VITAMIN D STATUS AND ITS ASSOCIATION WITH ANTIOXIDANT PROFILES IN DIABETIC PATIENTS: A CROSS-SECTIONAL STUDY IN IRAN

A. SAEDISOMEOLIA¹, EHSANEH TAHERI¹,², M. DJALALI¹, A. DJAZAYERI¹, M. QORBANI³,⁴, A. RAJAB⁵, B. LARIJANI²

ABSTRACT

BACKGROUND AND OBJECTIVES: There are increasing evidences about the relationship between vitamin D status and the control of diabetes. Several studies showed that vitamin D has an antioxidant property. In this study, we aimed to determine the relationship between serum levels of 25-hydroxy vitamin D (25-OH-D) and glycemic, antioxidant profile in diabetes compared to healthy groups.

MATERIALS AND METHODS: This cross-sectional study was conducted in 100 patients with type 2 diabetes mellitus (T2DM) and 100 healthy controls. Fasting serum levels of 25-OH-D, calcium, phosphorous, parathyroid hormone, glucose, HbA1C, insulin, homeostasis model assessment of insulin resistance index, total antioxidant capacity (TAC), activities of superoxide dismutase (SOD), glutathione reductase (GR), and glutathione peroxidase (GSH-PX) were measured. RESULTS: Eighty-two percent of type 2 diabetic patients and 75% of healthy subjects were suffering from vitamin D deficiency or insufficiency. The activities of GR and GSH-PX were higher in diabetic patients compared to control. There was a negative relationship between 25-OH-D and activity of GR, GSH-PX. Also, 25-OH-D had a positive association with activity of SOD in diabetic patients. In the control group, 25-OH-D had an inverse relationship with SOD, GSH-PX, and positively with GR activities. INTERPRETATION AND CONCLUSIONS: Vitamin D deficiency has a high prevalence among Iranian adult population with and without type 2 diabetes. Our results showed that vitamin D may have a beneficial effect on the control of glycemic profiles and oxidative stress in T2DM patients.

Key words: Diabetes mellitus, oxidative stress, vitamin D

¹Department of Cellular and Molecular of Nutrition, School of Nutritional Sciences and Dietetic, Tehran University of Medical Sciences, Tehran, ²Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Research Institute, Tehran University of Medical Science, Tehran, ³School of Public Health, Alborz University of Medical Sciences, Karaj, ⁴Department of Epidemiology, Iran University of Medical Science, Tehran, ⁵Iranian Diabetes Society, Tehran, Iran

Address for correspondence:
Miss. Ehsaneh Taheri,
Endocrinology and Metabolism Research Center,
Endocrinology and Metabolism Research Institute, Tehran University of Medical Science, Tehran, Iran
E-mail: ehsaneh_taheri@yahoo.com

Access this article online

Quick Response Code:
Website: www.indianjmedsci.org
DOI: 10.4103/0019-5359.120695
INTRODUCTION

Diabetes mellitus is a metabolic disorder with a high prevalence across the world. It is reported that 7.7% of Iranian adults (2 million adults) and 16.8% (4.4 million) have diabetes and impaired fasting glucose, respectively.

Vitamin D has a variety of non-skeletal functions including neuromuscular function to prevent psoriasis, multiple sclerosis, colorectal and prostate cancers, and to decrease the risk of cardiovascular disease, hypertension, dyslipidemia, and diabetes. Multiple cross-sectional studies have indicated that a low circulating concentration of 25-hydroxy vitamin D (25-OH-D) is associated with higher fasting serum glucose, reduced insulin sensitivity, and an increased risk of type 2 diabetes. Although, interventional studies to investigate the effect of vitamin D supplementation on glycemic profile have had controversial results.

Chronic hyperglycemia is accompanied with an increased generation of reactive oxygen species (ROS) as a result of glucose auto-oxidation. Oxidative stress is defined as an increased generation of free radicals and/or impaired compensatory response of endogenous antioxidant defenses, both of which were observed in type 2 diabetes. There is considerable evidence that the oxidative stress plays a key role in insulin resistance, impaired insulin secretion and many of the complications of diabetes such as micro-/ macro-vascular damage. Some experimental studies showed that vitamin D may have antioxidant properties by modifying some of the antioxidant enzymes. However, some others found that 25-OH-D had no beneficial effect on antioxidant defense. Therefore, the aim of this study was to determine the relationship between serum concentration of 25-OH-D and glycemic profile, serum concentration of total antioxidant capacity (TAC), activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), and glutathione reductase (GR) in diabetic patients compared to healthy subjects.

