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Are Serum Levels of F2-Isoprostane and Oxidized-LDL Related to Vitamin D Status in Type 2 Diabetic Patients? A Case-Control Study

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Abstract

Background: Considerable evidence suggests that oxidative stress affects diabetes mellitus (DM) and contributes to its complications. Vitamin D has been shown to possess antioxidant properties. The aim of this study was to determine the association between serum levels of calcifediol (25-OH-D), an indicator of vitamin D status, and lipid profiles with oxidative stress in patients with type 2 diabetes mellitus (T2DM).

Methods: In this case-control study, 57 T2DM patients with low vitamin D status (< 30 ng/mL) and 48 T2DM patients with normal vitamin D status (> 30 ng/mL) were enrolled. Fasting concentrations of 25-OH-D, calcium, phosphorus, parathyroid hormone (PTH), lipid profiles, fasting blood sugar (FBS), glycosylated hemoglobin (HbA1c), F2-isoprostane, and oxidized-low-density lipoprotein (ox-LDL) were measured.

Results: The mean fasting serum concentrations of 25-OH-D, calcium, and phosphorus in patients with low vitamin D status were significantly lower than in controls ($p < 0.001$). The mean concentrations of ox-LDL, F2-isoprostane, total cholesterol, and LDL were significantly higher in patients with low vitamin D status than in controls. There was a negative correlation between vitamin D levels and F2-isoprostane ($r = 0.647$and $P = 0.0001$), LDL ($r = -0.218$ and $P = 0.030$), and ox-LDL ($r = -0.637$ and $P = 0.0001$).

Conclusions: The results of present study indicated that serum concentrations of 25-OH-D were inversely correlated with F2-isoprostane, LDL, and ox-LDL. Therefore, vitamin D may have a beneficial effect on the control of lipid profiles and oxidative stress in T2DM patients.

Keywords: Diabetes mellitus type 2, F2-isoprostane, Oxidative stress, Ox-LDL, Vitamin D

Introduction

The increasing prevalence of diabetes mellitus (DM) is considered a global public health concern. According to the World Health Organization, the global prevalence of type 2 DM (T2DM) will increase from 171 million people in 2000 to 366 million in 2030 (1). Type 2 DM is more prevalent than type 1 DM, and is responsible for 90% of DM cases (2). Genetic and environmental factors play a pivotal role in the etiology and pathogenesis of DM (3). Low Vitamin D status, as an environmental risk factor, is associated with many non-skeletal diseases, including DM (4), hypertension (5), pemphigus (6), cardiovascular disease (7), and

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autoimmune diseases (8). Vitamin D deficiency is associated with decreased insulin synthesis and secretion (4). Many studies have shown that diabetic patients are likely to be vitamin D deficient, suggesting that vitamin D participates in the development of diabetes (9, 10). In addition, in diabetic patients with vitamin D deficiencies, serum levels of total cholesterol, triglycerides (TGs), and LDL were greater and HDL was less than in patients with vitamin D sufficiency (11).

The imbalance between reactive oxygen species (ROS) generation and the antioxidant system results in oxidative stress and cell damage, which is involved in the pathogenesis of many diseases (12). Type 2 DM is associated with excess generation of highly reactive free radicals, largely due to hyperglycemia, which causes oxidative stress (13).

Considerable documentation indicates that oxidative stress plays an important role in DM and its complications (14, 15). Some experimental studies showed that vitamin D can prevent oxidative damage-induced DM, possibly through its antioxidant effect (16, 17). In a cross-sectional study, it was found that vitamin D may have a beneficial effect on the control of glycemic profiles and oxidative stress in T2DM patients (18). However, other studies found no correlation between vitamin D levels and the antioxidant status of diabetic rats (19). In addition, population studies suggest that vitamin D may play a considerable role in boosting β-cell function and insulin sensitivity (4).

Because of the anti-oxidative effect of vitamin D and high prevalence of vitamin deficiencies, we aimed to compare the serum levels of vitamin D via 25-OH-D, and glycemic and lipid profiles, F2-isoprostane, and oxidized-low-density lipoprotein (ox-LDL) in vitamin D-deficient and vitamin D-sufficient T2DM patients.

