Inflammatory biomarkers and endothelial dysfunction among obese type 2 diabetic patients: A correlational study

Mohammed H. Saiem Al-Dahr

ABSTRACT

Background: The vascular complications of diabetes mellitus impose a huge burden on the management of this disease. Inflammation is one of the major factors in the formation of endothelial dysfunction. Endothelial dysfunction is a major contributor to the complications of diabetes mellitus.

Objective: The aim of the present study was to investigate the possible relationship between inflammation and endothelial dysfunction in obese type 2 diabetic patients.

Materials and Methods: Forty obese type 2 diabetic patients (24 males and 16 females) with body mass index (BMI) ranged from 31 to 35 Kg/m², non-smokers, free from respiratory, kidney; liver, metabolic and neurological disorders were recorded in this study. Their age ranged from 40 to 55 years were included in this study as the first group (A). While a forty non-diabetic subjects (23 males and 17 females) not suffering of any disease, their age ranged from 40 to 52 years were included in this study as the second group (B) and considered as a control group.

Results: Diabetic patients showed significantly higher values of TNF-α, IL-6, CRP, HBA1c, ICAM-1, VCAM-1 and E-selectin in comparison to controls. Also, there was significant positive correlation between inflammatory and endothelial dysfunction biomarkers among obese diabetic patients.

Conclusion: There was a strong association between elevated biomarkers of inflammation and endothelial dysfunction among type 2 diabetic patients.

Key Words: Endothelial Dysfunction; Inflammatory Cytokine; Obesity and Non-Insulin Dependent Diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a major medical problem worldwide. The World Health Organization (WHO) stated that about 350 million diabetic subjects all over the world and this number will be the double by 2030 (1), these numbers proved that there is a need for novel therapeutic intervention for both diabetes prevention and treatment (2). However, obesity and diabetes are becoming pandemic and pose a major risk for several comorbidities as cardiovascular disorders. Adipose tissue is considered as an active endocrine tissue that secretes cytokines which contribute to atherosclerosis and systemic inflammation which may contribute to the etiology of type 2 diabetes (3).

Obesity is associated with low-grade of systemic inflammation and disorder in production of adipocytokines that were involved in the pathogenesis of type 2 diabetes related cardiovascular disorders (4-5) which increased the mortality rate among the diabetic patients (6). Increased serum levels of markers of systemic inflammation e.g. interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α) and C-reactive protein (CRP) are usually associated with increased morbidity and mortality among type 2 diabetic patients (7-11).

Elevated serum levels of inflammatory markers in type 2 diabetic patients lead to endothelial cell dysfunction (12-13), which may be induced by hyperlipidemia, hyperinsulinemia and pancreatic B-cell failure leading to (13). Insulin regulates vascular function by stimulation of the expression of vascular cell adhesion molecule (soluble vascular cell adhesion molecule-1 (VCAM-1), soluble intercellular cell adhesion molecule -1 (ICAM-1) and E-selectin on endothelium. So, endothelial dysfunction is associated with insulin resistance (14). Endothelial dysfunction is characterized by prothrombic properties, pro-inflammatory state and reduced vasodilation (15,16).

The aim of this study was to detect the relationship between inflammations markers, endothelial dysfunction in obese type 2 diabetic patients in Saudi population.

MATERIALS AND METHODS

Subjects

Forty (40) adult obese type 2 diabetic patients (24 males and 16 females) with body mass index (BMI) ranged from 30 to 35 Kg/m², nonsmokers, free from kidney, respiratory, liver, neurological disorders, considered as the first group (A). Forty (40) age-matched non-diabetic subjects ranging in age from 40 - 52 years old were collected from diabetic clinic at King Abdulaziz University Hospital, Jeddah, Saudi Arabia and control group (B). All participants signed a consent form before participation in the study.
Methods

1. Sample Processing: Blood samples were collected in the morning after at least 10h fasting hemolyzed and lipemic samples were excluded. Whole blood samples were centrifuged (at 3500 rpm for 5 minutes), to separate the serum and plasma. All samples were stored after centrifugation at -80°C until time of processing. These samples were collected in three different vacutainer tubes (BD vacutainer). One tube for measuring CRP levels, glucose concentration and IL-6, the second tube contains Lithium Heparin to measure, soluble E-selectin (sE-selectin) and soluble inter-cellular adhesion molecule 1 (sICAM-1, VAM-1), TNF-α, and where the third tube contains EDTA K2, EDTA K3 for measuring Glycosylated Hemoglobin (HbA1).

A: Biochemical Parameters: Biochemical parameters including serum Glucose, Glycosylated Hemoglobin (HbA1C) and CRP all were measured at the same time after collection. Fasting serum glucose concentration, HbA1C and CRP were quantified by enzymatic-colorimetric methods using commercially available kits (Roche Diagnostics, Mannheim, Germany) at king Abdul-Aziz University Hospital. For postprandial protocol, after a 10 h overnight fasting, the subjects in both groups consumed a liquid made of 50g Dextrose (Thermo scientific, USA), blood samples were collected in the fasting state and after 2 h. Plasma and serum were immediately separated by centrifugation and processed as before.

B. Measurement of Biomarkers of Endothelial and Inflammatory Cytokines: Biomarkers of endothelial function included adhesion molecules (ICAM-1 and VCAM-1), soluble E-selectin, and inflammatory cytokines such as 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 soluble levels of ICAM-1 and VCAM-1, sE-selectin, (TNF-α), and IL-6 (GE Healthcare Amersham, Biotrak Easy ELISA), which employs the quantitative sandwich enzyme immunoassay technique.

