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The Association between Alfacalcidol (1-alpha-hydroxyvitamin D3) and Oxidative Stress in Patients with Type II Diabetic Nephropathy

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ABSTRACT
Diabetic nephropathy is the major risk factor for cardiovascular disease among young adults with DM Type (II), one of the more common microvascular complications, which influence the survival and quality of life of the patient. Research currently is underway to establish if there is a link between the effect of 1-alpha (OH) D3 and Type II Diabetic Nephropathy patients and whether the Type II regulated diabetic nephropathy patient is related to 1-alpha (OH) D3 and to oxidative stress conditions. In diabetic patients, no relationship has been established between the effect of alfacalcidol and antioxidant and/or prooxidant. The clinical applicability of our research will be to identify a correlation between diabetic nephropathy and oxidative stress. This will lead to the development of new guidelines to assess if this vitamin can be substituted or supplemented to treat this high mortality disease in patients with type II diabetes mellitus.

Keywords: Alfacalcidol, Oxidative stress, SOD, Type 2 Diabetic Nephropathy, Al-Sader Teaching Medical City.

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INTRODUCTION
Diabetic nephropathy (DN) is one of the most common microvascular complications of DM, affecting the quality of life and survival of patients, being the major cause of morbidity and mortality, particularly linked to cardiovascular disease among young adults with type II DN. Parameters such as microalbuminuria and decreased glomerular filtration (GFR) are used to diagnose diabetic nephropathy. Based on growing urinary albumin values, diabetic nephropathy has traditionally been categorised into phases-normoalbuminuria, microalbuminuria, and macroalbuminuria. Around one-third of DM II patients may develop microalbuminuria and 15-20% may develop macroalbuminuria within 20 years of disease diagnosis.[1]

Vitamin D and Diabetic Nephropathy
Vitamin D deficiency is associated with increased urinary albumin excretion and mortality. The kidney's proximal tubular cells recycle 1-α-(OH) D3, from the glomerular ultrafiltrate, back into the circulation[2]. In patients with end-stage renal disease, both 1-α-(OH) D3 and 25 (-OH) D3 levels are reduced. Low concentrations of calcitriol were correlated with higher risks of diabetes and higher albuminuria / creatinuria ratio. High rates of angiotensin II are found in injured kidneys, and it has been proposed that it is involved in the mechanism that leads to kidney destruction.

Alfacalcidol: The form of active vitamin D is **alfacalcidol.** It is used in patients who have renal failure because they are unable to produce those vitamins. Daily controls should be carried out on minerals of calcium and phosphorus and their hormones. Alphacalcidol, also referred to as alfacalcidol, a vitamin D3 derivative, will bypass the 1-α-hydroxylase of the renal because of the pre-existing hydroxylic group of the C1. This compound is used in Europe and effectively suppresses PTH in patients with dialysis. Of course, the six-week alphacalcidentol therapy in five dialysis patients did not inhibit iPTH levels but could elevate 1,25(OH)2 D levels, even with CKD patients. No variations in PTH suppression or hypercalcemia incidence and hyperphosphatemia between alphacalcidol and paricalcitol occurred during 16 weeks in 80 chronic hemodialysis patients in the largest study to date in ESRD patients[3]. Alfacalcidol is considered a most beneficial type of vitamin D supplication, primarily because of a long half-life of an individual and the lower renal load. This vitamin D supplement is the most widely administered for end-stage renal illnesses, as impaired renal function alters the capacity for dry medicine. alfacalcidol has important effects on the immune system including regulatory T cells. Active vitamin D3 metabolite is alfacalcidol and consequently the second stage of hydroxylation is not required in the kidney. The active agent alfacalcidol is contained in **One-Alpha**. One-Alpha is in a category of medicines called analogues for vitamin D. It's a vitamin D type. The amount of two substances inside the body is regulated by vitamin D. These compounds are known as phosphate and calcium. One-Alpha increases vitamin D levels in the human body. This ensures that the body also raises its levels of calcium and phosphate. One-Alpha is used to treat conditions such as kidney failure-related bone changes (osteodystrophy). Alfacalcidol and eldecalcitol therapies also decrease eGFR in the treatment cycle for both chronic kidney disease (CKD) stage 1 and 2 patients; however, they have a minor reduction and eGFR return to baseline soon after treatment has been stopped. In stage 3A and 3B patients through both therapists there are almost no improvements in eGFR[4].

Oxidative stress in Diabetic Nephropathy
State of imbalance caused by sustained hyperglycemia has several sources, both non-enzymatic and enzymatic. In diabetic nephropathy there is an increase in oxidative stress in all structures of the kidney. Free radicals mainly have the objective of causing peroxidation in membrane lipids, thereby causing a loss of integrity and dysfunction of the membranes. The body has an antioxidant defense system for its elimination. Studies carried out during the last decade have revealed that the development of diabetic nephalropathy begins in the first years after the onset of diabetes. There are laboratory elements that

