SERUM 25-HYDROXYVITAMIN D(3) LEVELS IN TYPE 2 DIABETIC PATIENTS WITH NORMO-, MICRO-, AND MACROALBUMINURIA

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Abstract

Background. The aim of this study was to observe the differences in serum 25-hydroxyvitamin D(3) levels in nondiabetic healthy control subject and type 2 diabetic patients, and to investigate the differences in serum 25-hydroxyvitamin D(3) levels in type 2 diabetic patients with normo-, micro- and macroalbuminuria.

Patients and Methods. Total 140 nondiabetic healthy controls and 384 type 2 diabetic patients (156 normoalbuminuric, 152 microalbuminuric and 76 macroalbuminuric) were included in the study. 25-hydroxyvitamin D(3) levels were measured *in sera* with the method of electrochemiluminescence using modular immunoassay analyzer.

Results. Vitamin D deficiency was detected in 70.85% and 22.9% of type 2 diabetic patients and nondiabetic healthy controls, respectively. Serum 25-hydroxyvitamin D(3) levels were significantly lower in type 2 diabetic patients compared to nondiabetic healthy controls (16.4 ± 9.5 ng/mL vs. 28.2 ± 11.6 ng/mL, $p=0.0001$). Serum 25-hydroxyvitamin D(3) levels were lower in albuminuric and nonalbuminuric diabetic patients (14.3 ± 7.9 ng/mL vs. 19.6 ± 10.9 ng/mL, respectively, $p=0.013$). Serum 25-hydroxyvitamin D(3) levels were 19.6 ± 10.9 ng/mL in normoalbuminuric, 14.9 ± 8.8 ng/mL in microalbuminuric and 12.9 ± 5.8 ng/mL in macroalbuminuric diabetic patients. While lower serum 25-hydroxyvitamin D(3) levels were detected both in microalbuminuric ($p=0.028$) and macroalbuminuric diabetic patients ($p=0.014$) compared to normoalbuminuric diabetic patients, 25-hydroxyvitamin D(3) levels did not change significantly between microalbuminuric and macroalbuminuric diabetic patients ($p=0.67$). Serum 25-hydroxyvitamin D(3) levels correlated negatively with urinary albumin excretion ($r=-0.24$, $p=0.016$) in patients with diabetes mellitus.

Conclusion. Findings of the present study demonstrated reduced serum 25-hydroxyvitamin D(3) levels which were significantly related with albuminuria in type 2 diabetic patients.

Key words: Vitamin D, diabetes mellitus, albuminuria.

INTRODUCTION

Vitamin D is a steroid prohormone synthesized in the skin following UV exposure or else acquired by supplemental or dietary intake. Originally, it was described as a regulator of calcium homeostasis. Furthermore, vitamin D status may influence the risk of developing metabolic diseases such as metabolic syndrome, hypertension, cardiovascular disease, insulin resistance and diabetes mellitus (1-4). Results from previous longitudinal and epidemiological studies about an association between circulating 25-hydroxyvitamin D(3) and diabetes mellitus have been heterogeneous (4-10).

Diabetes mellitus has become a major public health problem in the world. Diabetic nephropathy is the most common microvascular complication and is a major cause of end-stage renal disease that requires dialysis and/or renal transplantation (11). Albuminuria is the early sign of diabetic nephropathy. Albuminuria is defined as urine albumin-to-creatinine ratio (UACR) ≥ 30 mg/g according to American Diabetes Association Standards of Medical Care in Diabetes (12). Albuminuria can be divided into two phases, micro- and macroalbuminuria. Patients with macroalbuminuria, which is a more serious stage of diabetic nephropathy, have a poor prognosis (11, 13-15).

