STATUS OF VITAMIN D AMONG TURKISH ADULTS WITH TYPE 2 DIABETES MELLITUS IN PRIMARY HEALTH CARE

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ABSTRACT

Aims: *The aim of our study was to investigate the prevalence and correlates of 25-hydroxy vitamin D (25(OH)D) deficiency and the relationship between 25(OH)D and the type 2 diabetes mellitus (T2DM) in primary health care.*

Materials and methods*: One hundred and thirty four Turkish adults with T2DM from the Family Medicine outpatient clinic and 134 non-diabetic, healthy controls were included in the study. We measured serum 25(OH)D, calcium, low density lipoprotein (LDL), insulin, parathormone (PTH) and blood fasting glucose and compared the results between the T2DM group and matched healthy control subjects.*

Results*: Both the T2DM and healthy groups had vitamin D deficiency. The mean age for those with T2DM was 58.00 ± 12.03 years versus 46.76 ± 15.70 years among the controls. The mean levels of 25(OH)D were significantly lower in the T2DM adults than in the controls (11.85 ± 8.51 ng/ml versus 15.33 ± 17.42 ng/ml). In the T2DM adults, 35.8% were mildly (10-20 ng/ml), 35.8% were moderately (5-10 ng/ml), and 17.2% were severely (<5 ng/ml) vitamin D deficient as compared with 26.1% (mildly), 41.8% (moderately), and 11.9% (severely) in the control group. Overall, 88.8% of the T2DM patients and 79.9% of the healthy controls were vitamin D deficient.*

Conclusion*: This study demonstrated prevalent vitamin D deficiency in patients with T2DM and normal subjects. The population in our study was generally deficient in 25(OH)D indicating a greater need for vitamin D supplementation.*

Key words*: Type 2 diabetes mellitus, vitamin D; HbA1c, insulin sensitivity, 25(OH)D.*

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Introduction

Diabetes mellitus is a metabolic disorder with a high prevalence across the world[1]. Diabetes affects more than 300 million individuals worldwide with significant morbidity and mortality[2]. In the United States, it has been estimated that the incidence is about 1 million new cases per year[3]. It was detected that prevalence of diabetes in Turkish population was 13.7% according to TURDEP II study[4].

Vitamin D deficiency has recently emerged as one of the factors contributing to the development of both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM)[5-11]. It has been suggested that vitamin D reduces the risk of diabetes and antithetically vitamin D deficiency might play a role in occurrence of diabetes mellitus[12]. The finding that 25-hydroxy vitamin D (25(OH)D) deficiency is associated with impaired cell function and insulin resistance in animals[13, 14] and humans[15, 16] is in line with that hypothesis. Many cross-sectional studies have reported inverse associations between the serum 25(OH)D level and both insulin sensitivity and T2DM[17-22]. In a study of 700 individuals at risk for T2DM and/or metabolic syndrome, it was shown that the serum 25(OH)D level was independently associated with insulin sensitivity and β-cell function[23]. Longitudinal cohort studies have also established an association between 25(OH)D status and the risk of incident T2DM[24-27].

In the largest prospective study, with 11 000 participants at baseline and 6500 who returned for a follow-up evaluation after five years, it was

found that the baseline 25(OH)D concentration was inversely correlated with the fasting plasma glucose level and the homeostatic model of assessment for insulin resistance (HOMA-IR) at fifth year[28].

In recent years, studies have shown that using 25(OH)D can help decrease the incidence of diabetes and adjustment of insulin and glucose[29-31]. Surveys demonstrated that consuming supplements of calcium with 25(OH)D will significantly reduce fasting blood glucose level[32]. So, using supplements of 25(OH)D alone can prevent the incidence of diabetes[33]. Recently, some studies have shown that the prevalence of vit D deficiency has increased and more than 50% of adult age suffer from this deficiency(34). The prevalence of vit D deficiency in the USA and Turkish adult age was reported to be about 25% to 54% and 51.8%, respectively[35, 36].