MATERIALS AND METHODS

Study subjects

In this cross-sectional study, 200 subjects (aged between 20 and 80 years) including 100 type 2 diabetic patients from Iranian Diabetes Association and National Iranian Oil Company (NIOC) – Central Hospital and 100 healthy subjects from the staff members of Tehran University of Medical Sciences Tehran, Iran were selected via random sampling methods. Exclusion criteria included pregnancy, lactation, use of drugs which affect lipid profile or calcium and bone metabolism, chronic disorders of liver and kidney, endocrinology disorders such as hypo- or hyper-thyroidism and hyper-parathyroidism, smoking, insulin injection, use of anti-convulsion drugs, vitamin D, and calcium supplementation.

A written, informed consent was taken from each participant after the full explanations about the study according to the Tehran University of Medical Sciences Ethics Committee's procedure.

Sample collection

After an overnight fasting, 10 ml of peripheral blood was taken by a trained nurse. The blood samples were collected in three trace
element-free tubes, one for serum separation, two tubes with ethylene diaminetetraacetic acid (EDTA) for plasma separation, and Hb measurement. The plasma samples were separated from cells by centrifugation at 3000 rpm for 10 min. The buffy coat was removed, and the remaining erythrocytes were drawn from the bottom, washed three times in cold saline (9.0 g/l NaCl), and hemolyzed by the addition of an equal volume of ice-cold demineralized ultrapure water (MilliQ Plus Reagent Grade; Millipore) to yield a 50% hemolysate. The activities of erythrocyte CuZn-SOD, GPX, and GR were obtained for the fresh hemolysates. Cell membranes were removed by centrifugation at 1200 rpm for 5 min at 4°C. The hemolysates were used to determine antioxidant enzymes. Serum was separated by centrifugation of tubes containing the coagulated blood for 10 min at 1000 rpm at 4°C. Then all the samples were stored at −79°C.

**Laboratory analysis**

Fasting plasma glucose was measured using glucose-oxidase with Pars Azmoon Kit (Pars Azmoon Co., Tehran, Iran) and plasma insulin was measured using radioimmunoassay method (Biosorce Kit, Denmark). HbA1C was determined using Nyco Card Reader II analyzer according the procedure provided. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: Fasting insulin (µIU/ml) × fasting glucose (mmol/l)/22.5.

The serum concentration of 25-OH-D was measured using chemiluminescence method. Serum level of PTH was measured using RIA Kit (CIS Biointernational, France) with the normal range defined to be 8-79 pg/ml. Serum calcium and phosphorus were analyzed using Pars Azmoon Kit (Pars Azmoon Co., Tehran, Iran). Normal ranges of calcium and phosphorous were defined to be 8.6-10.3 mg/dl and 2.5-5 mg/dl, respectively. GSH-PX activity of erythrocytes was measured spectrophotometrically by Randox Laboratory Kit (Ransel, manual) according to the method described by Paglia and Valentine. In this method, GSH-PX enzyme oxidizes glutathione by the action of cumene hydro-peroxidase. In the presence of GR and nicotinamide adenine dinucleotide phosphate (NADPH), the oxidized glutathione was immediately converted to the reduced from with a concomitant oxidation of NADPH to nicotinamide adenine dinucleotide phosphate (NADP+). The decrease in absorbance at 340 nm and 37°C was measured. GR activity of erythrocytes was measured spectrophotometrically by Randox Laboratory Kit (Ransel, manual) at 340 nm and at 37°C by the method of Calberg and Mannervik. The activity of SOD was assayed by the spectrophotometer method of Marklund and Marklund. In this method, the xanthine–xanthine oxidase system was used as a superoxide radical-generator. The absorbance of the reduced product (Formazone) was measured at 505 nm. SOD activity was measured as the degree of inhibition of this reaction. Fasting serum TAC was measured using azino ethyl benz thiazoline-6-sulfonic acid.