Materials and Methods

Study Subjects

In this case-control study, we studied 57 T2DM patients with low vitamin D status (< 30 ng/mL) and 48 T2DM patients with normal vitamin D status (> 30 ng/mL) (controls) from the Iranian Diabetes Association. Exclusion criteria included pregnancy, lactation, chronic liver or kidney disorders, endocrinology disorders such as hypo- or hyper-thyroidism and hyper-parathyroidism, smoking, use of anti-convulsion drugs, and use of mineral or vitamin supplements.

All subjects were informed about the purposes and procedures of the study and all signed written consent forms before entering the study. The research protocol was approved by the Ethics Committee of Tehran University of Medical Sciences.

Anthropometric Measurements

Body weight (with minimal clothing) and height (without shoes and standing upright) were measured using a scale (Seca GmbH & co KG, Hamburg, Germany) with accuracies of 0.1 kg and 0.5 cm, respectively.

Dietary Assessment

The dietary data of the subjects were collected by three 24-h dietary recalls for one weekend and two weekdays. The average daily nutrient intake was calculated by modified Nutritionist IV software (version 3.5.2, First Data Bank; Hearst Corp, San Bruno, California).

Blood Collection

After receiving written consent from all subjects, blood samples were collected from the antecubital vein in the morning after 12-14 hour fasts, and centrifuged at 1000 RCF for 10 min at 4 ˚C. The blood samples were collected in two trace element-free tubes, one for serum separation, and one with ethylene diamine tetra acetic acid (EDTA) for plasma separation and hemoglobin A1C (HbA1C) measurement. The separated plasma was immediately stored at -80 ˚C until biochemical analysis.

Laboratory Analysis

Fasting plasma glucose was measured using glucose-oxidase with a ZistShimi Kit (ZistShimi Co., Iran). HbA1C was determined using a Nyco Card Reader II analyzer according the procedure provided (20). Calcium, phosphorus, TGs, cholesterol, high-density lipoprotein (HDL), and LDL were measured by a colorimetric method using biochemical kits (ZistShimi CO., Tehran,

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Iran). The serum concentration of 25-OH-D was measured using chemiluminescence. Normal ranges of calcium and phosphorous were defined as 8.6-10.3 mg/dL and 2.5-5 mg/dL, respectively. The ox-LDL was measured by a sandwich ELISA using a commercial kit (Mercodia-Sweden). F2-isoprostane levels were measured with an ELISA kit (Cusabio Biotech Co., China). Vitamin D sufficiency was defined as a serum 25-OH-D concentration > 75 nmol/L (30 ng/mL) and vitamin D deficiency was defined as serum 25-OH-D concentration < 75 nmol/L (30 ng/mL).

Statistical Analysis

All data were expressed as means ± standard deviations (SD). Normality of data distribution was analyzed by the Kolmogorov-Smirnov test. Groups were compared with independent sample t or chi square tests for continuous and categorical data, respectively. Correlations between vitamin D levels and lipid profiles, F2-isoprostane, and ox-LDL of diabetic patients were determined using Spearman's rho. P values < 0.05 were considered to be statistically significant. Data were analyzed using the Statistical Package for the Social Sciences (Version 11.5, SPSS Inc, Chicago, IL, USA).

Results

A total of 105 diabetic patients participated in this study. Fifty-six subjects (53%) were females, and 49 (47%) were males. The greatest duration of diabetes was 16 years. The mean age of the patients was 56.34 ± 8.16 years. Their mean BMI was 29.68 ± 5.89. Demographic and biochemical characteristics among case and control groups are shown in Table 1. No significant differences were observed in mean scores for ages, weights, or BMIs between groups. The mean fasting serum concentration of 25-OH-D, calcium, and phosphorus in patients with low vitamin D status was significantly less than that of controls *($p < 0.001$)*. The mean total cholesterol and LDL were significantly greater in patients with low vitamin D status than in controls *($p < 0.01$)*. However, the mean TG and HDL were not significantly different between the two groups (Table 1). The mean serum ox-LDL and F2-isoprostane were significantly greater in patients with low vitamin D status than in controls *($p < 0.001$)*.

