metalloproteinase [42]. Moreover, Jablonski et al. reported that the lower levels of 25-hydroxyvitamin D enhanced by inflammation related to nuclear factor κ B leads to impairment of the vascular endothelium [43]. We also studied the levels of blood glucose and HBA1c and the inflammatory markers. There was a significant difference in the levels of blood glucose and HBA1c and the inflammatory markers among different groups of included patients. These findings could be explained by the prolonged uncontrolled hyperglycemia enhancing the formation of glycated substances in addition to activation of macrophages, which enhance the production of TNF-α that leads to the formation of CRP [44].

We studied the levels of HIF-1 α in our included diabetic patients compared to controls. HIF-1 α was found to be significantly related to the severity of diabetic renal nephropathy. It was increased in patients with macroalbuminuria compared to other diabetic and control groups. A significant negative correlation was noted in our study between vitamin D deficiency and HIF-1 α. It was reported early that vitamin D prevents the HIF-1 α protein synthesis and its inducers as endothelin-1 (ET-1) and VEGF, thereby postponing the occurrence of DKD [40,45].

Oxidative stress induced by hyperglycemia is considered the principal pathological condition for vascular complications in diabetes and DKD [46]. Vitamin D was observed to play a vital role in decreasing the renal oxidative stress and inflammation in diabetic rats and diminishing renal damage that might explain the connection between DKD and vitamin D deficiency [47]. In the current study, logistic regression analysis was used to determine that the risk factors for prediction of albuminuria in patients with type 2 diabetes mellitus were older age, increased levels of blood glucose, CRP, and HIF in addition to low HDL and vitamin D.

In conclusion, from our obtained data, vitamin D deficiency and high serum level of HIF-1α is related significantly to the severity of diabetic nephropathy. It can be used as a biochemical marker for the DKD severity.

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Ethical Statement:

Our study was approved by the Research Ethics Committee (No. 42-0010), Taif University, Taif city, Saudi Arabia. All patients provided written informed consent prior to enrollment in the study.

Declaration of Interest:

All authors declare no conflict of interest.

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