Epidemiological studies have shown that low 25-hydroxyvitamin D(3) levels are common in patients with albuminuria (10, 17-21). Whether serum 25-hydroxyvitamin D(3) levels can indicate the severity of diabetic nephropathy in patients with micro- and macro-albuminuria has been controversial (10, 17, 20, 21). The aim of this study was to observe the differences in serum 25-hydroxyvitamin D(3) levels in nondiabetic healthy control subject and type 2 diabetic

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patients, and to investigate the differences in serum 25-hydroxyvitamin D(3) levels in type 2 diabetic patients with normo-, micro- and macroalbuminuria.

PATIENTS AND METHODS

Total 140 (78 female and 62 male) nondiabetic healthy controls and 384 (199 female and 185 male) type 2 diabetic patients (156 normoalbuminuric, 152 microalbuminuric and 76 macroalbuminuric) living in the same geographically area (Antalya, South-west Anatolia, between the longitudes 29°20'-32°35' East and altitudes 36°07'-37°29' North) were included in the study. All patients in the study were recruited from the outpatient clinic of Nephrology Department of the Antalya Education and Research Hospital and Endocrinology Department of the Akdeniz University Hospital during September and October 2013. Demographic and clinical data, including age, sex, and duration of diabetes mellitus, weight, height, and medication were recorded. Age, gender and body mass index matched healthy subjects living in the same geographically area were included to the study as a control group. Subjects of control group were selected from individuals who did not have chronic diseases such as diabetes mellitus, hypertension, dyslipidemia and malignancy. Patient and healthy control subjects who use tobacco and alcohol were excluded from the study. Subjects using a medication which may affect vitamin D metabolism were also excluded. Subjects having any comorbidities affect patients' ability to spend time outside. Subjects were informed about the study, and their written consents were obtained. The study was performed in accordance with the principles set out in the Declaration of Helsinki.

25-hydroxyvitamin D(3) and iPTH levels were measured in sera with the method of electrochemiluminescence using modular immunoassay analyzer (Roche Diagnostics GmbH, Mannheim, Germany). For 25-hydroxyvitamin D(3) analysis, intraassay coefficients of variation (CVs) were 5.1% (control value 15.0 ng/ml) and 1.7% (control value 67.0 ng/mL) and the interassay CVs were 8.5% (control value 15 ng/mL) and 2.2% (control value 67.0 ng/ml). HbA1c was estimated by the method of turbidimetric inhibition immunoassay (Tina-quant® II) method on the Cobas 8000 autoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Serum levels of creatinine and calcium were measured with Roche Modular P otoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany)

using modified Jaffe and o-kresoltalein endpoint colorimetric methods, respectively. The urine albumin concentration was evaluated by immunoturbimetry (Tina-quant Albumin Gen.2 assay; Roche Diagnostics GmbH, Mannheim, Germany). The estimated glomerular filtration rate (GFR) was calculated in mL/min per 1.73 m² using the simplified Modification of Diet in Renal Disease equation $(186.3 \times (\text{serum creatinine})^{-1.154} \times \text{age}^{-0.203})$, multiplied by 0.742 for women (22).

25-hydroxyvitamin D(3) levels below 20 ng/mL were accepted as vitamin D deficiency, levels between 21-29 ng/mL as insufficiency, and above 30 ng/mL as sufficient levels (23).

All patients with type 2 diabetes were instructed to collect a 24-hour urine sample to measure the urinary albumin excretory rate. According to the urinary albumin excretory rate, patients were divided into three groups. Normo-, micro- and macroalbuminuria were defined as urinary albumin excretion rates of <30 mg/24 hours, between 30 and 300 mg/24 hour, and ≥300 mg/24 hours, respectively (14).

Statistical Analysis

Statistical analysis was performed using SPSS version 21.0. Continuous variables were tested for normality according to the Kolmogorov-Smirnov test. Differences in various clinical parameters between different groups were analysed using the chi-square, Anova and Mann-Whitney U tests. Correlation analysis was performed by Spearman's correlation test. Differences were considered statistically significant for p values less than 0.05.

