Association between insulin resistance and inflammatory cytokines among obese Saudi type 2 diabetic with vitamin D deficiency

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ABSTRACT

Background: Vitamin D deficiency is now considered a public health problem around the world that is strongly associated with all-cause and cardiovascular mortality. However, vitamin D deficiency may play a role in mediating low-grade inflammation and insulin resistance among type 2 diabetic patients. Objective: The aim of the present study was to investigate the possible relationship between inflammation, insulin resistance among obese Saudi type 2 diabetic with vitamin D deficiency. Materials and Methods: One hundred obese Saudi patients with T2DM (60 males and 40 females). Their age was 46.38 ± 7.53 year, and a control group included one hundred healthy volunteers, who was gender and age matched. Results: Obese T2DM patients showed significantly higher glucose, insulin, glycosylated hemoglobin (HBA1c), Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and C-reactive protein(CRP) in addition to significantly lower values of the quantitative insulin-sensitivity check index (QUICKI) and 25(OH) vitamin D levels in comparison to controls. Serum levels of TNF-α, IL-6 and CRP showed an inverse relationship with QUICKI and a direct relationship with HOMA-IR and HBA1c among obese Saudi type 2 diabetic with vitamin D deficiency. Conclusion: Within the limit of there is an association between insulin resistance and inflammatory cytokines among obese Saudi type 2 diabetic with vitamin D deficiency.

Keywords: insulin resistance, type 2 diabetes, obesity, inflammatory cytokines, vitamin D deficiency

INTRODUCTION

Vitamin D deficiency is a worldwide epidemic health related problem (1,2) and potentially playing a role in the development of type 2 diabetes mellitus (T2DM) as In 2008, it was estimated that 1 billion individuals presented with vitamin D insufficiency or deficiency (3) and cardiovascular disease (CVD) (4), as well as with an increased overall mortality risk[5]. The pathogenesis of T2DM remains unknown as there are many malfunctioning mechanisms that occur simultaneously which can contribute to the development of the disease (6); however increasing evidence suggests that vitamin D deficiency (as measured by serum 25-hydroxyvitamin-D3 concentration) may also contribute to the pathogenesis of T2DM (7-9). One follow-up study, through 20 years on 4,843 patients with T2DM, showed that vitamin D intake was associated with reduced prevalence of the T2DM (10).

Type 2 diabetes mellitus (T2DM) is a major global health problem. About six people approximately die every minute from the disease worldwide; a rate that will soon portray T2DM as one of the most prevalent health problems in the world (11). The number of people with diabetes is around 285 million and expected to reach 438 million by 2030 worldwide (12), ninety percent of which will have T2DM (13). T2DM is associated with increased morbidity and mortality due to its predisposing factor for cardiovascular disease and stroke (14,15). Cardiovascular disease is the leading cause of death in individuals with T2DM (16).

Clinical studies have demonstrated a positive correlation between circulating vitamin D (25-hydroxyvitamin D; 25(OH)D) levels and insulin sensitivity, they indicate that vitamin D deficiency may predispose to glucose intolerance, altered insulin secretion and type 2 diabetes (17), either through a direct action via vitamin D receptor (VDR) activation or indirectly via calcemic hormones and also via inflammation (18,19). Chronic low-grade inflammation, frequently observed in obese individuals, is involved in the development of insulin resistance, which increases the risk of type 2 diabetes (20). Hotamisligil and colleagues were the first who stated the first link between obesity, inflammation and insulin action (21). The relations between 25(OH)D concentrations and inflammatory markers have been investigated in several studies, with most of these studies based on small samples or specific patient groups (22,23).

There is strong evidence that activation of inflammatory pathways interferes with normal metabolism and disrupts proper insulin signaling resulting in increased expression of pro-inflammatory cytokines (24). These cytokines can target cell membrane receptors, feeding into inflammatory response and exacerbating insulin resistance (25). Another important molecular mediators that link pro-inflammatory cytokine to inhibition of insulin signaling are suppressors of cytokine signaling (SOCS) 1 and 3, induced by IL-6, which lead to ubiquitinylation and degradation of insulin receptor substrate (IRS) proteins (26). There is increasing evidence from clinical and observational studies that a systemic, sub-clinical, low-intensity inflammatory reaction not only co-exists, but also precedes the development of T2DM (27,28). However, the inflammatory markers that have shown the strongest predictive capacity in the development of T2DM are C-reactive protein (CRP) and IL-6 (29, 30). Epidemiological studies have shown that TNF-α, CRP and IL-6 are positively correlated with BMI and percentage body fat (31, 32). Several recent human studies
have associated vitamin D status with type 2 diabetes development [27].

The aim of the present study was to investigate the possible relationship between inflammation, insulin resistance among obese Saudi type 2 diabetic with vitamin D deficiency.

MATERIALS AND METHODS

Subjects

One hundred Saudi obese T2DM patients (60 males and 40 females) with body mass index (BMI) ranged from 31 to 35 Kg/m², were selected from the out-patient diabetic clinic of the King Abdulaziz Teaching Hospital. They were checked for fasting/random glucose levels. Only participants have fasting blood sugar levels more than 5.6 mmol/L or random blood sugar level more than 7.8 mmol/L (impaired blood sugar) were included in this study and were further checked for type 2 diabetes mellitus as per recent American Diabetes Association criteria i.e. fasting blood sugar ≥7.0 mmol/L or post-prandial blood sugar ≥11.1 mmol/L [2-h plasma glucose 11.1 mmol/L during an oral glucose tolerance test] and glycosylated hemoglobin (HbA1c%) > 6.5% [33]. Exclusion criteria included smokers, kidney insufficiency, congestive heart failure, pregnant female patients, hepatitis and respiratory failure. A detail clinical history and physical examinations were conducted which included the age, sex, symptoms suggestive of diabetes and family history of diabetes. Physical examinations included anthropometric measurements such as height, weight, body mass index (BMI) and waist circumference. Also, One hundred apparently healthy, medically free, and treatment naive individuals were recruited to serve as non-diabetic control. Informed written consent was obtained from each included subjects.