Table1. Demographic and biochemical characteristics among case and control groups[a]

Variables	Patients with low vitamin d status	Patients with normal vitamin d status	P value
Age (year)	55.63±8.3	57.17±8.15	0.34
Weight (kg)	80.08±14.15	76.63±12.8	0.2
BMI (kg/m^2)	30±5.92	29.6±5.8	0.73
FBG	136.81±36.98	150.54±92.45	0.32
HbA1c	9.33±1.55	8.98±1.16	0.26
25-OH-D (ng/mL)	13.03±5.24	47.74±15.35	<0.001
Calcium (mg/dL)	8.15±0.62	9.59±0.84	<0.001
Phosphorous (mg/dL)	3.21±0.56	4.04±0.47	<0.001
TG (mg/dL)	146.08±54.96	147.2±75.96	0.93
LDL-C (mg/dL)	140.57±54.88	114.43±64.04	0.01
HDL-C (mg/dL)	41.43±5	39.33±8.25	0.12
TC (mg/dL)	211.28±56.73	188.45±49.99	0.03
OX-LDL (IU/L)	88.80±21.16	47.92±16.91	<0.001
F2-isoprostane (pg/mL)	283.9±111.38	93.23±42.02	<0.001

[a]Data were expressed as mean±SD, P value was resulted from independent sample t-test, FBG: fasting blood glucose, HbA1c: glycosylated hemoglobin, TG: Triglyceride, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, TC: Total cholesterol,Oxidized Low-Density Lipoprotein.

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Table 2 shows the dietary intakes of diabetic patients with low and normal vitamin D status. The mean energy, protein, fat, and carbohydrate intake in patients with low vitamin D status were not significantly different from those in controls. Because the distribution of the data was not normal, we transformed the data into vitamin D logarithms. The vitamin D logarithms between the two groups were significantly different *(P = 0.004)*. Interestingly, a negative correlation was observed between the vitamin D level and F2-isoprostane (r = 0.647and P = 0.0001), LDL (r = -0.218 and P = 0.030), and ox-LDL (r = -0.637 and P = 0.0001). However, no statistically significant correlation was seen between vitamin D and TG, cholesterol, or HDL (Table 3).

Table 2. Dietary Intake Among the patients with low vitamin D status, and Control Groups[a]

Variables	Patients with low vitamin D status	Patients with normal vitamin D status	P value
Energy(kcal/day)	1309.66±81.30	1310.03±72.23	0.99
Protein (g/day)	53.79±3.68	63.43±4.78	0.12
Fat (g/day)	139.03±15.96	210.32±18.09	0.86
Carbohydrate(g/day)	36.90±4.84	35.02±3.21	0.65
Vitamin D (mg/day)	0.59±0.22	1.35±0.70	0.76
Calcium (mg/day)	617.46±50.26	688.26±66.12	0.39
Phosphorus (mg/day)	837.63±77.15	833.76±63.11	0.97

[a] Data are expressed as mean ± SD, P value was resulted from independent sample t tests.

Table 3. Correlations of vitamin D with lipid profiles, F2-isoprostane and ox-LDL in diabetic patients

Variables		F2-isoprostane	ox-LDL	TG	TC	LDL-C	HDL-C
Serum vitamin D (ng/mL)	r	-0.647	-0.637	-0.033	-0.107	-0.218	0.87
	p-value	0.0001	0.0001	0.734	0.274	0.030	0.373

r= correlation coefficient, P-value was resulted from Spearman correlation, TG: Triglyceride, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, TC: Total cholesterol, ox-LDL: Oxidized Low-Density Lipoprotein.

Discussion

Evidence obtained from many studies indicates that insufficient vitamin D can be considered an environmental factor that contributes to the pathophysiology of the disease. The key immunomodulatory role of vitamin D is well known. Low serum vitamin D has been observed in several autoimmune diseases including systemic lupus erythematosus (SLE) (21-23), insulin-dependent DM (IDDM) (24), rheumatoid arthritis (RA) (25), and multiple sclerosis (MS) (26).

