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RESEARCH ARTICLE

Comparison of vitamin D levels in Diabetic nephropathy and Non-diabetic chronic kidney disease

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Abstract

Background and Aims: Diabetic Nephropathy is the most common renal complication of diabetes mellitus and a leading cause of end-stage renal disease. A deficiency in 25(OH) D has been linked to cardiovascular disease, diabetes and the progression of chronic kidney disease (CKD). Our aim was to determine vitamin D levels in different stages of diabetic nephropathy and non diabetic CKD and to compare it with the control subjects. **Methods:** This study was conducted in summer 2013 on 75 subjects divided into 3 groups: group (1): 15 healthy controls, group (2): 15 non diabetics with CKD and group (3): 45 type 2 diabetics who were subdivided into 3 groups according to albumin-to-creatinine ratio (ACR); (3a): 15 patients with normoalbuminuria; (3b): 15 patients with microalbuminuria and (3c): 15 patients with macroalbuminuria. Laboratory tests included FBS, HbA1c, CBC, urea, serum creatinine, ACR, eGFR and 25-hydroxyvitamin D. **Results:** There was a significant decrease in vitamin D levels in groups 2, 3a, 3b and 3c compared to control group (31.13±7.60, 64.20±10.70, 49.19±12.02, 20.40±9.48 vs. 79.20±19.06 nmol/L respectively, $p<0.001$). Also, there was significant difference between patient groups. Vitamin D levels were negatively correlated with age, duration of diabetes, FBS, HbA1c, creatinine, urea and ACR and positively correlated with hemoglobin and creatinine clearance in diabetics and all studied groups ($p<0.001$). Blood urea was the most important predictor of vitamin D levels followed by HbA1c and creatinine. **Conclusion:** Vitamin D levels were decreased in diabetics and in CKD. The higher the proteinuria the lower was the vitamin D levels.

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Introduction

Type 2 diabetes is a chronic and progressive condition associated with the risk of invalidating micro- and macrovascular complications (Miccoli et al., 2011). Diabetes is the major cause of end-stage renal disease (ESRD) in the world and has enormous medical, social and economic consequences (Dabla, 2010). Diabetic nephropathy is a risk factor for cardiovascular disease and the leading cause of chronic kidney disease in patients starting renal replacement therapy (Gross et al. 2005). The first sign of renal involvement in patients with diabetes is microalbuminuria (Graham et al., 2010). Vitamin D appears to play an extensive role as a cell differentiating and antiproliferative factor with actions in a variety of tissues, including the renal, cardiovascular, and immune systems (Al-Badr and Martin, 2008). A deficiency in 25(OH) D has been linked to cardiovascular disease, diabetes, the progression of chronic kidney disease (CKD) and immune system disorders (Villarreal et al., 2011). The kidney

appears to be a major target organ for both the classical and non-classical actions of vitamin D, with the vitamin D receptor being appropriately highly expressed in this site. In patients with CKD, the new non-classical role of vitamin D also encompasses regulation of the rennin angiotensin system (RAS) and the nuclear factor (NF) kB pathway, both promoting progressive renal damage (Villarreal et al., 2011). Activation of the NF-kB pathway triggers a cascade of events yielding cytokines, chemokines and other inflammatory factors, which exacerbate tissue injury in the renal disease process. In diabetic nephropathy, angiotensin II appears to activate NF-kB, which in turn activates angiotensinogen expression in renal cells when hyperglycemia is present. This cycle is likely partly responsible for the local accumulation of angiotensin II in diabetic nephropathy, is likely to have deleterious effects on blood pressure and the vasculature, and may contribute to renal parenchymal damage (Williams et al., 2009). Recently the VITAL study, a randomized controlled clinical trial with 281 type 2 diabetic patients with diabetic nephropathy, has shown that administration of a vitamin D receptor agonist (paricalcitol) in addition to blockade of the RAS causes sustained reduction in albuminuria and thereby potentially has clinically relevant renoprotective effects in patients with diabetic nephropathy (Agarwal et al., 2005). Adequate levels of vitamin D are also associated with decreased insulin resistance and reduced blood pressure, the two main, potentially modifiable risk factors for diabetic nephropathy initiation and progression (Holick, 2005).

Our aim was to determine vitamin D levels in different stages of diabetic nephropathy and non diabetic chronic kidney disease (CKD) and to compare it with the control subjects.