Laboratory Analysis

For the biochemical estimations, 5.0 ml fasting venous blood samples from the subjects were drawn after a minimum fasting period of 12 hours. Serum samples were stored at -80 °C till further use.

A. Serum concentrations of 25-OH vitamin D: Measurement of 25(OH) vitamin D for all patients and controls were done by the commercial kit RIA (Elisa Kit; DiaSorin, Stillwater, MN, USA). Plasma 25(OH) vitamin D concentrations of less than 20 ng/ml were defined as 25(OH) vitamin D deficiency and less than 30 ng/ml as 25(OH)D deficiency and insufficiency [34,35].

B. Serum glucose, insulin and insulin resistance tests: Glucose was measured on the Hitachi 912 Chemistry Analyzer using the hexokinase reagent from Boehringer Mannheim (Indianapolis, IN 46256). For the oral glucose tolerance test; after an overnight fast, subjects were given 75 g of oral glucose dissolved in 250 ml of water and blood sugar was quantified after 2 hours. Human insulin was measured with an insulin kit (Roche Diagnostics, Indianapolis, IN, USA) using a cobas immunoassay analyzer (Roche Diagnostics). Insulin resistance was assessed by homeostasis model assessment (HOMA-IR). HOMA-IR = (fasting blood glucose (mmol/l)– fasting insulin (mU/ml))/22.5 [36]. However, insulin sensitivity was assessed by the quantitative insulin-sensitivity check index (QUICKI) using the formula: QUICKI=1/[log(insulin) + log(glucose)] [37]. All serum samples were analyzed in duplicates.

C. Inflammatory cytokine measurements: Inflammatory cytokines included tumor necrosis factor-alpha (TNF-α) and Interleukin-6 (IL-6) levels were measured from frozen plasma samples stored at -80 °C. Enzyme-linked immunosorbent assays kits (ELISAs) were used to measure TNF-α and IL-6 (GE Healthcare Amersham, Biotrik Easy ELISA), which employs the quantitative sandwich enzyme immunoassay technique. However, C-reactive protein (CRP) level was quantified by enzymatic-colorimetric method using commercially available kits (Roche Diagnostics, Mannheim, Germany) at King Abdul-Aziz University Hospital.

Statistical Analysis

Independent t-test was used to compare mean differences between both groups. Statistical analysis of data was performed using SPSS (Chicago, IL, USA) version 17. The degree of correlation inflammation and insulin resistance among obese T2DM patients with vitamin D deficiency was detected by Pearson’s product moment correlation coefficients (r).

RESULTS

One hundred obese Saudi T2DM patients were enrolled including 60 men and 40 women, had a mean age of 46.38 ± 7.53 years and one hundred healthy subjects, had a mean age of 45.64 ± 8.15 years, there was no significant differences in body mass index between both groups (Table 1). Table 2 summarizes the comparison between T2DM patients and matched controls. Obese T2DM patients showed significantly higher glucose, insulin, glycosylated hemoglobin (HBA1c), Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and C-reactive protein(CRP) in addition to significantly lower values of the quantitative insulin-sensitivity check index (QUICKI) and 25(OH) vitamin D levels in comparison to controls (Table 3).

Table 1: Demographic and anthropometric characteristics of type 2 diabetic patients and control subjects

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>Female/ Male</th>
<th>Mean ±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)Vitamin D (ng/ml)</td>
<td>14.26 ± 3.51</td>
<td>32.45 ± 5.83</td>
<td>7.61</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>15.12 ± 2.84</td>
<td>10.36 ± 2.45</td>
<td>6.32</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>8.71 ± 1.36</td>
<td>4.22 ± 1.21</td>
<td>5.73</td>
</tr>
<tr>
<td>IL-6 (mg/mL)</td>
<td>8.13 ± 2.42</td>
<td>5.74 ± 1.74</td>
<td>5.22</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>15.56 ± 3.71</td>
<td>8.13 ± 2.82</td>
<td>7.15</td>
</tr>
<tr>
<td>HOMA-IR (%)</td>
<td>9.31 ± 2.80</td>
<td>6.17 ± 1.36</td>
<td>6.43</td>
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Table 2: Pearson’s correlation coefficients test value of the studied variables in the diabetic group

| TNF-α (pg/mL) | 0.71** | 0.67** | 0.94** |
| IL-6 (mg/mL) | 0.62** | 0.30** | 0.58** |
| CRP (mg/dl) | 0.58* | 0.92* | 0.61** |
| HBA1c (%) | 0.392 | 0.635** | 0.43* |

HOMA-IR : Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index, QUICKI : The quantitative insulin-sensitivity check index ; HOMA-IR = glycosylated hemoglobin; ICAM-1 - Inter-Cellular Adhesion Molecule ; VCAM-1 - Vascular Cell Adhesion Molecule; (*) indicates a significant difference between the two groups, P < 0.05; **: P < 0.01; ***: P < 0.001.
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