would allow the detection of risk factors and their early diagnosis and possible treatments that would delay or prevent its development. In Iraq, 9.2% of adults on a previous medical diagnosis were DM-7.2%, which showed a rise of 2.2%. It is extremely significant with regard to the existing demand for health care services in the health system and demonstrates the severity of the problem of Type II DNin Iraq. The prevalence of 16.1 percent, according to Ali et al (2016) [5], who conducted a survey in 2013 for type 2, patients who were diabetic at a diabetic centre in Baghdad, Iraq's capital. Ali J Hashim Al-Saedi (2009) [6] examined 80 adult Iraqi diabetic patients diagnosed as having glomerular disease from January 2000 until April 2008 in native kidney biopsies. In 32 patients (40 per cent), diagnostic diarrhoea was observed in 16 patients (20%) and diarrheal nephroliferative diarrhoea was observed in 20 patients (25%), Minor disease change in eight patients (10%), and renal amyloidosis in 4 patients (5%). Diabetic patients undergoing renal biopsy were diagnosed with membrane proliferative GN in the most common histologic diagnosis. The cross-sectional studying of 5,186 patients in the 3 provinces of Kurdistan in Iraq was performed by Nojdar Salahuddin M Ali et al (2019) [7]. Most diabetic patients suffered as a result of hyperlipidemia (38.4%) and hypertension (37.7%), while some (0.7%) patients suffered stroke. Some DM problems with demographic variables have been substantially associated with this. Differences among different variable groups have been highly significant during this research. Results showed a substantial increase (positive correlation) with disease duration, with the levels of HbA1c, fasting blood sugar (FBS), RBS and blood sugar (RBS). The prevalence of type 2 diabetes in Iraq was estimated at 2 million in 2007, or 7.43 percent of the total Iraqi population. Case control analysis on 66 Type II diabetes mellits, T2 DM and ND Mellitus subjects, Al-Najaf Area, Iraq has reported serum erythropoietin levels as significantly decreased in T2DM patients in March 2016 to May 2016 compared to safe control subjects, with and without the use of microalbuminuria. Zainab Abbas Jwad et al., (2018)[8] Concentrations of microalbuminuria have increased dramatically compared with those of healthy control subjects in patients with T2 DM. Iraqi doctors found that DM just about forty years ago wasn't so usual. In 1993, a survey of 9.3% of the population between the ages of 30–69 was conducted in the town of Mosul.6 Type 2 DM affects approximately 30% of the Bahraini population[9]. In contrast to Calcitriol, alfacalcidol is superior to calcitriol because of the low costs. The occasional oral administration of alfacalcidol to groups of Type II DNpatients might, in our hypothesis, be at least as successful as SHPT-control intravenous treatment (IV) in the sense of KDIGO guidelines[10]. In addition, IV moving to oral administration may also be a cost-effective solution. The present investigation aimed to determine the circulating levels of 1-alpha-hydroxyvitamin D3 in a group of patients with DM2 nephropathy, establish the state of oxidative stress in the study population, and to determine whether there is a relationship between serum concentrations of 1-alpha-hydroxyvitamin D3 and oxidative stress status in controlled Type II DNpatients of attended to Al-Sader Teaching Medical City,Najaf, Iraq.

METHODOLOGY

Type of Study: Descriptive cross-sectional study[11].
Period of Study: February 2020 until August 2020
Location of the Study: Al-Sader Teaching Medical City located in the city of Najaf, Najaf governorate, Iraq
Sample collection: This study included 38 patients with Type II Diabetic Nephropathy (group 1), recruited from the outpatient endocrinology service of Al-Sader Teaching Medical City located in the city of Najaf, Najaf governorate, Iraq. In addition, a control group composed of 36 volunteers from medical staff and relatives (group 2) was evaluated.
Inclusion Criteria: Patients diagnosed with Type II Diabetic Nephropathy for those who use alfacalcidol, 0.25 µg daily for a period of not less than six months. Age greater than or equal to 45 years and less than 65 years. Glycated haemoglobin less than 10%, in management with diet, physical exercise and / or metformin. Patients who accepted the conditions of the study signed the Free and Informed Consent Term (ICF) before any procedure; The control group, similar to the case group, was composed of people over 20 years of age, with normal serum creatinine levels, with no known diseases or use of medications, and who signed the ICF before any study procedure.
Exclusion Criteria: Patients with smoking, HBs Ag positive and those with Nephrotic syndrome were excluded from the current study except those on using of lipid-lowering agents and / or angiotensin converting enzyme inhibitors and / or angiotensin type I receptor agonists were not an exclusion criterion.
Blood sampling: After twelve hours of fasting, 5 mL of venous blood is extracted with the venoject system in 2 vacutainer tubes, which were centrifuged at 3500 revolutions for 5 minutes and the serum obtained was stored at -80°C for subsequent measurement of vitamin D and oxidative stress levels.
Determination of circulating levels of vitamin D: It was determined by the competitive chemiluminescence method in two phases; according to the previously described methodology[12]. The use of the test is intended for the quantitative determination of circulating levels of vitamin D in human serum and plasma, and allows evaluating the availability of vitamin D3 is considered when the levels are below 10 ng / mL, insufficiency between 11 and 29 ng / mL and optimal level when concentrations are higher than 30 ng / mL[13].
Determination of oxidative stress levels
Pro-oxidant state. The lipid peroxidation test was carried out by the colorimetric method of substances reactive to thiobarbituric acid according to the methodology previously described[14]. In this technique the proteins are precipitated with thiochloroacetic acid (TCA); malondialdehyde (MDA), produced during lipid peroxidation, reacts with thiobarbituric acid (TBA) to generate a pink coloration. Then the absorbance of the supernatant is measured spectrophotometrically at a certain wavelength according to the kit used. The results were expressed in micromolar of malondialdehyde per micro liter of serum µM / µL.
TBARS procedure: For the determination of TBARS method adopted from the previous study by Tarcin et al (2010)[15]
Antioxidant status: The superoxide dismutase (SOD) technique was used based on the method described by Chee Lee. et al (2018),[16] in which the enzymatically or photochemically generated O-radical is measured. The results are expressed in units per milliliter (U / ml).
Statistical Analysis: A mean determination was made to all variables. For the evaluation of the data on the levels

of vitamin D, the Fisher test was used since it presents a statistical goodness of fit similar to that of chi square or Pearson and is the most appropriate for statistical analysis when the sample size is little. The Spearman correlation (Rho) was used to estimate the strength of association between the variables, as an equivalent Pearson correlation test for non-parametric data.

Ethical Considerations: The present work was presented to the Ethics Committee of the Najaf Health Directorate. The patient signed an informed consent according to the requirements of the Ministry of Health of Iraq.

RESULTS

The clinical characteristics of patients with Type II DM and control are shown in Table 1. Regarding the prevalence of systemic arterial hypertension, 81% of patients with Type II DM were hypertensive, while 19% were normotensive. The laboratory characteristics of patients with Type II DM and the control group are shown in Table 2.

Table 1. Clinical characteristics of patients with Type II Diabetic Nephropathy (DN) and the control group.