Materials and methods:

This is a case control study was conducted on 75 subjects, collected from outpatient clinic of endocrinology unit and nephrology department of Ain Shams University Hospital. Exclusion criteria included infection, fever, congestive heart failure and exercise within 24 hours. Patients receiving drugs that affect vitamin D levels such as anti-epileptic drugs and corticosteroids were also excluded. Selected subjects **were divided into 3 groups: Group 1:** 15 healthy control subjects (8 males and 7 females). **Group 2:** 15 non-diabetics with chronic kidney disease on conservative treatment (5 males and 10 females). **Group 3:** 45 type 2 diabetic patients who were subdivided into 3 groups according to urinary albumin-to-creatinine ratio (ACR): (3a): 15 diabetic patients with normoalbuminuria (7 males and 8 females), (3b): 15 diabetic patients with micro-albuminuria (6 males and 9 females), and (3c): 15 diabetic patients with macro-albuminuria and renal impairment (6 males and 9 females). Gender distribution was comparable among all groups (X^2 1.61, p value < 0.50).

Screening for microalbuminuria can be performed by measurement of the albumin-to-creatinine ratio (ACR) in a random spot collection (µg albumin/ mg creatinine). Normal < 30, Microalbuminuria 30-300 and Macroalbuminuria > 300 (Dabla, 2010). All subjects were subjected to full medical history emphasizing the duration of diabetes mellitus, drug history and history of any medical condition. Clinical examination included blood pressure and lower limb edema. Laboratory tests included fasting blood glucose (FBG) was measured using an automated glucose oxidase method using Behring Diagnostics Reagents (SVR Glucose Test; Behring, La Jolla, CA). HbA1c is assayed by Stanbio Procedure No.0350 "Quantitative colorimetric determination of Glycohemoglobin in blood". Complete blood count (CBC), serum creatinine done by photometric colorimetric test and urea was done by modified urease-berthelot method (Fawcett and Scott, 1960). The serum creatinine should be used to estimate GFR and stage the level of chronic kidney disease (CKD), if present. Estimated GFR (eGFR) was estimated using Cockcroft- Gault formulae (Cockcroft and Gault, 1976). GFR (ml/min per 1.73 m^2 body surface area): Normal ≥ 90, mildly decreased 69-89, moderately decreased 30-59, severely decreased 15-29 and kidney failure < 15 or dialysis (Kramer and Molitch, 2005).

Serum 25 hydroxyvitamin D levels were measured by ELISA (this test kit is a competitive protein binding assay for the measurement of 25 hydroxy vitamin D. Immunodiagnostic AG, Stubenwald-Allee 8a, D-64625 Bensheim, Australian). Levels of serum 25(OH) D**:** Deficient <50 nmol/L, insufficient 50-75 nmol/L, Optimal 75-225 nmol/L (Holik et al., 2011).

This study was approved by the internal review board of Ain Shams University. All subjects provided written informed consent before the study.

Results:

The studied groups were age and sex matched. There was a statistically significant difference ($p < 0.05$) between groups regarding systolic and diastolic blood pressure, FBG, HbA1c, hemoglobin, serum creatinine, urea, creatinine

clearance, ACR and vitamin D using ANOVA test. There was a highly statistical significant decrease in vitamin D levels ($P < 0.01$) in groups 2, 3b and 3c compared to control group, while significant decrease ($P < 0.05$) in group 3a. Comparing vitamin D levels in all groups showed that the lowest level of vitamin D was in diabetics with macroalbuminuria.

In sub analysis of vitamin D status in all studied groups we found that in control group 53.33% was sufficient and 46.67% was insufficient, in CKD group all were deficient, in group 3a: 20% was sufficient, 66.77% was insufficient and 13.33% was deficient, in group 3b: 46.67% was insufficient while 53.33% was deficient and group 3c all were deficient ($X^2 = 22.26$, p value < 0.01).

A highly statistical significant difference ($p < 0.01$) was found between diabetic groups regarding duration of diabetes, serum creatinine, urea, creatinine clearance, ACR and vitamin D. A statistically significant difference ($p < 0.05$) was found regarding FBG and hemoglobin.

Vitamin D levels were negatively correlated with age, duration of diabetes, FBS, HbA1c, serum creatinine, urea and ACR and positively correlated with hemoglobin and creatinine clearance in diabetics and all studied groups ($p<0.001$). However, no significant correlation was found between vitamin D levels and blood pressure.