The aim of this study is to assess the 25(OH)D status among Turkish adults with T2DM in comparison to healthy controls, and the relationship with glycemic control in primary health care.

Materials and methods

The study sample included Turkish participants diagnosed with T2DM. This cross-sectional study was done from March 2014 to April 2014 in the outpatient clinics of Family Medicine department of the Bezmialem Vakif University Hospital in the largest city of Turkey, namely Istanbul, in the North-west region of the country. Participating 134 subjects with T2DM (more than 6 months duration), and age and sex matched 134 healthy control subjects with no acute or chronic problems were randomly selected and recruited in the study.

Patients taking multi-vitamin supplementation or having hepatic, renal or metabolic bone disorders (including parathyroid related problems) were excluded from the study. In addition, those patients having history of malabsorption syndromes such as celiac disease or active malignancy or with active infection and also patients suffering from HCV or HIV or other chronic infections were excluded from the study as it is known they correlate with vitamin D deficiency status. Written informed consent was taken from each subject fulfilling the mentioned criteria, before study inclusion. Study subjects were asked to complete a generalized questionnaire that contains demographic information including past and present medical history, and to return after fasting for more than 8 hours for anthropometry and blood withdrawal. At the screening visit, blood samples were examined for levels of glucose, lipid profile and some other bio-chemical tests. Subjects who had abnormal levels of these at bio-chemical laboratory tests were excluded.

Subjects were requested to visit the family medicine outpatient clinic following an overnight fasted state (≥8 hours) for anthropometry and blood withdrawal by the clinic nurse and physician on duty, respectively. Anthropometry included height (rounded to the nearest 0.5 cm), weight (rounded to the nearest 0.1 kg), and mean systolic and diastolic blood pressure (mmHg). Body mass index (BMI) was calculated as weight in kilograms divided by height in square meters. Fasting blood samples were collected and transferred immediately to a non-heparinized tube for centrifugation. Collected serum was then transferred to pre-labeled plain tubes and delivered to the bio-chemistry laboratory in Bezmialem Vakif University Hospital.

Fasting glucose, lipid profile, calcium, and phosphorous were measured using a chemical auto-analyzer (Toshiba, Japan). Serum 25(OH)D was measured with a Roche Elecsys modular analytics Cobas e601 using an electrochemiluminescence immunoassay (Roche Diagnostics, GmbH, Mannheim, Germany) and commercially available in vitro diagnostic systems kits (Cobas e601, Germany). The inter- and intra-assay coefficients of variation (CV) for 25 (OH) D enzyme-linked immunosorbant assay (ELISA) were 5.3% and 4.6%, respectively, with 100% cross-reactivity to 25(OH)D3 and 75% cross-reactivity to 25(OH)D2. It should be noted that TS EN ISO 15189 maintains the Quality Assurance (QA) standards, whereas the QA department audits the laboratory at regular intervals. Ethical approval was taken from the institutional review board (IRB) before beginning of the study.

T2DM is confirmed in those having fasting blood glucose above 126 mg/dl and postprandial blood glucose above 200 mg/dl or glycated hemoglobin (HbA1c) >6.5%, as performed on two different occasions according to American Diabetes Association (ADA) criteria. Hypovitaminosis D is defined as that, wherein the levels of 25(OH)D are below 20 ng/ml according to Endocrine Society.

Data were analyzed using the Statistical Package for the Social Sciences version 16.0

(SPSS, Chicago, IL, USA). Normal continuous variables were presented as mean ± standard deviation. Test of significance was calculated by unpaired student's t test between cases and controls. Correlation of 25(OH)D with other parameters was performed by two-tailed Pearson's correlation. Significance was set at $p < 0.05$.