	Gender (M / F)	**Age years**	**D-2DM[a] (years)**	**SBP[b](mmHg)**	**DBP[c] (mmHg)**	**BMI[d] (Kg/m^2)**
Type II **DN**	25/13	28.8 ± 7.9	13.2 ± 6.6	120.3 ± 13.6	76.1 ± 9.6	24.2 ± 3.9
Healthy Controls	17/19	24.5 ± 2.0	-	118.2 ± 10.5	72.3 ± 9.7	25.6 ± 4.6
p	not signiifcant	not signiifcant	-	not signiifcant	not signiifcant	p <0.05

[a]**D-2DM** = Duration of DNType 2; [b]SBP = Systolic Blood Pressure; [c]DBP = Diastolic Blood Pressure; [d]BMI = Body Mass Index.

The number of patients at different stages is evaluated in terms of vitamin D3 levels, even in patients with type II diabetes Mellitus nephropathy (Figure 1). Regarding diabetes nephropathy, 9 patients (22.6%) were found to be naturally albuminuric, 26 (68%) to have microalbuminuria, and 4 (11%) to have macroalbuminuria. In Type II **DN** and patients with normal and decreased levels of vitamin D, the average values of microalbuminuria converted into log 10 are compared in Table 3.

Table 2. Laboratory characteristics of patients with Type II Diabetic Nephropathy and the control group.

	% of Hb.A1c	Avaerage levels of Vitamin D (ng/mL)	G.F.R (mL/min)	Macro-albuminuria (mg / 24h)	Micro-albuminuria (log10mg / 24h) *
Type II **DN**	10.3 ± 3.2	24.2 ± 7.4	95 ± 20	236.2 ± 690.7	1.78 ± 0.36
Healthy Controls	5.3 ± 0.2	24.9 ± 10.2	93 ± 10	-	-
p	p <0.05	not signiifcant	not signiifcant	-	-

*($\log_{10}$) = transformation of micro-albuminuria values to $\log_{10}$; (G.F.R) = Glomerular Filtration Rate; Hb. A1c glycated haemoglobin.

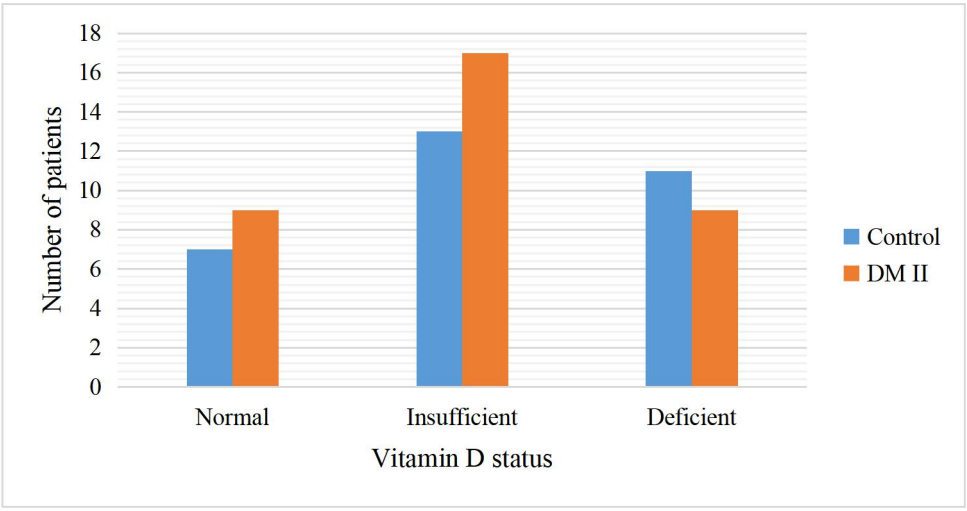

Figure 1. Status according to vit. D levels in patients with Type II **DN** and in the control group.

Table 3. Log_{10} of albuminuria and vitamin D levels in patients with Type II **DN**.

Vitamin D levels (ng / mL)

	Normal	Changed
Log_{10} Albuminuria	1.5 ± 0.08	1.93 ± 0.6

Table 4. Vit.D intensities and nephropathy phases in patients of Type II **DN**.

	Stages of nephropathy		
	Normo.albuminuria (mg/24h)	Micro-albuminuria (mg/24h)	Macro-albuminuria (mg/24h)
Vit. D (ng/mL)	27.01 ± 5.2	25.1 ± 5.9	14.9 ± 8.4

The levels of vitamin D are shown in Table 4 in the various stages of diabetic nephropathy. Comparing the levels of vitamin D of patients with Type II **DN**, only those with normoalbuminuria and microalbuminuria have no substantial difference.

In the analysis, different letters represent statistically significant differences ($p < 0.05$). When assessing vitamin D levels in relation to the degree of diabetic nephropathy, we found a progressive drop in these levels as the nephropathy worsens (Fig.2). When albuminuria levels were analyzed according to the status of vitamin D levels, we found similar behaviors (Fig. 3). Additionally, there was no correlation between vitamin D levels and GFR in patients with Type II **DN.**

Our simple linear regression model with dependent variable albuminuria and independent variable vitamin D showed an $R^2 = 0.24$ with $p < 0.01$. This indicates that at every 1 ng / mL vitamin D increases, the urinary albuminuria excretion would result in a 43 mg / g reduction of creatinine. The gradual reverse model revealed the independent predictor of urinary albumin release of vitamin D, HB1Ac and DDM level. We observed a rise in the predictive value of the urinary excretion albumin in contrast to 1-top-(OH) level D3 alone ($r2=0.34$ and $p<0.01$), when these three variables were used as predictors in a multiple linear regression model. Normal levels of vitamin D in normoalbuminuria patients. Mean levels of vitamin D in microalbuminuria patients. Normal levels of macroalbuminuria vitamin D in patients.