Stepwise regression analysis showed that the most important predictors of vitamin D levels in this study were blood urea followed by HbA1c and serum creatinine.

Table (1): Comparison between the different studied groups as regard their demographics and investigations:

	Group 1 (n=15) Control	**Group 2 (n=15)** CKD	**Group 3 (n=45)**		
			Group 3a (n=15) DM	**Group 3b (n=15)** DM + microalb.	**Group 3c (n=15)** DM+macroalb.+CKD
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
DDM(years)	--	--	11.87±3.78	16.27±5.59	19.47±5.82
FBG (mg/dl)	88.67±10.79	91.67±13.25	146.33±20.13 ** ++	204.27±72.77 ** ++	212.87±79.16 ** ++
A1c (%)	4.84±0.33	4.73±0.45	8.71±0.99 ** ++	8.55±0.99 ** ++	8.70±1.09 ** ++
Cr (mg/dl)	0.98±0.21	2.68±0.45 **	1.01±0.25 ++	1.02±0.25 ++	2.44±0.38 **
Urea (mg/dl)	26.27±4.64	84.73±21.55 **	25.47±7.03 ++	30.67±5.60 * ++	107.80±27.58 ** +
CrCl (ml/min)	93.11±13.92	36.48±13.44 **	92.07±12.19 ++	95.01±25.46 ++	39.82±12.25 **
ACR (mg/g)	2.48±0.56	9.13±3.46 **	2.48±0.45 ++	116.67±37.94 ** ++	1073±254.76 ** ++
D3 (nmol/l)	79.20±19.06	31.13±7.60 **	64.20±10.70 * ++	49.19±12.02 ** ++	20.40±9.48 ** ++

DDM: duration of DM, , FBG: fasting blood glucose, A1C: hemoglobin A1c, Cr: creatinine, CrCL: creatinine clearance, D3: 25 (OH) vitamin D.

* Significant difference when comparing to group 1 ($P < 0.05$).

** Highly significant difference when comparing to group 1 ($P < 0.01$).

\+ Significant difference when comparing to group 2 ($P < 0.05$).

++ Highly significant difference when comparing to group 2 ($P < 0.01$).

Table (2): Comparison between diabetic groups as regard their demographics and investigations by using ANOVA test:

	Group 3 (n=45)			F	P	Sig.
	Group 3a (n=15) DM	**Group 3b (n=15)** DM+ microalb.	**Group 3c (n=15)** DM + macroalb.+ CKD			
	Mean ± SD	**Mean ± SD**	**Mean ± SD**			
DDM(years)	11.87±3.78	16.27±5.59*	19.47±5.82**	8.246	< 0.01	**HS**
FBG (mg/dl)	146.33±20.13	204.27±72.77**	212.87±79.16*	3.794	< 0.05	**S**
A1c (%)	8.71±0.99	8.55±0.99	8.70±1.09	0.122	> 0.05	**NS**
Cr (mg/dl)	1.01±0.25	1.02±0.25	2.44±0.38** ++	109.877	< 0.01	**HS**
Urea (mg/dl)	25.47±7.03	30.67±5.60*	107.80±27.58** ++	113.699	< 0.01	**HS**
CrCl (ml/min)	92.07±12.19	95.01±25.46	39.82±12.25** ++	45.825	< 0.01	**HS**
ACR (mg/g)	2.48±0.45	116.67±37.94**	1073±254.76** ++	74.682	< 0.01	**HS**
D3 (nmol/l)	64.20±10.70	49.19±12.02*	20.40±9.48** ++	63.942	< 0.01	**HS**

DDM: duration of DM, FBG: fasting blood glucose, A1C: hemoglobin A1c, Cr: creatinine, CrCL: creatinine clearance, D3: 25 (OH) vitamin D.

* Significant difference when comparing to group 3a ($P < 0.05$).
** Highly significant difference when comparing to group 3a ($P < 0.01$).
++ Highly significant difference when comparing to group 3b ($P < 0.01$).