Results

We evaluated clinical and laboratory findings in 134 patients with T2DM. The characteristics of the whole group according to DM status and 25(OH)D levels are shown in Table 1.

n	Type 2 DM 134	Control 134	p value
Gender (Male/Female)	52/82	52/82	0.747
Age (years)	58.00 ± 12.03	59.82 ± 12.16	0.217
Duration of DM (years)	6.83 ± 4.38	-	
BMI (kg/m2)	28.95 ± 3.52	26.69 ± 3.08	0
LDL (mg/dL)	136.28 ± 35.39	123.38 ± 39.67	0.004
25(OH)D (ng/ml)	11.85 ± 8.51	15.33 ± 17.42	0.039
25(OH)D status	n (%)	n (%)	
>20 ng/ml	15 (11.2)	27 (20.1)	
10 - 20 ng/ml	48 (35.8)	35 (26.1)	
5 - 10 ng/ml	48 (35.8)	56 (41.8)	
<5 ng/ml	23 (17.2)	16 (11.9)	
Calcium (mg/dL)	9.50 ± 0.43	9.45 ± 0.31	0.226
Parathormone (pg/mL)	53.83 ± 30.83	46.52 ± 21.52	0.025
Insulin (µU/ml)	19.50 ± 19.17	13.34 ± 10.73	0.001
HOMA-index (µU/ml × mg/dL)	8.42 ± 8.96	3.39 ± 3.43	0
Fasting glucose (mg/dL)	208.20 ± 67.96	99.35 ± 11.65	0
HbA1c (%)	8.83 ± 1.85	5.42 ± 0.44	0
Creatinine, serum (mg/dL)	0.79 ± 0.26	0.70 ± 0.19	0.003
ALT (u/L)	28.44 ± 17.50	21.02 ± 13.44	0

Table 1: Basic characteristics of and 25(OH)D levels in diabetic and healthy control adults.
Data given as mean ± standart deviation.
BMI: Body Mass Index; LDL: Low density lipoprotein; 25(OH)D: 25 hydroxy vitamin D; HOMA-index: Insulin resistance index; HbA1c: Glycated hemoglobin; ALT: Alanine transaminase.

Of the total number of participants, 61.2 % were females. The mean age for the T2DM subjects was 58.00 ± 12.0 years versus 59.82 ± 12.16 years for the healthy controls. Both groups differed significantly in all anthropometric measures.

Adults with T2DM had significantly higher mean BMI ($p<0.001$) than the healthy adults. T2DM adults also had significantly higher fasting glucose concentrations ($p<0.001$) and LDL-cholesterol ($p<0.05$) than healthy adults. Calcium levels did not showed statistically significant differences between T2DM and non-diabetic subjects ($p>0.05$), and all subjects had calcium levels within the normal range. Calcium levels were 9.50 ± 0.43 mg/dL in the T2DM adults and 9.45 ± 0.31 mg/dL in the healthy controls.

Parameters	Pearson's correlation coefficient
Serum 25 (OH) Vitamin D levels with glucose levels	-0.216 *
Serum 25 (OH) Vitamin D levels with HbA1c levels	-0.258 **
Serum Glucose with insulin resistance (HOMA-IR)	0.250 **
Serum Glucose with HbA1c levels	0.778 **
Serum Insulin levels with insulin resistance (HOMA-IR)	0.740 **
Serum Calcium with age	-0.178 *
BMI with HbA1c levels	0.198 *

Table 2:Correlation studies between some biochemical variables of diabetic adults.
p<0.05, p<0.01*
HbA1c: Glycated hemoglobin; HOMA-IR: Insulin resistance index; BMI: Body Mass Index

We classified a level of 25(OH)D above 20 ng/mL as normal, 10-20 ng/mL as a mild deficiency, 5-10 ng/mL as moderate, and <5 ng/mL as severe deficiency[37].