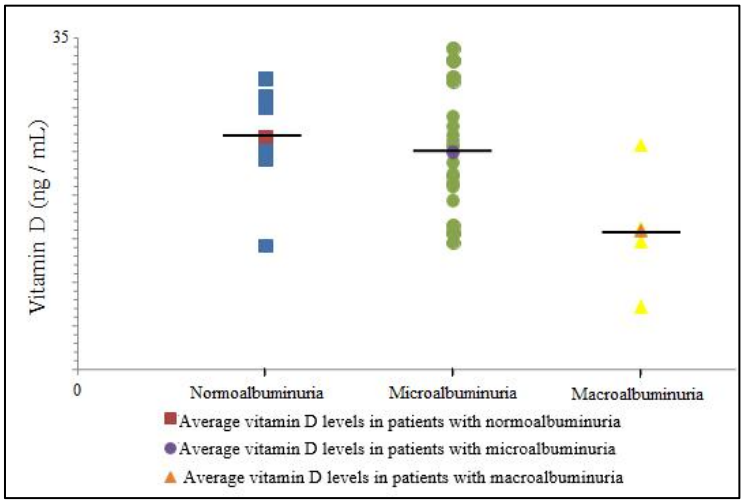

Figure 2. Correlation amongst Vit. D levels and the degree of diabetic nephropathy.

- **Status of vitamin D levels:** Average levels of albuminuria in patients with normal vitamin D levels. Average levels of albuminuria in patients with insufficient vitamin D levels. Average levels of albuminuria in patients with deficient vitamin D levels.

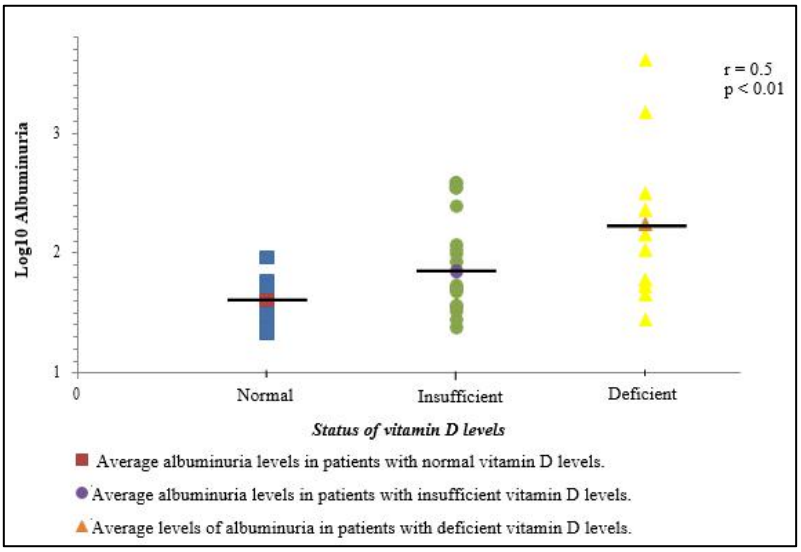

Figure 3. Correlation between albuminuria concentrations and vitamin D status in Type II **DN** patients.

Circulating levels of Vitamin D3 were used as markers of the status of vitamin D sufficiency[17], since these represent the sum of its cutaneous production and ingestion oral vitamin D3[18].

The average value of the levels of 1,25 α hydroxyvitamin D3 was 27.7 ± 5.7 ng / ml, 32% of the sample presented adequate levels of vitamin D (concentrations greater than 30 ng / ml) while 68% presented insufficient levels (Values between 10 - 29.9 ng / ml). On the other hand, there were no cases of vitamin deficiency for control group (Table 5.).

Table 5. Classification of The Levels of 1,25 α hydroxyvitamin D3 ng / mL

	Insufficiency Levels between 10-29.9 ng / ml	TOTAL
DM II nephropathy parientes	26	38
Control	Nil	26

- **TBARS levels (Proxidant State):** The values of the pro-oxidant status indicator TBARS are shown in table 6. The average level of malondialdehyde concentration (MDA) was 15.45±0.48μM / μL in **Type II** DN patients and 30.44±1.25 μM / μL in control

Table 6. TBARS Values for control and **Type II** DN Patients **(MDA μM / μL levels)**

Patient No	Gender	TBARS μM/μL	Patient No	Gender	TBARS μM/μL	Control Number	Gender	TBARS μM/μL	Control Number	Gender	TBARS μM/μL
1	M	8.9	20	F	8.81	1	F	17.09	20	M	16.92
2	M	7.9	21	M	7.98	2	F	15.17	21	M	15.32
3	M	8.9	22	M	8.81	3	F	17.09	22	M	16.92
4	F	10.9	23	M	11.01	4	F	20.93	23	M	21.14
5	F	9.9	24	M	9.80	5	F	19.01	24	M	18.82
6	M	9.9	25	F	10.00	6	F	19.01	25	M	19.20
7	M	11.9	26	M	11.78	7	F	33.21	26	M	22.62
8	M	6.4	27	M	6.34	8	F	12.29	27	M	12.17
9	M	9.9	28	M	10.00	9	F	19.01	28	M	19.20
10	F	7.9	29	F	7.98	10	F	15.17	29	M	15.32
11	M	7.9	30	M	7.98	11	F	15.17	30	M	15.32
12	M	13.9	31	M	14.04	12	F	26.69	31	M	26.95
13	M	9.9	32	F	10.00	13	F	19.01	32	M	19.20
14	M	8.9	33	M	8.81	14	F	17.09	33	M	16.92
15	M	11.4	34	F	11.51	15	F	21.89	34	M	22.11

16	M	15.9	35	M	16.06	16	F	30.53	35	M	30.83
17	F	13.4	36	F	13.27	17	F	25.73	36	M	25.47
18	F	8.4	37	M	8.48	18	F	16.13			
19	F	13.4	38	F	13.27	19	F	25.73			

6.3 SOD levels (Antioxidant Status): The values of the antioxidant status (SOD activity) are shown in table 7. The average level of SOD activity was 52.32±1.22 U / ml in Type II **DN** patients and 28.97±1.07 U / ml in control

Table 7. SOD Activity Values for control and Type II **DN** Patients SOD activity U / ml