Our findings revealed that the serum concentration of 25-OH-D was significantly less in patients with low vitamin D status than in control subjects. In a cohort study, as a part of the Mini-Finland Health Survey, a significant inverse association was found between serum 25-OH-D and the risk of T2DM in a simple model.

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However, the association was attenuated in the multivariate analysis, adjusting for potential risk factors of T2DM (9). Saedisomeolia et al. reported results similar to ours (11). Few studies have measured micronutrients other than vitamin D in diabetic patients, but our results showed that the mean fasting levels of calcium and phosphorus in patients with low vitamin D status were significantly less than in controls. In a cross-sectional study of diabetic patients, the mean serum level of calcium was significantly greater in diabetic patients with vitamin D deficiency than in patients with sufficient vitamin D, but an association was not seen between phosphorus and vitamin D. Also, serum levels of total cholesterol, TG, and LDL were greater, and HDL was less in diabetic patients with vitamin D deficiency than in patients with sufficient vitamin D levels (11). In our study, mean total cholesterol and LDL were significantly greater in patients with low vitamin D status than in controls, and the mean TG and HDL were not significantly different between the two groups.

It is worth noting that we hypothesized that some other lipid profiles, such as ox-LDL and F2-isoprostane, are also associated with vitamin D status in diabetic patients. Hence, we initially collected data on these variables to investigate a possible association between them and vitamin D status in diabetic patients, because we found no remarkable research that assessed F2-isoprostane and ox-LDL relative to vitamin D levels in these patients. Our study indicated that in T2DM patients, TG, cholesterol, LDL, and specially, ox-LDL and F2-isoprostane, were negatively correlated with the serum vitamin D level, but a positive correlation was observed between HDL and serum vitamin D. However, in another study, an inverse, but not significant, association was seen between levels of 25-OH-D with TG and total cholesterol, and a positive correlation with HDL-C and LDL-C (11). A review of 22 cross-sectional and 10 placebo-controlled interventional studies found that serum levels of 25-OH-D are directly related to HDL cholesterol. In addition, all studies reported an inverse association between serum levels of 25-OH-D and TG. No general agreement exists regarding the effects of 25-OH-D on serum levels of TG in interventional studies with vitamin D supplementation. Although a positive association was observed in some studies, other studies showed an inverse relationship between serum levels of 25-OH-D and TG (27). These findings are consistent with our results.

Diabetes mellitus is associated with oxidative stress, defined as increased oxidative stress and simultaneous defects in the antioxidant defense system. Oxidative stress plays a key role in the onset and progression of diabetes complications, including macro/micro vascular damage. Vitamin D possesses antioxidant properties, so that vitamin D3 can inhibit the lipid peroxidation induced by iron in brain liposome; therefore, vitamin D could serve as a cellular membrane antioxidant. Anticancer activity of vitamin D is also attributable to its antioxidant properties (18, 28).

Little attention has been given to the effect of environmental and genetic factors, such as place of residence, ethnicity, and demographical characteristics on vitamin D serum levels in diabetic patients. The authors of a study of environmental factors in the etiology of Type 1 DM found that differences in the genetic predisposition between various populations may at least partly explain the conspicuous variation in incidence rates between different ethnic groups (29). Further research is needed to understand the impact of these factors on vitamin D in diabetic patients and their mechanisms of action.

The strength of this study is that it measures of micronutrients associated with 25-OH-D in diabetic patients with sufficient and insufficient vitamin D. The study was limited in that: (1) blood samples were collected only once, and this took place only in one season of the years of study; hence, 25-OH-D levels for the patients might not be fully comparable, however, adjustment for time when blood samples were collected did not notably change the results, (2) considering the case-control design in this study, recall bias should be recognized when findings from dietary intake of macronutrients are interpreted, and (3) small samples in both groups were another limitation for generalization of our findings.

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In summary, the results support the hypothesis that high serum 25-OH-D concentrations may be associated with a decrease in some lipid profiles, F2-isoprostane, and ox-LDL in diabetic patients with sufficient vitamin D but not in those with vitamin D deficiencies. Further research is needed to confirm the association and to distinguish between the independent role of vitamin D and the role of healthy dietary and lifestyle patterns in diabetic patients.

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