Vitamin D insufficiency was defined as serum 25-OH-D level between 50 and 75 nmol/l (20-30 ng/ml) and vitamin D deficiency was defined as serum 25-OH-D > 50 nmol/l (20 ng/ml).
Statistical analysis
Descriptive statistics were tested to be normally distributed using histograms and Kolmogorov–Smirnov method before statistical analysis. If the data distribution was not normal, log-transformed variables were used. All values are expressed as mean ± SD. The Student’s t-test was employed to compare the differences between the mean of continuous variables and the χ² test was used for categorical data. A value of $P < 0.05$ was considered to be statistically significant. All data were analyzed using Statistical Package for the Social Sciences (SPSS) software (SPSS version 16.0, Chicago, USA) compatible with Microsoft Windows.

RESULTS

Two hundreds subjects including 100 patients with type 2 diabetes (50 men, 50 women) and 100 healthy subjects (50 men, 50 women) participated in present study. Baseline demographic and clinical characteristics of the population are presented in Table 1. There was no significant difference in age, weight, and body mass index between type 2 diabetic patients and healthy controls. The fasting serum concentration of calcium was highly significant in controls than diabetic patients [Table 1].

Fasting Blood Sugar (FBS), Glycosylated Haemoglobin (HbA₁C), and Homeostasis Model of Assessment - Insulin Resistance (HOMA-IR) index were significantly higher in type 2 diabetic patients compared to healthy subjects. However, fasting serum concentration of insulin had no significant statistical difference between two groups [Table 1].

The activity of GR and GSH-PX were significantly higher in diabetic patients than healthy controls, whereas the activity of SOD and TAC showed no significant difference among the two groups [Table 1].

Distribution of vitamin D status including vitamin D deficiency (25-OH-D < 50 nmol/l), vitamin D insufficiency (25-OH-D between 50 and 75 nmol/l), and sufficiency (25-OH-D > 75 nmol/l) were 55.8%, 26.3%, 17.9% in diabetic patients and 57%, 18.4%, and 24.6% in healthy subjects, respectively.

We also found a positive trend between serum levels of 25-OH-D and activities of SOD,

<p>| Table 1: Baseline demographic and biological characteristics of the overall population |</p>
<table>
<thead>
<tr>
<th>Variables</th>
<th>Type 2 diabetes (n=100)</th>
<th>Control (n=100)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>51.3±11.18</td>
<td>51.5±13.4</td>
<td>0.88</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80±13.8</td>
<td>73.2±13.0</td>
<td>0.09</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2±9.3</td>
<td>26.3±4.5</td>
<td>0.98</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.0±0.6</td>
<td>9.1±0.5</td>
<td>0.029</td>
</tr>
<tr>
<td>Phosphorous (mg/dl)</td>
<td>3.7±0.03</td>
<td>3.7±0.04</td>
<td>0.59</td>
</tr>
<tr>
<td>PTH (pmol/l)</td>
<td>46.0±26.8</td>
<td>40.7±18.8</td>
<td>0.10</td>
</tr>
<tr>
<td>25-OH-D (ng/ml)</td>
<td>22.1±15.2</td>
<td>24.2±10.0</td>
<td>0.05</td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>174.9±64.2</td>
<td>87.7±9.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>HbA₁C (%)</td>
<td>7.5±1.9</td>
<td>5.0±0.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>11.8±8.9</td>
<td>13.7±18.1</td>
<td>0.13</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>5.17±4.20</td>
<td>3.11±1.73</td>
<td>0.001</td>
</tr>
<tr>
<td>SOD activity (U/grHb)</td>
<td>1097.8±341.0</td>
<td>1159.4±378.2</td>
<td>0.31</td>
</tr>
<tr>
<td>GR activity (U/grHb)</td>
<td>6.5±5.9</td>
<td>3.9±2.9</td>
<td>0.001</td>
</tr>
<tr>
<td>GSH-PX activity (U/grHb)</td>
<td>61.1±36.4</td>
<td>24.6±9.7</td>
<td>0.001</td>
</tr>
<tr>
<td>TAC (mg/dl)</td>
<td>3.2±0.7</td>
<td>3.6±0.6</td>
<td>0.052</td>
</tr>
</tbody>
</table>

BMI=Body mass index, PTH=Parathyroid hormone, 25-OH-D=25-hydroxy vitamin D, FBS=Fasting blood suger, HbA₁C=Glycosylated haemoglobin, HOMA-IR=Homeostasis model assessment of insulin resistance, SOD=Superoxide dismutase, GR=Glutathione reductase, GSH-PX=Glutathione peroxidase, TAC=Total antioxidant capacity, *Values are expressed as mean±SD
GSH-PX, and GR in type 2 diabetic patients. Among the diabetic patients also, 25-OH-D has a significant positive correlation with TAC \( (P = 0.05) \) [Table 2]. In healthy subjects, our data indicate that 25-OH-D has a negative trend with the activities of SOD, GSH-PX, and GR activity [Table 3].