Patient No	Gender	SOD U/ml	Patient No	Gender	SOD U/ml	Control Number	Gender	SOD U/ml	Control Number	Gender	SOD U/ml
1	M	33.60	20	F	33.26	1	F	18.82	20	M	18.63
2	M	36.75	21	M	37.12	2	F	20.58	21	M	20.79
3	M	35.70	22	M	35.63	3	F	19.99	22	M	19.95
4	F	30.45	23	M	29.84	4	F	17.05	23	M	16.71
5	F	35.70	24	M	35.34	5	F	19.99	24	M	19.79
6	M	32.55	25	F	32.88	6	F	18.23	25	M	18.41
7	M	34.65	26	M	34.58	7	F	19.40	26	M	19.37
8	M	31.50	27	M	30.87	8	F	17.64	27	M	17.29
9	M	33.60	28	M	33.26	9	F	18.82	28	M	18.63
10	F	45.15	29	F	45.60	10	F	25.28	29	M	25.54
11	M	38.85	30	M	38.77	11	F	21.76	30	M	21.71
12	M	27.30	31	M	26.75	12	F	15.29	31	M	14.98
13	M	33.60	32	F	33.26	13	F	18.82	32	M	18.63
14	M	33.60	33	M	33.94	14	F	18.82	33	M	19.00
15	M	30.45	34	F	30.39	15	F	17.05	34	M	17.02
16	M	32.55	35	M	31.90	16	F	18.23	35	M	17.86
17	F	28.35	36	F	28.07	17	F	15.88	36	M	15.72
18	F	37.80	37	M	38.18	18	F	21.17			
19	F	51.45	38	F	51.35	19	F	28.81			

- **Glycosylated Haemoglobin Values:** The percentages of glycated hemoglobin are presented in Table 8. The average value of glycated hemoglobin (Hb A1c) is 9.96% ± 0.24 for DB II Patients and 5.50 ± 0.11 for control public. It should be remembered that an inclusion criterion was presenting an Hb A1c <10%.

Table 8. Percentage of Glycosylated Haemoglobin for control and Type II **DN** Patients %HG A1c

Patient No	Gender	**%HG A1c**	Patient No	Gender	**%HG A1c**	Control Number	Gender	**%HG A1c**	Control Number	Gender	**%HG A1c**
1	M	6.67	20	F	7.00	1	F	3.73	20	M	3.92
2	M	7.07	21	M	6.93	2	F	4.24	21	M	4.16
3	M	6.36	22	M	6.68	3	F	3.18	22	M	3.34
4	F	6.57	23	M	6.89	4	F	3.61	23	M	3.86
5	F	6.46	24	M	6.79	5	F	3.62	24	M	4.07
6	M	6.67	25	F	6.53	6	F	4.00	25	M	3.27
7	M	7.07	26	M	7.42	7	F	3.54	26	M	4.16
8	M	6.77	27	M	7.11	8	F	3.72	27	M	4.26

9	M	5.86	28	M	6.15	9	F	3.28	28	M	3.08
10	F	6.06	29	F	5.94	10	F	3.64	29	M	3.33
11	M	6.87	30	M	7.21	11	F	3.43	30	M	4.33
12	M	6.67	31	M	7.00	12	F	3.67	31	M	3.50
13	M	6.26	32	F	6.58	13	F	3.51	32	M	3.68
14	M	6.77	33	M	6.63	14	F	4.06	33	M	3.98
15	M	6.97	34	F	7.32	15	F	3.48	34	M	3.66
16	M	6.46	35	M	6.79	16	F	3.56	35	M	4.07
17	F	5.96	36	F	6.26	17	F	3.34	36	M	3.13
18	F	6.57	37	M	6.43	18	F	3.94			
19	F	6.87	38	F	7.21	19	F	3.43			

- **Vitamin D and TBARS**: The relationship between vitamin D concentrations and TBARS levels is presented in figure 4. According to Spearman's Rho test of 0.03, it is not possible to establish a dependency relationship between the variables.

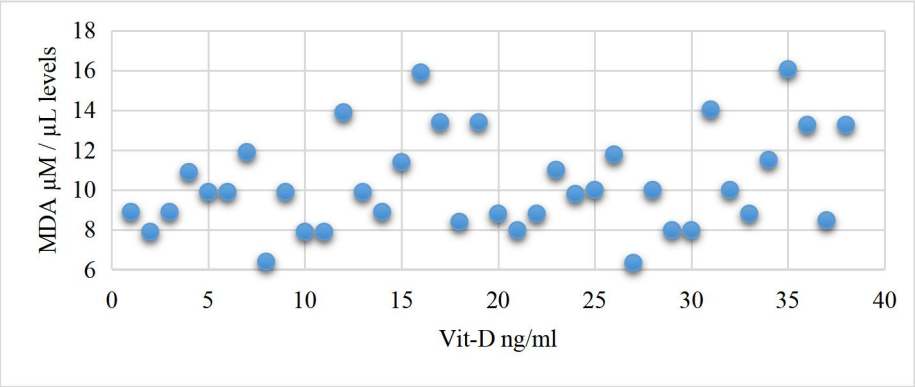

Figure 4: Relationship between Vitamin D and TBARS

- **Vitamin D and SOD:** The behavior of the relationship between vitamin D concentrations and SOD activity is presented in figure 5, where the Spearman Rho test yields a value of 0.22 which indicates that there is no relationship between these two variables.

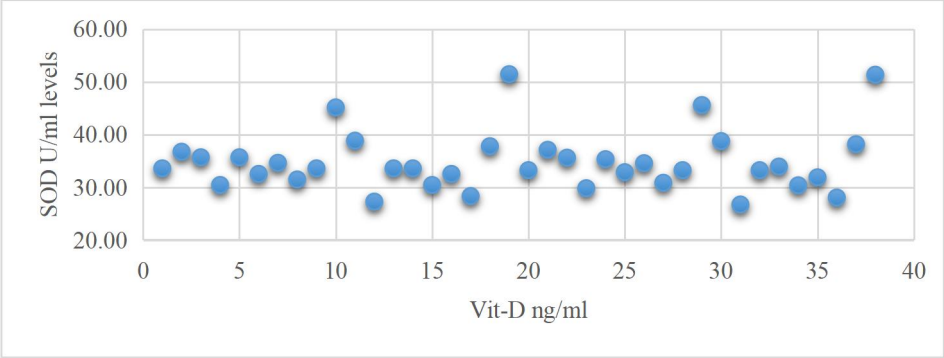

Figure 5: Relationship between vitamin D concentrations and SOD activity

- **Glycosylated Hemoglobin and Vitamin D:** Vitamin D and glycated hemoglobin are independent variables (Rho spearman 0.10)
- **Glycosylated hemoglobin and TBARS:** Spearman's Rho between glycated hemoglobin and oxidative stress was - 0.09, which does not indicate a relationship between these two variables.
- **Glycosylated hemoglobin and SOD**: Glycated hemoglobin and SOD activity did not show a relationship (Rho Spearman 0.12)
- **Glycosylated hemoglobin and vitamin D**: Vitamin D and glycated hemoglobin are independent variables (Rho spearman 0.10)
- **Glycosylated hemoglobin and TBARS**: Spearman's Rho between glycosylated

hemoglobin and oxidative stress was -0.09, which does not indicate a relationship between these two variables.