According to this classification, both groups had 25(OH)D deficiency and 25(OH)D levels were significantly lower among T2DM cases compared with healthy adults ($p<0.05$). Mean 25-hydroxyvitamin D levels were 15.33 ± 17.42 ng/ml in the normal controls and 11.85 ± 8.51 nmol/L in the T2DM group ($p<0.05$). Overall, 88.8 % of the T2DM and 79.9 % of the healthy adults were 25(OH)D deficient. Pearson's Correlation co-efficient of different parameters are shown in Table 2.

Discussion

To the best of our knowledge, there are no population-based studies that have examined the association between 25(OH)D and T2DM in primary care in Turkey. Nearly nine tenth of the participants with T2DM had 25(OH)D deficiency. The study revealed that 25(OH)D deficiency was higher in T2DM adults (88.8%) compared with non-diabetic patients (79.9%). A significant difference in the mean value of 25(OH)D between the T2DM and healthy participants was found ($p<0.05$). Although 25(OH)D deficiency was prevalent in both groups, it was much higher among the diabetic patients. 25(OH)D has been attributed roles in the pathogenesis and prevention of diabetes(38). Furthermore, 25(OH)D might inhibit the autoimmune reaction targeted toward cells of the pancreas(39). The deficiency or insufficiency of 25(OH)D is highly prevalent and this observation is more profound in patients with T2DM[40-42].

In our study, 25(OH)D level was significantly low among T2DM than healthy controls ($p = 0.03$). There was hypovitaminosis D among T2DM cases than healthy controls. As 25(OH)D level was having negative correlation with insulin resistance, so decrease in 25(OH)D level is associated with increase in insulin resistance. We detected that HOMA-IR levels of patients with T2DM were significantly higher than healthy controls ($p < 0.001$). 25(OH)D affects the insulin level as well as insulin sensitivity. The presence of 25(OH)D receptors on different tissues explains its diversity of action[43]. Hence, reduced levels of 25(OH)D are associated with insulin resistance and may thus be linked to poor glucose control observed in T2DM patients[44, 45].

Increasing evidence supports the hypothesis that 25(OH)D may play a key role in the pathophysiology of glucose metabolism. Although these mechanisms are not fully understood, pathways may include impaired pancreatic β-cell function, insulin resistance, and systemic inflammation[46].

The results of our study are consistent with previous reports that depicted how hypovitaminosis D in adults may influence the risk of developing diabetes[47, 48]. In an animal model active 25(OH)D can increase islet insulin secretion upon glucose stimuli(49). In a previous study it has been shown that 25(OH)D levels are positively correlated with insulin sensitivity and negatively correlated with HbA1c levels, indicating 25(OH)D deficiency as a predisposing factor in the pathogenesis of diabetes mellitus and cardiovascular diseases[50].

It is well known that severe hypovitaminosis D causes a compensatory increase in the secretion of parathormone (PTH); this inspired the hypothesis that low 25(OH)D levels exert their action on insulin resistance indirectly through PTH. For example, a cross-sectional study revealed that the PTH level, but not the 25(OH)D level, is an independent predictor of the 'metabolic syndrome' in morbidly obese men and women[51]. Poor vitamin D levels induce higher PTH concentrations to finally increase calcium resorption from the skeleton and reabsorption in the kidneys. Elevated PTH concentrations have been shown to inhibit insulin synthesis and secretion from β -cells[52] and to reduce insulin sensitivity[53,54]; thus, hypovitaminosis D-induced secondary hyperparathyroidism may contribute to the development of metabolic syndrome. In line with these studies, in the present study, a significant difference was detected when PTH levels were compared between patients with T2DM and non-diabetics ($p < 0.05$). In addition, PTH correlates with several of the features included in the metabolic syndrome, such as systolic and diastolic blood pressure, waist circumference, BMI, and insulin sensitivity, as estimated by HOMA[55]. The mechanisms underlying the relationship between PTH and insulin resistance are still unclear; some author[s][56, 57] again hypothesized a correlation between intracellular calcium affected by PTH levels and insulin resistance, while others suggested that increase in PTH levels encourage intracellular flux of calcium within adipocytes, leading to increased lipogenesis and, therefore, weight gain[58].