**DISCUSSION**

In our study, the prevalence of vitamin D deficiency in diabetic patients and healthy subjects were 82.1% and 75.4%, respectively. According to other studies carried out in the Iranian population, a high prevalence of vitamin D deficiency in either diabetic patients or healthy controls is observed.\(^{[16,17]}\) In other sunny countries in the Middle East such as Turkey, India, Saudi Arabia, Kuwait, and Pakistan, vitamin D deficiency is also prevalent.\(^{[18,19]}\) The most probable causes for high prevalence of vitamin D deficiency in Iran include: poor sunlight exposure, skin pigmentation, and inadequate dietary intake of vitamin D, lack of food fortification program, and probably specific polymorphism in vitamin D receptor (VDR), and vitamin D-binding protein. Moreover, Awumey found that the activity of vitamin D 24-hydroxylase, the enzyme which destroys vitamin D is higher in Indians compared to Americans.\(^{[20]}\) which may play a role in the Iranian population as well.

Accumulating evidence suggests that hypovitaminosis D may play a key role in outbreak and development of impaired glucose tolerance, type 2 diabetes mellitus, and metabolic syndrome. Diabetes mellitus is accompanied by oxidative stress, which is defined as increased oxidative stress and simultaneously defects in antioxidant defense system.\(^{[21]}\) Oxidative stress plays a key role in the onset and progression of diabetes complications including macro/micro vascular damage.\(^{[22]}\) Our results showed the lower activity of SOD and higher activities of GSH-PX and GR in diabetic patients compared to control subjects. There is contradiction about the activity of antioxidant enzymes in diabetes mellitus. Both increased and decreased activities of these enzymes are reported in experimental and trial studies.\(^{[23]}\) It is expected

### Table 2: Regression analysis for 25-hydroxy vitamin D and studied independent variables in diabetic patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR crude (CI 95%)</th>
<th>P value</th>
<th>OR adjusted* (CI 95%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/grHb)</td>
<td>1.00 (0.999-1.002)</td>
<td>0.62</td>
<td>1.001 (0.999-1.003)</td>
<td>0.22</td>
</tr>
<tr>
<td>GSH-PX (U/grHb)</td>
<td>1.002 (0.992-1.013)</td>
<td>0.69</td>
<td>1.004 (0.999-1.017)</td>
<td>0.55</td>
</tr>
<tr>
<td>GR (U/grHb)</td>
<td>0.92 (0.84-1.002)</td>
<td>0.05</td>
<td>0.90 (0.79-0.99)</td>
<td>0.05</td>
</tr>
<tr>
<td>TAC (mg/dl)</td>
<td>0.88 (0.77-1.00)</td>
<td>0.66</td>
<td>0.83 (0.70-0.95)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

OR=Odds ratio, CI=Confidence interval, SOD=Superoxide dismutase, GSH-PX=Glutathione peroxidase, GR=Glutathione reductase, TAC=Total antioxidant capacity, \( P<0.05 \) is statistically significant, *Adjusted for age and gender

### Table 3: The regression analysis for 25-hydroxy vitamin D and studied independent variables in healthy subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR crude (CI 95%)</th>
<th>P value</th>
<th>OR adjusted* (CI 95%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/grHb)</td>
<td>1.00 (0.99-1.002)</td>
<td>0.70</td>
<td>1.002 (0.99-1.005)</td>
<td>0.29</td>
</tr>
<tr>
<td>GSH-PX (U/grHb)</td>
<td>0.95 (0.92-1.03)</td>
<td>0.33</td>
<td>1.002 (0.88-1.18)</td>
<td>0.13</td>
</tr>
<tr>
<td>GR (U/grHb)</td>
<td>1.07 (0.90-1.27)</td>
<td>0.39</td>
<td>1.002 (0.779-1.68)</td>
<td>0.05</td>
</tr>
<tr>
<td>TAC (mg/dl)</td>
<td>1.05 (0.53-2.08)</td>
<td>0.88</td>
<td>0.99 (0.66-1.88)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