RESULTS

There was a similar male/female ratio in diabetic and control groups ($p > 0.05$). In addition, 25-hydroxyvitamin D(3) levels were similar in male and female subjects in diabetic and control groups ($p > 0.05$). Vitamin D deficiency was detected in 70.85% and 22.9% of type 2 diabetic patients and nondiabetic healthy controls, respectively, in our study (Table 1). Serum 25-hydroxyvitamin D(3) levels were significantly lower in type 2 diabetic patients compared to nondiabetic healthy controls (16.4 ± 9.5 ng/mL vs. 28.2 ± 11.6 ng/mL, $p = 0.0001$) (Table 2).

Serum 25-hydroxyvitamin D(3) levels were lower in albuminuric and nonalbuminuric diabetic patients (14.3 ± 7.9 ng/mL vs. 19.6 ± 10.9 ng/mL, respectively, $p = 0.013$). Serum 25-hydroxyvitamin D(3)

Table 1. 25-hydroxyvitamin D(3) levels in diabetic and control groups

25-hydroxyvitamin D(3) levels	Diabetes mellitus N (%)	Control N (%)	P value
<20 ng/mL	272 (70.85)	32 (22.9)	0.001
20 – 30 ng/mL	80 (20.85)	44 (31.4)	0.01
<30 ng/mL	352 (91.7)	76 (54.3)	0.001
>30 ng/mL	32 (8.3)	64 (45.7)	0.001

Table 2. Comparison of diabetic and control groups

	Diabetes mellitus N=384				Control N=140	P value*
	Total N=384	Normoalbuminuria N=156	Microalbuminuria N=152	Macroalbuminuria N=76		
Age (years)	55.5 ± 10.9	56.1 ± 10.1	54.5 ± 10.4	55.7 ± 9.4	51.7 ± 11.3	0.08
HbA1c (%)	8.2 ± 1.9	8.3 ± 1.6	8.1 ± 1.0	8.2 ± 2.2	5.5 ± 0.4	0.0001
Creatinine (mg/dL)	0.9 ± 0.3	1.0 ± 0.4	0.9 ± 0.2	1.0 ± 0.5	0.8 ± 0.1	0.01
Glomerular filtration rate (mL/min/1.73 m ²)	86.4 ± 20.4	90.4 ± 21.9	84.4 ± 24.9	85.8 ± 32.4	91.1 ± 17.4	0.22
Calcium (mg/dL)	9.8 ± 0.9	9.9 ± 0.8	9.7 ± 0.7	9.8 ± 9.9	9.9 ± 0.8	0.9
PTH (pg/mL)	49.9 ± 35.4	46.1 ± 32.5	51.6 ± 35.1	52.0 ± 29.7	49.2 ± 15.4	0.8
25OH-D (ng/mL)	16.4 ± 9.5	19.6 ± 10.9	14.9 ± 8.8 ^a	12.9 ± 5.8 ^b	28.2 ± 11.6	0.0001
Albuminuria (mg/day)	188.5 ± 283.0	15.5 ± 8.3	222.5 ± 77.0	459 ± 315.0	17.5 ± 8.2	0.0001

*. P value for comparison of diabetic and control groups.

a: p=0.028, for comparison of microalbuminuric and normoalbuminuric diabetic patients.

b: p=0.014, for comparison of macroalbuminuric and normoalbuminuric diabetic patients.

levels were 19.6 ± 10.9 ng/mL in normoalbuminuric, 14.9 ± 8.8 ng/mL in microalbuminuric, and 12.9 ± 5.8 ng/mL in macroalbuminuric diabetic patients. While lower serum 25-hydroxyvitamin D(3) levels were detected both in microalbuminuric ($p=0.028$) and macroalbuminuric diabetic patients ($p=0.014$) compared to normoalbuminuric diabetic patients, 25-hydroxyvitamin D(3) levels did not change significantly between microalbuminuric and macroalbuminuric diabetic patients ($p=0.67$).