- **Glycosylated Haemoglobin and SOD**: Glycated haemoglobin and SOD activity did not show a relationship (Rho Spearman 0.12)

DISCUSSION

Our data show that decreased vitamin D levels are associated with diabetic nephropathy and its existence and severity[19]. Few studies[20] have evaluated vitamin D levels during diabetic nephropathy in early stages and have reported contradictory results in their relationship with the existence of microalbuminuria. In other cases, in three groups (23 patients with DM nephropathy, 25 patients with Type II DN, micro-albuminuria and 24 checks), Verroti et al (1999) [21] compared vitamin D levels and urine albumin excretion. In patients with microalbuminuria, their results showed lower levels of 1- α- (OH) D3 than in other categories. Our results support Verroti et al. (1999) definition. Though we have not discerned a difference among normal type II DM nephropathy patients with 1- α- (OH) D3 mean levels with microalbuminurics, our study has shown a good connexion between decrease in vitamin D levels and the development of diabetical nephropathy stages. Moreover, our data show the lack of a correlation between vitamin D and GFR-tested renal function similar to the figures mentioned in Pradeep, Dabla. (2010). [22] In our sample the hypovitaminosis D prevalence was very high (78%) among control subjects and there was no difference between the patients with DM II with 73%. There were no variations between the 1- α- (OH) D3 means and the vitamin D status of the two classes. In patients with newly diagnosed DM II, Xiao et al (2016) [23] showed high rates of vitamin D deficiency in comparison with controls in his study. Our control subjects considered overweight, mean BM I levels, with higher levels than the type II nephropathy patients. It has been stated that overweight is associated with hypovitaminosis D[24], so that the fact that vitamin D levels between the two groups were not different could not be justified. We do not have comprehensive studies to determine hypovitaminosis D prevalence in Iraq at present. The town of Najaf data indicate low vitamin D levels in postmenopausal women (43%)[25], whereas Ibrahim (2018) [26] found no cases of vitamin D deficiency when assessing 142 blood samples from the same population and area. However, there are no studies that will indicate the optimal supplemental level and the likelihood of a potential therapeutic impact on diabetic nephropathy during the early stages could be explained by increasing vitamin D levels to constant levels (> 30 ng, per prolonged time), regardless of the variability of the assay. The loss of vitamin D carrier proteins in these patients is one aspect which has not been studied and might raise hypotheses about the role of vitamin D in diabetes nephropathy. Hang et al., (2008)[27] studied the renal loss of BPD of diabetic patients and found a higher degree of loss of this protein in the urine in order to increase the albuminurics. It may be asked, therefore, whether a risk marker for kidney damages or a cause of the loss of this protein in the urine is present. Our regression results indicate a clear mechanism of vitamin D action independent of glycemic diabetic regulation on the excretion of urinary albumins.

A cohort study with normoalbuminuric patients that is seriously controlled, with an examination of serum vitamin D levels to verify development of nephropathy for these patients, would be an interesting design to assess the role of vitamin D as an environmental factor in diabetic nephrophathy. However, this is an ethically unsustainable research because vitamin D deficiency must be replaced after it has been detected. Via treatments of individuals with newly diagnosed microalbuminuria, even in the absence of hypovitaminosis D, the closest solution to this question will be to check the likelihood of a reversal of this disorder in order to preserve the levels of this vitamin above 30 ng / mL. A big move in treating the early stages of diabetic nephropathy will be taken when this theory had been confirmed. The low number of Normoalbuminuric patients compared to microalbuminuria was part of the deficiencies of our research. This may have arisen from the fact that there was no differenzation between the mean levels of 1- α- (OH) D3 in these two groupings, in conjunction with the great variability in albuminuria levels in patients with DM type II nephropathy.

Our work was a cross-sectional study, from which we are developing a replacement and supplementation protocol, with higher doses of vitamin D, in patients with type 1 diabetes mellitus and microalbuminuria. The circulating levels of 1,25 dihydroxyvitamin D3 resulting from the sum of the dermal production of it and the oral ingestion of vitamin D3 (Alfacalcidol) indicate the sufficiency of vitamin D. Despite a supposed higher exposure to the sun in our environment, insufficient concentrations of vitamin D have been reported in Iraq,[28] where inadequate concentrations were found in: 42% of the elderly, 24% of women with osteoporosis, 50% of adolescents without pathologies and in 60% of young adults. Vitamin D deficiency has long been shown to be common in the diabetic population and that affects mainly cardiovascular complications; It has even been considered to be a factor that increases the risk of mortality from cardiovascular disease. The mean value of hydroxyvitamin D in the present study was 27.7 ng / mL; 68% of the patients had vitamin D insufficiency; this insufficiency was independent of gender and metabolic control. It should be noted that despite the low average found, there were no cases of vitamin D deficiency, that is, levels less than 20 ng / mL of hydroxyvitamin D (which according to the categorization directly classifies deficiency).

The risk of developing cardiovascular disease has been reported to be twice as high in diabetic patients with low levels of vitamin D as in diabetics with adequate levels of vitamin D.. This could be an explanation of why some patients with Type II DNdevelop greater complications than others despite maintaining adequate long-term glycemic control. The results obtained in this study confirm that despite good long-term Metabolic control more than half of the studied group presents a degree of hypovitaminosis D that very possibly increases the risk of presenting mainly cardiovascular complications.