OR=Odds ratio, CI=Confidence interval, SOD=Superoxide dismutase, GSH-PX=Glutathione peroxidase, GR=Glutathione reductase, TAC=Total antioxidant capacity, \( P<0.05 \) is statistically significant, *Adjusted for age and gender
that the activity of antioxidant enzymes such as SOD, GSH-PX, and GR are increased as a compensatory response to increasing oxidative stress. SOD is the first line of the cellular antioxidant system, which converts the superoxide radical to hydrogen peroxide. On the other hand, hyperglycemia is accompanied by the loss of Cu⁺, which is an essential cofactor for the activity of SOD.

The main new finding of this study was determining the association between serum levels of 25-OH-D and activity of antioxidant enzymes in diabetic patients and healthy controls. Results of our study indicate an inverse relationship between serum levels of 25-OH-D and activities of GSH-PX and GR and a positive trend with SOD in diabetic patients. In healthy subjects, 25-OH-D associated inversely with SOD, GSH-PX, and positively with GR activities. Positive association between the serum level of 25-OH-D and GR activity in healthy controls can be described by the role of glutathione (GSH) in maintenance of intracellular redox balance. GSH-PX is the enzyme that converts hydrogen peroxide to water using GSH as a hydrogen donor. Then GSH recycles GR reaction that simultaneously converts NADPH to NADP⁺.

In addition to the indirect effect of vitamin D to increase GSH concentration, we can conclude that by increasing the activity of GR and simultaneously decreasing GSH-PX activity in healthy subjects, vitamin D can enhance the GSH pool. Furthermore, serum concentration of 25-OH-D has a positive association with TAC. Several studies on experimental models showed that vitamin D has antioxidant property. Wiseman reported that vitamin D₃ can inhibit the lipid peroxidation induced by iron in brain liposome, therefore, vitamin D could serve as cellular membrane antioxidant. Anticancer activity of vitamin D is also attributable to its antioxidant property. Sarder also conducted a study in Murine lymphoma and concluded that the anticancer effect of vitamin D₃ is mainly via it's antioxidant potential. Vitamin D can be considered as a casual antioxidant which scavenges ROS in the first stage before activation of other stress-sensitive response pathways. In the other hand, vitamin E or other antioxidants may be considered a more “symptomatic” rather than a causal treatment for oxidative stress because this antioxidants scavenge already-formed oxidants.

Tse demonstrated that vitamin D up-regulates Iκβ which is an inhibitor of nuclear factor kappa B (NF-κβ). NF-κβ is the nuclear transcription factor that regulates a large number of genes including inflammation and activation of stress-sensitive signaling pathway. Phosphorylation of Iκβ leads to the activation of NF-κβ. Wong founded a vitamin D response element in Iκβα gene promoter. Therefore, vitamin D may have a direct effect on the regulation of Iκβα. In addition, Sun reported that the basal level of Iκβα is higher in VDR⁺ mouse embryonic fi broblasts (MEFs) compared to VDR – situations.

This study had some limitations. First, the main limitation was the cross-sectional design of this study. Therefore, the casual association between vitamin D and other factors cannot be determined. Second, compared to the other large cross-sectional studies, our sample size was smaller; therefore, it may decrease the power of study to detect a significant
association between parameters. Third, a random measurement error may be arising by the use of a single measurement of glycemic and antioxidant markers.

In conclusion, vitamin D deficiency has a high prevalence among the adult population with and without type 2 diabetes. A serum level of 25-OH-D has an inverse association with fasting serum levels of glycemic profiles in diabetic patients and controls. Moreover, for the first time, we look for association between serum concentration of 25-OH-D and antioxidant markers and showed that vitamin D may have beneficial effects on control of oxidative stress among diabetic patients. However, the underlying molecular mechanism remains unclear. Larger cross-sectional or intervention studies are recommended to clarify the exact mechanism.

ACKNOWLEDGMENTS

The protocol of this study was approved by the Ethics Committee of Tehran University of Medical Science (TUMS). This study has been financially supported by Tehran University of Medical Science (TUMS) Research Grant (Grant No.: 10091).

REFERENCES


34. Beg AA, Baldwin AS Jr. The I kappa B proteins:


Source of Support: Nil. Conflict of Interest: None declared.