Serum 25-hydroxyvitamin D(3) levels correlated negatively with urinary albumin excretion ($r=-0.24$, $p=0.016$) and serum PTH levels ($r=-0.33$, $p=0.001$) in patients with diabetes mellitus. On the other hand, serum 25-hydroxyvitamin D(3) levels did not correlate significantly with glomerular filtration rate ($r=0.07$, $p=0.4$). Albuminuria correlated positively with HbA1c ($r=0.24$, $p=0.018$) and serum creatinine levels ($r=0.3$, $p=0.002$) and negatively with glomerular filtration rate ($r=-0.3$, $p=0.004$) in patients with diabetes mellitus.

To further investigate the relationship between 25-hydroxyvitamin D(3) categories (<20, 20-30 and >30 ng/mL, respectively) and albuminuria categories (normo, micro and macroalbuminuria), low 25-hydroxyvitamin D(3) levels were related to albuminuria ($X^2=12.763$, $p=0.012$).

DISCUSSION

It has been estimated that more than 1 billion people worldwide have vitamin D deficiency or insufficiency. Minorities are more likely to be deficient in vitamin D because of low dietary intake, low exposure to solar ultraviolet B radiation and reduced vitamin D production. In the present study, serum 25-hydroxyvitamin D(3) levels were markedly low both in type 2 diabetic patients (16.4 ± 9.5 ng/mL) and nondiabetic healthy control subjects (28.2 ± 11.6 ng/mL). 25 hydroxyvitamin D(3) levels of 91.7% of our diabetic patients and of 54.3% of our nondiabetic healthy control subjects were below the level of 30 ng/mL.

Low vitamin D status, measured by serum 25-hydroxyvitamin D(3) levels, for a long time is known to be a risk factor for osteoporosis, and it has recently been linked to premature death and the occurrence variety of other chronic diseases such as obesity, metabolic syndrome and diabetes mellitus (1-4). Apart from inflammatory mechanisms, an increased inhibition of pancreatic B-cell function and an intensified insulin resistance in peripheral cells in the state of vitamin D deficiency have been documented by experimental and clinical studies (24, 25).

Majority of epidemiological evidence is suggestive for an association between circulating 25-hydroxyvitamin D(3) and diabetes mellitus (3-

10). Our findings also demonstrated lower serum 25-hydroxyvitamin D(3) levels in type 2 diabetic patients regardless of their kidney function, which were in accordance with the majority of previous epidemiological studies. Furthermore, findings of the present study highlighted the need to improve screening for vitamin D deficiency in patients with type 2 diabetes mellitus, especially in minority populations, because vitamin D is known to have a role in decreasing the risk of many chronic illness, including cancer, cardiovascular diseases, and infectious disease (26). This is especially pertinent for individuals with diabetes, who are at a higher risk of developing these conditions than the non diabetic population and thus may receive a greater benefit from having higher vitamin D levels (27-29).

Lack of sun exposure is one of the primary causes of epidemic vitamin D deficiency worldwide. Yilmaz *et al.* (30) reported Vitamin D deficiency in 73% of type 2 diabetic patients in Ankara region of Turkey. In the present study we evaluated vitamin D levels in a specific period of the year (September to November) at a particular geographical area (Antalya, South-west Anatolia, between the longitudes 29°20'-32°35' East and latitudes 36°07'-37°29'North) and only in type 2 diabetic patients from a specific nation (Turkish) and determined Vitamin D deficiency in 70.85% of our type 2 diabetic patients.

Type 2 diabetes mellitus may give rise to many microvascular and macrovascular complications, leading to high morbidity and mortality. Low blood 25OH-D concentrations are associated with an increased risk of macrovascular and microvascular disease events in type 2 diabetes (31).