Table (3): Stepwise regression analysis; Dependent variable: Vitamin D:

Model	Non-standardized Coefficients	Standardized Coefficients	T	P	Sig.
	B	**Beta (β)**			
Urea	-0.480	-0.745	-9.529	< 0.01	**HS**
Urea **A1c**	-0.472 -2.415	-0.732 -0.203	-9.839 -2.709	< 0.01	**HS**
Urea **A1c** **Cr**	-0.257 -3.288 -11.596	-0.398 -0.277 -0.391	-2.911 -3.667 -2.852	< 0.01	**HS**

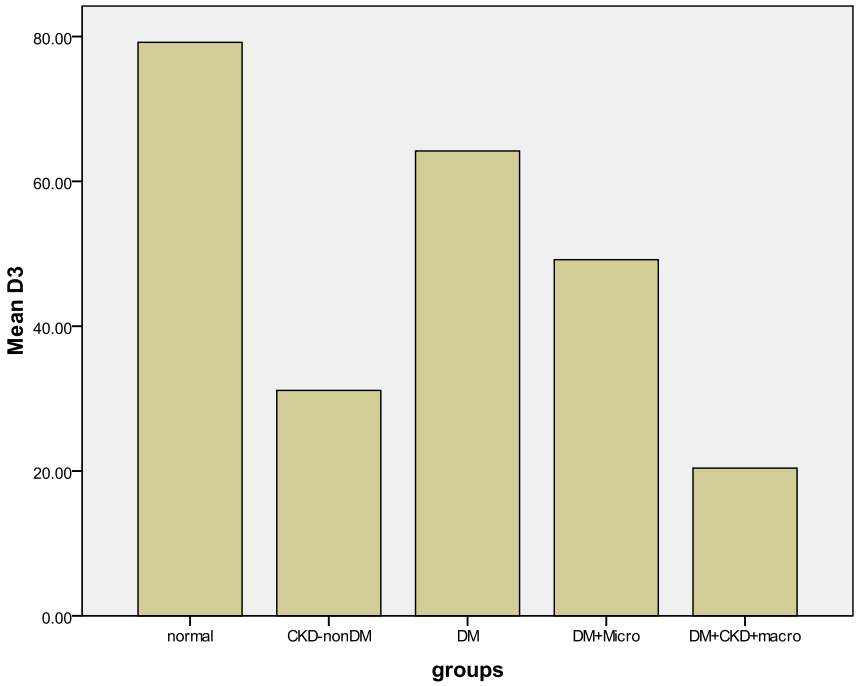

Figure 1: Comparison of vitamin D levels in all groups

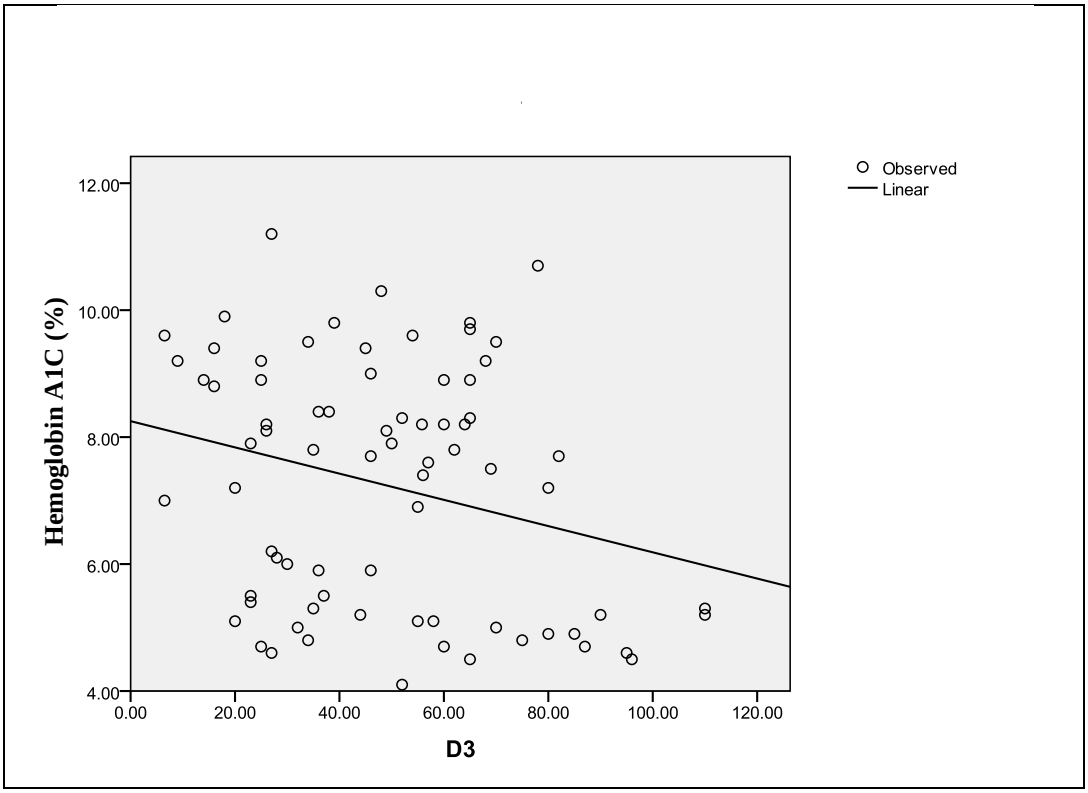

Figure 2: Correlation between vitamin D and A1C in all studied groups showing a significant indirect correlation (r = -0.264).

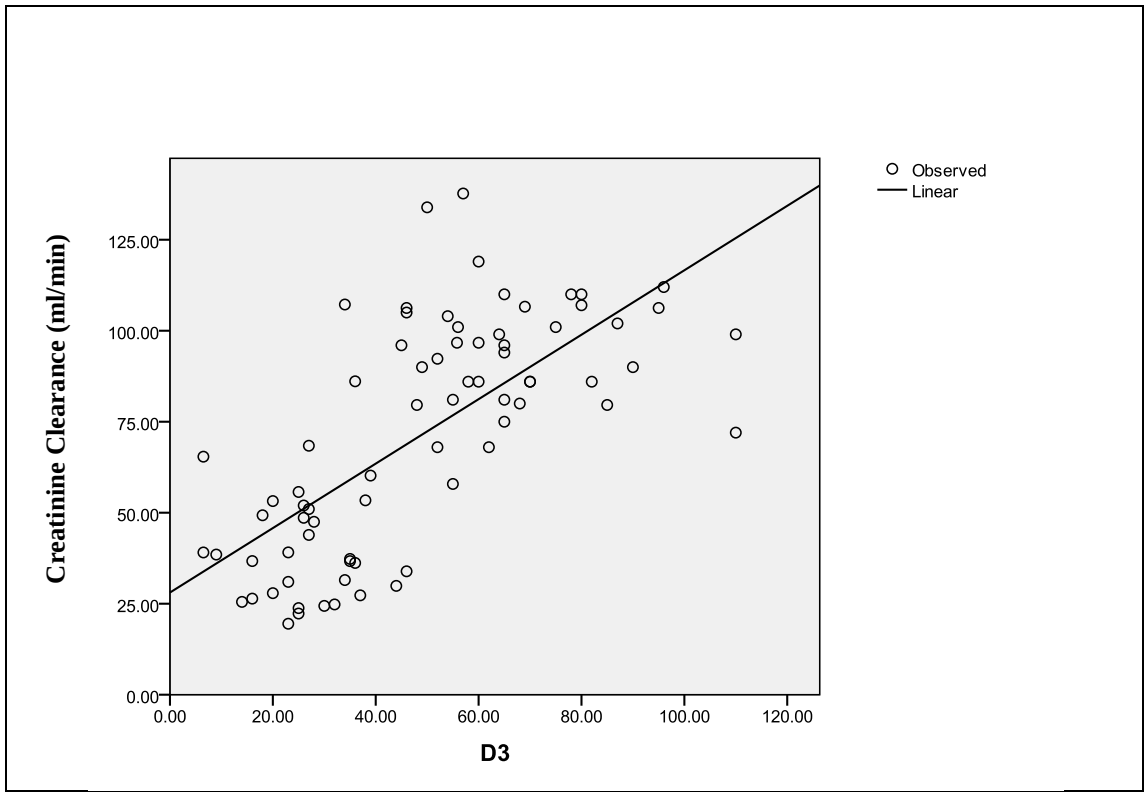

Figure 3: Correlation between vitamin D and creatinine clearance in all studied groups showing a high significant direct correlation (r = 0.692).

Discussion:

In the present study we found that vitamin D was significantly decreased in diabetic subjects than normal control and the prevalence of vitamin D insufficiency and deficiency were significantly higher in diabetic groups. Our results were in agreement with Isaia et al., who found that 25(OH) D levels were significantly lower in diabetic patients than in control subjects and the prevalence of 25(OH) D deficiency was significantly higher in diabetic patients than in control subjects (Isaia et al., 2001). This is also in agreement with Li et al. who found that type 2 diabetic patients have a high prevalence of vitamin D deficiency and vitamin D deficiency is independently associated with diabetic nephropathy (Li et al., 2013).