On the other hand, studies in patients with diagnosed Type II DNhave not shown that adequate levels of vitamin D improve insulin resistance or glucose metabolism (Shapses and Manson. 2011). In this study, glycemic control, measured by glycated hemoglobin, was adequate (<7%) even in patients who presented insufficient levels of vitamin D. Although in previously published studies it has been reported that in healthy individuals there is an inverse relationship between the low levels of vitamin D,

glucose concentration and insulin resistance, vitamin D supplementation does not modify blood glucose or insulin sensitivity. Oxidative stress can be defined as the loss of balance between the production of reactive oxygen species (ROS) and the antioxidant system. Several studies have shown that hyperglycemia alters various metabolic mechanisms, some such as insulin sensitivity and lipid metabolism, favoring oxidation reactions that increase the production of reactive oxygen species (ROS), molecules that have been implicated in the pathophysiology of chronic micro and macrovascular complications of DM2 nephropathy. It is important to clarify that in an environment of high reactivity and the short half-life of the radicals, it is difficult to estimate the pro-oxidant and antioxidant balance. TBARS has been accepted and widely used as an indirect but valid indicator in the study of radicals in serum and tissues. In various studies[29], hyperglycemia has been reported as a possible factor for increasing the production of reactive oxygen species. Oxidative stress has also been proposed to be an important cause of the development of insulin resistance, carbohydrate intolerance, damage to pancreatic beta cells, and concomitant activation of pro-inflammatory signalling pathways. A study carried out in rats with diabetes showed a greater development of oxidative stress in relation to normal rats and showed that the early control of hyperglycemia reduces oxidative stress in the long term (evaluated by the TBARS method and reduced glutathione) compared to that observed in the group of diabetic rats that did not undergo early glycemic control[30]. In the present study carried out in humans with Type II DN(with adequate control), the measurement of free radical activity is reported by the TBARS method; the levels found are higher than those described in a population of healthy individuals aged 58 ± 15 years (5 ± 1.9 µM / µL)[31].

To our knowledge, the relationship between antioxidant status and vitamin D levels in humans with type 2 diabetic nephropathy has not been evaluated in the selected area Al-Najaf, Iraq. It has been reported in rat models with diabetes that the administration of vitamin D increases oxidative stress levels and reduces antioxidant activity and on the other hand, in humans without diabetes it has been reported that vitamin D supplementation D significantly decreases oxidative stress[32]. However, in our work in humans with Type II DN (with adequate glycemic control), no relationship was found between vitamin D levels and the antioxidant status evaluated through SOD activity. The antioxidant status measured in our patients was similar to that described in patients with Type II DN and lower in normal subjects. It should be noted that the plasma concentrations of vitamin D to obtain an effect on oxidative stress are not necessarily the same to trigger other actions attributed to this vitamin. The results obtained in this study could be used as baseline to assess whether the correction of the insufficiency is accompanied by either an increase or decrease in oxidative stress in people with DM2 nephropathy. It is important to consider that neither of the two indicators of antioxidant and pro-oxidant status (SOD and TBARS) is expressed as categorical variables, the studies that use these techniques do not suggest it, but are used as a reference or base.

CONCLUSION

This study carried out in Al-Sader Teaching Medical City, Iraq, who belong to a chronic disease program, showed a deficiency of vitamin D in a high proportion of them (68%). In this cross-sectional study, in a group of patients with controlled DM2 nephropathy, the levels of pro-oxidant status indicators (TBARS) were higher than the average reported for the healthy population. In this study, in a group of patients with controlled DM2 nephropathy, the levels of antioxidant status indicators (SOD) were similar to those reported in a study with type 2 diabetic patients and lower than healthy controls. No relationship was found between vitamin D levels and antioxidant and pro-oxidant status in the diabetic patients in the study.

REFERENCES

1 Sigdel, Manoj & Rajbhandari, Nirakar & Basnet, Sandeep & Nagila, A & Basnet, Purusotam & Tamrakar, Bishal. (2008). Microalbuminuria among type-2 diabetes mellitus patients in Pokhara, Nepal. Nepal Medical College journal: NMCJ. 10. 242-5.

2 Wang, Yongji & Zhu, Jinge & DeLuca, Hector. (2014). The vitamin D receptor in the proximal renal tubule is a key regulator of serum 1α,25-dihydroxyvitamin D 3. American journal of physiology. Endocrinology and metabolism. 308. ajpendo.00422.2014. 10.1152/ajpendo.00422.2014.

3 Bhan, Ishir et al. "Nutritional vitamin D supplementation in dialysis: a randomized trial." *Clinical journal of the American Society of Nephrology: CJASN* vol. 10,4 (2015): 611-9. doi:10.2215/CJN.06910714

4 BoydJonesCarmel TMooney, Chapter 20 - Drugs used in the management of thyroid and parathyroid disease,Small Animal Clinical Pharmacology (Second Edition), 2008, Pages 498-508

5 Ali AA, Al Lami FH. Prevalence and determinants of microalbuminurea among type 2 diabetes mellitus patients, Baghdad, Iraq 2013. Saudi J Kidney Dis Transpl. 2016; 27:348–55

6 Hashim Al-Saedi AJ. Pathology of nondiabetic glomerular disease among adult Iraqi patients from a single center. Saudi J Kidney Dis Transpl 2009; 20:858-61

7 Ali NSM, Allela OQB, Salih HM, Ahmed IH. Prevalence of Type 2 Diabetes Associated Complications ion Kurdistan Region Iraq. J Basic Clin Pharma 2019; 10: 1-6

8 Zainab Abbas Jwad et al., Serum Erythropoietin Level and Microalbuminuria, Journal of Clinical and Diagnostic Research. 2018 Jun, Vol-12(6): BC05-BC08

9 Al-Sayegh AH. "Prevalence rates of cardiovascular diseases and diabetes in Mosul using an extended version of WHO (rose) Questionnaire." Ann Coll Med Mosul. 1995; 21:9-17.