In this study, we obtained a significant negative association between 25(OH)D level and glucose and HbA1c levels. 25(OH)D deficiency can be caused by the accumulation of body fat and jeopard normal metabolic functioning, favoring obesity and T2DM. Adipose tissue, in turn, can act by lowering serum levels of 25(OH)D. This fat soluble vitamin

can be stored in adipose tissue[59]. Thus, low levels of serum 25(OH)D would be a result of obesity and not its cause[60]. Obesity is common among T2DM subjects and 25(OH)D stored in adipose tissue causes decrease in bioavailability[61]. This fact places obese T2DM patients at greater risk of developing 25(OH)D deficiency[62]. However, in this study participants with T2DM were having BMI lower than 30.

While sunlight is an excellent source of 25(OH)D, in healthy subjects, 25(OH)D deficiency occurs mainly because of low dietary intake and less exposure to sunlight, clothing, decreased outdoor activities, and use of sunscreens affect the 25(OH)D synthesis in skin and, therefore, 25(OH)D status, too[63]. Seasonal variation also affects the 25(OH)D status, mainly in winter. Certain other causes such as malabsorption, liver or kidney disorder and obesity also increase the risk for development of 25(OH)D deficiency(64-66). 25(OH)D synthesis requires specific wavelength of sunlight UVB rays (290-310 nm) which is predominantly more during morning hours and in summer days[67].

The prevalence of 25(OH)D deficiency was higher in our study both in participants with T2DM (88.8 %) and in healthy subjects (79.9 %) than in previous Western studies[68-70] and consistent with studies performed in healthy subjects in Turkey and among patients with T2DM in a neighbor country, 80.8 % and 89.2 %, respectively[71, 72]. These variations in the results of different studies might be explained by differences in dietary intake, sun avoidance behaviors, geographical environment, skin color, or genetic predisposition.

Despite several important findings in the present study, relatively small sample size is considered as a limitation of it. Another limitation was the time period the study was performed, since data were collected during pre-summer months (in March and April). We have to bear in mind the effect of variation in seasons in 25(OH)D levels[63, 73]. In our study, mean 25(OH)D levels in diabetic adults were relatively lower than those published in Western studies[68-70]. This might reflect the high prevalence of 25(OH)D deficiency and insufficiency in our normal population. The high prevalence of 25(OH)D deficiency might be related to decreased sun exposure. Despite the fact that there is sunlight in two thirds of the year in Istanbul (according to data taken from Turkish State Meteorological Service), time spent outdoors is severely limited. Thus, 25(OH)D deficiency was highly prevalent in Turkish population. It has been indicated in some studies[74, 75] that 25(OH)D status showed a strong relationship with especially type of clothing worn. Status of 25(OH)D was much better in women with modern clothing than in women with Islamic traditional wearing with the arms and legs covered. Deficiency of 25(OH)D was particularly prevalent among women in near-east and middle-east countries[76].

This study revealed that 25(OH)D deficiency was higher in patients with T2DM compared with non-diabetic healthy controls. Furthermore, 25(OH)D deficiency was found to be widespread among Turkish population. The low levels of 25(OH)D in the population have been attributed mainly to social habits, particularly the avoidance of sunlight and 25(OH)D supplements and also diary and marine foods. The increase in prevalence of T2DM among Turkish population in recent years might be related to our findings about widespread 25(OH)D deficiency. We further suggest that decreasing the prevalence of obesity by obesity control efforts to be handled nation-wide in primary health care would help enable the population to decrease the prevalence of T2DM. We advise all T2DM patients to take 25(OH)D regularly and to increase sun exposure. We also suggest developing strategies to supply the population with 25(OH)D especially in primary health care.

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