The relationship of vitamin D status with serum ox-LDL in diabetes mellitus type 2

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ABSTRACT: The antioxidative role of vitamin D has been reported in previous studies. This study investigated the association between vitamin D status and serum ox-LDL in patients with diabetes type 2. This study conducted on 105 patients with diabetes type 2 including 57 ones with insufficient vitamin D status as cases and 48 ones with sufficient vitamin D status as controls. Weight and height were measured and body mass index (BMI) was calculated. Nutrients intake and daily sun exposure were assessed by questionnaire. Serum levels of lipid profile, oxidized-LDL (ox-LDL), calcium, phosphorus and 25-hydroxy vitamin D were measured. Total cholesterol (P=0.03), LDL-C (p=0.01) and LDL-OX (P=0.01) levels were significantly higher in patients with insufficient vitamin D status (P<0.001). No significant differences in triglycerides, HDL-C, intake of nutrients, daily sun exposure were found between two groups. Vitamin D may have a role in reduction of oxidative stress and free radicals in diabetes.

Keywords: Diabetes mellitus, Vitamin D, Oxidative Stress, ox-LDL

INTRODUCTION

Diabetes mellitus is a common metabolic disorder disease that is following with insulin resistance. Therefore insulin secretion from β cells of pancreas will be increased as a compensatory mechanism. This compensatory response due to normal range of fasting blood sugar in 3.9-5.6mmol/L (70-100mg/dl) in the first stage of diabetes (Reaven et al. 2000). Stress of β cells is raised following insulin secretion increasing. In human with susceptibility of diabetes and disability of β cells in increasing insulin secretion, fasting blood glucose and blood glucose after a meal will rose because hemostasis of glucose could not remain normal for long term and finally diabetes mellitus type 2 will appear (McNamara et al. 1987). Hyperglycemia is one of the most important risk factor of oxidative stress. Therefore production of free radicals is increased and anti-oxidant defense system is impaired in diabetes (Maritim et al. 2003). In healthy individuals anti-oxidant defense system inhibits free radicals and prevents their injurious effects (Tesfamariam. 1997). Vitamin D in one of the nutrients that was investigated as an antioxidant recently and antioxidant effects of vitamin D were shown in previous experimental and in vitro studies (Nowson et al. 2002). Low density lipoprotein (LDL) is involved in transportation and metabolism of lipids. LDL has high affinity with free radicals in oxidative stress condition and form oxidized LDL. Therefore levels of oxidized LDL are associated with severity of oxidative stress. Increases of oxidized LDL worst the diabetes and its subsequent diseases like cardiovascular diseases (CVD) (Jffrey et al. 2006). Therefore it seems oxidized LDL could be an accurate biomarker for assessment of antioxidant effects of vitamin D in patient with type 2 diabetes. Moreover its not be used in Iranian population for determination of antioxidant effects of vitamin D previously.

METHODS AND SUBJECTS

This case control study was performed on 105 patients with diabetes type 2. The sample included patients that suffering from diabetes as least for 3 years and received medication with lowering glucose drugs. Exclusion criteria included the following: 1) insulin injection 2) inflammatory diseases 3) kidney diseases 4) liver diseases 5) parathyroid diseases 6) pregnancy and lactation 7) consumption of anticonvulsant and steroids 8) a history of tobacco abuse 9) supplementation with vitamins and minerals. All patients signed out consent forms. Ten milliliter
venous blood samples were collected from all patients after a 12 hour fast. Seca Instruments were used to measuring anthropometric indexes. Height was measured without shoes with an accuracy of 0.5cm and weight was measured with light cloths with an accuracy of 100g. The daily intakes of energy, macronutrients, calcium, phosphorus and vitamin D were also assessed through 24-hour questionnaire and were analyzed with N4 software. Serum levels of 25-hydroxy vitamin D was measured and the patients were divided in two groups based on vitamin D status: 1) patients with serum levels of 25-hydroxy vitamin D less than 30ng/ml (insufficient vitamin D status) as control group 2) patients with serum levels of 25-hydroxy vitamin D higher than 30ng/ml (sufficient vitamin D status) as case group. Forty eight patients were as controls and cases included 57 patients. Two groups matching up based on age, sex and body mass index (BMI). Seven milliliter of each samples without anticoagulant were centrifuged with 3000rpm/min for 10 minutes and the serum was stored at -70°C. Total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglycerides (TG), calcium and phosphorus serum levels were measured using routine assays (Zistshimi, Iran). Commercial ELISA kit was used to measuring serum levels of oxidized LDL (Mercodia, Germany). Serum levels of 25-hydroxy vitamin D were also measured using luminance method with Elecsysand commercial kit (Roch, Germany). Measurement of HbA1c was performed on 3ml of samples with EDTA. Data were analyzed using the SPSS version 20 software package. All quantitative data have been presented as the mean ± standard deviation. Independent student t-test was used for comparison of two groups. P values <0.05 were considered significant.