10 Kidney Disease: Improving Global Outcome (KDIGO) CKD-MBD Work Group. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral

and Bone Disorder (CKD-MBD) Kidney Int. 2009;113: S1–S130

11 Golbahar, Jamal & Al-Saffar, Nida & Diab, Diab & Al-Othman, Sara & Darwish, Abdulla. (2013). Vitamin D Status in Adults: A Cross Sectional Study. Bahrain Medical Bulletin. 35. 17-22. 10.12816/0000510.

12 Brandi, Lisbet. (2008). 1alpha (OH)D3 One-alpha-hydroxy-cholecalciferol--an active vitamin D analog. Clinical studies on prophylaxis and treatment of secondary hyperparathyroidism in uremic patients on chronic dialysis. Danish medical bulletin. 55. 186-210.

13 Noorulamin, Muhammad. (2019). Biochemical Analysis and Frequency of Vitamin D Deficient People in Different Age and Gender. 260-265.

14 Kumar, Bairapogu & Itaggappa, Maliyannar & Thimmaraju, Kamandalagondi. (2017). Association of vitamin-D deficiency with oxidative stress in newly diagnosed type 2 diabetes. International Journal of Research in Medical Sciences. 10.18203/2320-6012.ijrms20175132.

15 Tarcin, Ozlem & Gogas Yavuz, Dilek & Ozben, Beste & Telli, Ahu & Velioglu Ogunc, Ayliz & Yüksel, Meral & Toprak, Ahmet & Yazici, Dilek & Sancak, Seda & Deyneli, Oğuzhan & Akalin, Sema. (2010). Effect of Vitamin D Deficiency and Replacement on Endothelial Function in Asymptomatic Subjects. Obstetrical & Gynecological Survey. 65. 173-174. 10.1097/01.ogx.0000369678.64828. 55..

16 Chee Lee, Wee & Mokhtar, siti safiah & Munisamy, Seetha & Yahaya, Sahran & Rasool, Aida Hanum. (2018). Vitamin D status and oxidative stress in diabetes mellitus. Cellular and Molecular Biology. 64. 60-69. 10.14715/cmb/2018.64.7.11.

17 Issa, Ahmed & Ibraheem, Sana. (2007). Alteration of Vitamin "D" level in Sera of Iraqi Population. Journal of Kerbala University. 5. 58-64.

18 Abdulrahman, Rabar & Rahman, Balen. (2018). Prevalence of vitamin D level in the serum of patients living in Erbil city, Iraq, referred to private clinical laboratory and effect of age and sex on it. Journal of Biological Research - Bollettino della Società Italiana di Biologia Sperimentale. 91. 10.4081/jbr.2018.6916.

19 Derakhshanian, Hoda & Shab Bidar, Sakineh & Speakman, John & Nadimi, Hoda & Djafarian, Kurosh. (2015). Vitamin D and diabetic nephropathy: A systematic review and meta-analysis. Nutrition. 31. 10.1016/j.nut.2015.04.009.

20 Agarwal, Rajiv. (2009). Vitamin D, Proteinuria, Diabetic Nephropathy, and Progression of CKD. Clinical journal of the American Society of Nephrology: CJASN. 4. 1523-8. 10.2215/CJN.02010309.

21 Verrotti, A.; et al. Calcium metabolism in adolescents and young adults with type 1 diabetes mellitus without and with persistent Microalbuminuria. Journal of endocrinological investigation. v. 22, p.198-202. 1999.

22 Dabla, Pradeep. (2010). Renal function in diabetic nephropathy. World journal of diabetes. 1. 48-56. 10.4239/wjd. v1.i2.48.

23 Xiao, Xiaoyan & Wang, Yajuan & Hou, Yanlian & Han, Feng & Ren, Jianmin & Hu, Zhao. (2016). Vitamin D deficiency and related risk factors in patients with diabetic nephropathy. Journal of International Medical Research. 44. 10.1177/0300060515593765.

24 Ahmadieh, Hala & Azar, Sami & Lakkis, Najla & Arabi, Asma. (2013). Hypovitaminosis D in Patients with Type 2 Diabetes Mellitus: A Relation to Disease Control and Complications. ISRN endocrinology. 2013. 641098. 10.1155/2013/641098.

25 Muhsin, Hydar & Almsaid, Hayder & Alsahlanee, Rasha. (2019). Relationship between vitamin D3 levels and marital status: A random study in females suffering from bone disorders in Al-Najaf province. 1. 65-70.

26 Ibrahim, Sanaa. (2018). Vitamin D Level in Patients with Bone Diseases and Dental Caries.

27 Huang, C & Ma, G & Tao, M & Ma, X & Feng, J & Liu, Q. (2008). The Relationship between Renal Injury and Change in Vitamin D Metabolism in Aged Rats with Insulin Resistance or Type 2 Diabetes Mellitus. The Journal of international medical research. 36. 289-95. 10.1177/147323000803600211.

28 Ahmed, Sakar. (2018). Vitamin D level study within the population in Sulaymaniah City – IRAQ. 10.14500/icpas2018.mim32.

29 Saif-Elnasr, Mostafa & Ibrahim, ImanM & Alkady, Manal. (2017). Role of Vitamin D on glycemic control and oxidative stress in type 2 diabetes mellitus. Journal of Research in Medical Sciences. 22. 22. 10.4103/1735-1995.200278.

30 Enciso, Patricio & Wang, Lixiang & Kawahara, Yuta & Sakamoto, Shohei & Shimada, Shingo & Takeichi, Yukina & Takayanagi, Ryoichi & Nomura, Masatoshi. (2015). Dietary vitamin D3 improves postprandial hyperglycemia in aged mice. Biochemical and biophysical research communications. 59. 10.1016/j.bbrc.2015.04.008.

31 Abdel-rahman, Emaad & Saadulla, Lawand & Reeves, William & Awad, Alaa. (2011). Therapeutic Modalities in Diabetic Nephropathy: Standard and Emerging Approaches. Journal of general internal medicine. 27. 458-68. 10.1007/s11606-011-1912-5.

32 El-Sherif, Hanaa & Al-Beladi, Hanaa & Bahamash, Khadijah & Khoja, Sawsan & Jambi, Ebtihaj & Alshubaily, Fawzia. (2019). Vitamin D status and different oxidative stress markers in diabetic patients. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 9. 1101-1113.