Diabetic nephropathy is the most common microvascular complication and also risk factor for cardiovascular disease and leading cause of chronic kidney disease. Epidemiological studies have shown that low 25-hydroxyvitamin D(3) levels are common in patients with albuminuria (10, 17-21). Whether serum 25-hydroxyvitamin D(3) levels can indicate the severity of diabetic nephropathy in patients with micro-and macro-albuminuria has been controversial (10, 17, 20, 21). Huang *et al.* (17) reported that low serum 25-hydroxyvitamin D(3) levels were common in type 2 diabetic patients with albuminuria, particularly in patients with macroalbuminuria, but not in those with microalbuminuria. In contrast to Huang *et al.*, but in agreement with our findings, Damasiewicz *et al.* (20) reported an independent association of 25-hydroxyvitamin D(3) deficiency with

microalbuminuria, but not with macroalbuminuria. The small proportion of participants with macroalbuminuria may be an important factor in the observed contrasting findings.

A factor that may impact the differential development of diabetic nephropathy is vitamin D. The role of the kidney in the hydroxylation of the vitamin D metabolite 25-hydroxycalciferol from the liver to the biologically active form of 1,25(OH)₂D(3) is well established (1, 32). Studies suggest that, in addition to the impact that chronic renal failure has on increasing the likelihood of 1,25(OH)₂D(3) deficiency and insufficiency caused by the kidney's role in vitamin D metabolism, vitamin D deficiency and insufficiency also have an active role in the progression of kidney disease (33-39). Inhibition of the renin-angiotensin-aldosterone system and blocked Wnt/β-catenin signaling pathway by the vitamin D have been demonstrated; animal studies suggest that receptor-mediated vitamin D actions have a renoprotective role in diabetic nephropathy (33, 34, 38). These findings suggest that 1,25(OH)₂D(3) administration attenuates the development of glomerulosclerosis and interstitial fibrosis, and the progression of proteinuria through parathyroid hormone-independent antiproliferative actions and decreases in podocyte loss and podocyte hypertrophy (35, 36). Adequate levels of vitamin D are also associated with decreased insulin resistance and reduced blood pressure, potentially modifiable risk factors for diabetic nephropathy (11, 26). However, little information is available about the association between diabetic nephropathy and vitamin D levels in humans.

In our study, serum 25-hydroxyvitamin D(3) levels were low in type 2 diabetic patients with albuminuria and there was a negative correlation between serum 25-hydroxyvitamin D(3) levels and urinary albumin excretion. Low serum 25-hydroxyvitamin D(3) levels were detected both in microalbuminuric and macroalbuminuric diabetic patients as compared to normoalbuminuric diabetic patients. Surprisingly, 25-hydroxyvitamin D(3) levels did not differ significantly between microalbuminuric and macroalbuminuric diabetic patients. These results should be interpreted with caution given that we had a relatively small sample size of macroalbuminuric patients.

Because of the cross-sectional nature of the present study, we were unable to determine whether the observed association was present because the vitamin D deficiency increased the risk of albuminuria or

because the albuminuria increased the risk of vitamin D deficiency. Previous studies suggested that both these interactions may occur simultaneously. Our study evaluated 25-hydroxyvitamin D(3) which is the circulating metabolite produced in the liver metabolized later in kidneys to 1,25 (OH)₂ D(3). Based on this well-established pathway, it might be proposed that albuminuria might not be the reason for the observed low levels of 25 hydroxyvitamin D(3) in the present study.

Present study had some limitations. As previously mentioned the cross-sectional nature of the data limited inference and only allowed for the identification of associations. We were only able to measure 25 hydroxyvitamin D(3) levels and could not evaluate the role of the kidney in metabolizing this form of vitamin D to the biologically active metabolite 1,25(OH)₂ D(3). Nevertheless, it should also be noted that measurement of 25 hydroxyvitamin D(3) levels is an accepted approach for the evaluation of vitamin D status since only a small amount of 25 hydroxyvitamin D(3) is metabolized in the kidney (1, 32).

In conclusion, findings of the present study demonstrated reduced serum 25-hydroxyvitamin D(3) levels which were significantly related with albuminuria in type 2 diabetic patients.

Conflict of interest

There was no any disclosing and financial or personal relationship with other people or organisations that could inappropriately influence our work.

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