RESULTS

Serum levels of 25-hydroxy vitamin D, calcium and phosphorus were significantly lower in case group (P<0.001). Total cholesterol (P=0.03), LDL-C (P=0.01) and LDL-OX (P<0.001) were also higher in case group compared with control group. Serum levels of triglycerides, HDL-C and daily sun exposure were not significantly different between two groups. There were no significant differences in intake of energy, macronutrients, calcium, phosphorus and vitamin D between the groups.

Table 1. Comparison of clinical characteristics between case with Serum levels of 25-hydroxy vitamin D less than 30ng/ml and control with serum levels of 25-hydroxy vitamin D higher than 30ng/ml groups

<table>
<thead>
<tr>
<th>Factors</th>
<th>Cases (n=57)</th>
<th>Controls (n=48)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>vitamin D, ng/ml</td>
<td>13.03±5.2</td>
<td>44.74±15.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>calcium, mg/dl</td>
<td>8.15±0.62</td>
<td>9.59±0.84</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>phosphorus, mg/dl</td>
<td>3.21±0.56</td>
<td>4.04±0.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sun exposure, min/day</td>
<td>67.4±84.2</td>
<td>47.81±67.17</td>
<td>0.2</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>211.28±56.73</td>
<td>188.45±49.99</td>
<td>0.03</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>146.08±54.96</td>
<td>147.2±75.96</td>
<td>0.93</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>140.57±54.88</td>
<td>114.43±64.04</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>41.43±5</td>
<td>39.33±8.25</td>
<td>0.12</td>
</tr>
<tr>
<td>LDL-OX, IU/L</td>
<td>88.8±21.16</td>
<td>47.92±16.91</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are Mean±SD or frequency counts, as appropriate.

DISCUSSION

We have found that levels of oxidized LDL was significantly higher in patients with diabetes type 2 and serum levels of 25-hydroxy vitamin D less than 30ng/ml compared with patients with serum levels of 25-hydroxy vitamin D higher than 30ng/ml. Since serum LDL-C was also lower in control group it seems vitamin D may decrease oxidative stress in these patients. Free radicals are increased in diabetic patients and subsequently attack to LDL-C. Therefore levels of oxidized LDL are raised in the patients (Nakhjavani et al. 2010). Levels of oxidized LDL are associated with atherosclerosis (Davi et al. 2005). Vitamin D is soluble in fat and accumulates in cell membrane. Vitamin D decreases peroxidation of lipids and increases membrane fluidity (Mukhopadhyay et al. 2000). Vitamin D may through reduction of lipid peroxidation decreases oxidized LDL and prevent cardiovascular diseases in diabetic patients. Association between Vitamin D and endothelial function, blood pressure and cardiovascular diseases was shown in former studies. Vitamin D deficiency correlated with hypertension, increased vascular resistance and coronary artery calcification (Sugden et al. 2008). Moreover Vitamin D may improve antioxidant enzymes function. Hyperglycemia could increases oxidative stress through glucose autoxidation, protein glycosylation, formation of advanced glycosylation end products and activation of poliol pathway. Carbonyl group of monosaccharides is converted to alcohol in poliol pathway that leads to accumulation of sorbitol and galactitol in lens of eye and cataract. Sorbitol depletes NADPH therefore inhibits glutathione reductase (GR) activity.
There are some reports that show vitamin D could increase expression of glucose 6-phosphate dehydrogenase (G6PD). This enzyme produces NADPH in pentose phosphate pathway which could increase activity of the enzyme. Oxidative stress hemolysis red blood cells in patients with G6PD deficiency (Bao B et al. 2008). Increases of vitamin D reduced glutathione (GSH) that leads to increases of extracellular and intracellular thiol. Thiol hemostasis guarantees defense mechanisms against xenobiotics and oxidants (Karmakar et al. 2002).

**CONCLUSION**

In the present study it was found serum levels of total cholesterol, LDL-C and LDL-OX were significantly higher in diabetic patients with type 2 and insufficient vitamin D status than patients with sufficient vitamin D status. Oxidative stress could leads to diseases associated with diabetes specially CVD. Therefore, improvement of vitamin D status may reduce the risk of the diseases.

**ACKNOWLEDGMENT**

This MSc thesis was financially supported by Tehran University of Medical Sciences. Research assistant of school of public health, Tehran University of Medical Sciences, who provided field of research, is appreciated. It is appreciated of participants that collaborate us during the study.

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