Vitamin D was significantly decreased in diabetics with microalbuminuria and macroalbuminuria than other groups. This is in agreement with the result obtained by Mehrotra et al., who studied 146 patients with diabetic nephropathy and found that Suboptimal 25OHD levels are very common in patients with albuminuria and diabetes. They suggested that urinary protein losses may be more important than loss of GFR in inducing vitamin D depletion (Mehrotra et al., 2008). Joergensen et al., found that urinary albumin excretion rate (mg/day) was significantly higher in a group with vitamin D deficiency than sufficient group (Joergensen et al., 2010).

In our study vitamin D levels were significantly lower in patients with chronic renal disease than control subjects. This is in agreement with Mehrotra et al., who found that vitamin D deficiency is more common in CKD subjects (stage I-V) than non-CKD subjects ((Mehrotra et al., 2008).

We found that vitamin D levels were significantly negatively correlated with HbA1c in all studied groups. This is in agreement with Dalgard et al., who found that the HbA1c concentration decreased at higher serum 25(OH)D3 concentrations independent of covariates ($\beta = -0.026$, $P < 0.026$)(Dalgard et al.,2011). Also, we found that vitamin D levels in diabetic patients were negatively correlated with HbA1c, but of no significant value. Zoppini et al., found that in type 2 diabetic patients, high A1C levels were associated with low concentrations of serum 25(OH) D independently of duration of diabetes, diabetic treatment and nephropathy (Zoppini et al., 2013).

Vitamin D levels were significantly and negatively correlated with urea, serum creatinine, ACR, and duration of diabetes in diabetic patients. It was significantly positively correlated with creatinine clearance in all studied

subjects and in diabetic patients. This is in concordance with Li et al. who found that vitamin D concentration was significantly negatively correlated with urinary albumin excretion rate ($r = -1.783$, $P < 0.001$)(Li et al.,2013). Also, Villarreal et al., found that vitamin D was indirectly correlated with proteinuria (Villarreal et al., 2011). For diabetic patients with diabetic nephropathy, proteinuria should be a factor to be considered when the 25 (OH) D deficiencies found. As vitamin D circulates mostly bound to proteins (vitamin D binding protein**)** hence, the higher the proteinuria, the lower the 25(OH) D levels. A deficiency in 25(OH) D has been documented in other protein loss states, such as nephrotic syndrome and treatment with peritoneal dialysis (Villarreal et al., 2011).
A cross-sectional analysis of the NHANES III data revealed a correlation between vitamin D insufficiency and increased prevalence of albuminuria in the US adult population, suggesting that vitamin D has an intrinsic antiproteinuric activity (De Boer et al., 2007).

Findings of this study show an association between vitamin D deficiency and nephropathy in a sample of Egyptian adults with type 2 diabetes mellitus. As a result of the cross-sectional nature of this study, we were unable to determine whether this association is present because vitamin D deficiency increases the risk of nephropathy or because nephropathy increases the risk of vitamin D deficiency.

Previous studies suggest that the relationship between these 2 variables is such that both of these interactions may be occurring simultaneously. This study also described the high prevalence of vitamin D deficiency in patients with diabetes. This finding highlights the need to improve screening for vitamin D deficiency in patients with diabetes, since vitamin D is known to have a role in decreasing the risk of many chronic illnesses, including cancer, cardiovascular disease, and infectious diseases (Holick, 2005). There are limitations to this study that should be considered. We were only able to measure 25-OH-D in this study, which did not allow us to evaluate the role of the kidney in metabolizing this form of vitamin D to the biologically active metabolite 1,25(OH)2 D3. However, this is an accepted approach to the evaluation of vitamin D status because only a small amount of 25-OH-D is metabolized in the kidney (Holick, 2007).

Vitamin D replacement in CKD patients has a renoprotective role in addition to its role in treatment of secondary hyperparathyroidism. The renoprotective and therapeutic potentials in diabetic nephropathy is through targeting the RAS (Klaus, 2008).

Large prospective controlled studies may be needed to evaluate the renoprotective role of vitamin D supplementation in diabetics with normo or macroalbuminuria, or non-diabetics with CKD.

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