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, endothelial dysfunction in obese type 2 diabetic patients was detected by Pearson’s product moment correlation coefficients (r).

RESULTS

The mean values of the clinical and laboratory parameters variables for participants in the two groups have a statistical significant difference as presented in (Table 1). The data that have been computed in (Table 2) summarizes the comparison between patients and matched controls. Diabetic patients showed significantly higher values of TNF-α, IL-6, CRP, Hba1c, ICAM-1, VCAM-1 and E-selectin in comparison to controls. However, (Table 3) represents correlation coefficient (r) between studied parameters in diabetic patients, significant positive correlations were found between TNF-α, IL-6, CRP, Hba1c and ICAM-1, VCAM-1, E-selectin. transplant-related and clinical data of the randomized patients are presented in Table 1 and 2. No differences in either demographic or transplant data were observed between the 2 groups. No between-group differences were found as regards clinical and laboratory parameters as shown in Table 2. All patients in both groups completed the 3-year follow-up and were included in the final intention-to-treat analysis.

Table 1: This table demonstrates the mean value of clinical and laboratory parameters for all participants in the two groups.

<table>
<thead>
<tr>
<th></th>
<th>Diabetic group</th>
<th>Control group</th>
<th>T-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>Male 47±6.87</td>
<td>Male 35±0.65</td>
<td>5.42</td>
<td>P &lt;0.05</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>33.11±2.25</td>
<td>26.58±2.97</td>
<td>4.52</td>
<td>P &lt;0.05</td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>180.32±14.45</td>
<td>87.63±6.75</td>
<td>9.87</td>
<td>P &lt;0.05</td>
</tr>
<tr>
<td>PPS (mg/dl)</td>
<td>253.67±21.46</td>
<td>105.92±15.21</td>
<td>8.95</td>
<td>P &lt;0.05</td>
</tr>
</tbody>
</table>

Table 2: This table illustrates the mean value and significance of inflammatory markers, Glycosylated Hemoglobin, and cellular adhesion molecules TNF-α, IL-6, CRP, Hba1c (%), ICAM-1, VCAM-1, E-selectin in both diabetic (A) and control group (B).

<table>
<thead>
<tr>
<th></th>
<th>Mean ±SD</th>
<th>T-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic group</td>
<td>Control group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>5.93 ± 1.57</td>
<td>10.10 ± 1.52</td>
<td>5.43</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>16.34 ± 3.22</td>
<td>10.16 ± 2.61</td>
<td>6.25</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>5.27 ± 1.25</td>
<td>5.11 ± 1.85</td>
<td>5.76</td>
</tr>
<tr>
<td>Hba1c (%)</td>
<td>9.13 ± 2.15</td>
<td>6.05 ± 0.87</td>
<td>6.13</td>
</tr>
<tr>
<td>ICAM-1 (ng/ml)</td>
<td>94.65 ± 11.23</td>
<td>81.17 ± 9.16</td>
<td>7.45</td>
</tr>
<tr>
<td>VCAM-1 (ng/ml)</td>
<td>832.31 ± 46.11</td>
<td>724.16 ± 38.22</td>
<td>9.27</td>
</tr>
<tr>
<td>E-selectin (ng/ml)</td>
<td>14.89±6.02</td>
<td>8.71±3.51</td>
<td>5.84</td>
</tr>
</tbody>
</table>

Table 3: Pearson’s correlation coefficients test value of the studied variables in the diabetic group.

<table>
<thead>
<tr>
<th></th>
<th>ICAM-1 (ng/ml)</th>
<th>VCAM-1 (ng/ml)</th>
<th>E-selectin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/ml)</td>
<td>0.875***</td>
<td>0.767***</td>
<td>0.618***</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>0.635**</td>
<td>0.792***</td>
<td>0.753***</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.914***</td>
<td>0.584**</td>
<td>0.881***</td>
</tr>
<tr>
<td>Hba1c (%)</td>
<td>0.473*</td>
<td>0.519**</td>
<td>0.476*</td>
</tr>
</tbody>
</table>

DISCUSSION

Currently, the steady rising medico-social burden of type 2 diabetes is positively correlated to the vascular system comorbidities, there is 4-fold increase in the rate of ischemic heart disease, a 10-fold increase in rate of peripheral vascular disorders, and a 3- to 4-fold increase of mortality rate with about 75% of diabetic patients dying by vascular disorders (17, 18). The principal findings of our study are that higher levels of BMI, Hba1c, IL-6, TNF-α, CRP were associated with higher levels of E-selectin levels in type 2 diabetics. Our findings are consistent with many studies which reported a significant association between diabetes and elevated CRP (19, 20). However, Swellam et al. stated that elevated levels of CRP can be used for early diagnosis of non-insulin dependent diabetes mellitus and can predict diabetic complications (21). Also Pradhan et al. concluded that elevated levels of CRP and IL-6 predict the development of type 2 diabetes mellitus (22). Also, Liu et al., confirmed the role played by TNFα, IL-6 and CRP as an etiologic factor for non-insulin dependent diabetes (23).
In this study VCAM-1, ICAM-1 and E-selectin level were significantly higher in diabetic group than the control group. Therefore, the results in this study are consistent with Meigs et al., who reported that endothelial dysfunction predicts non-insulin dependent diabetes among women (19). Also, Thorand et al., supported the role of endothelial dysfunction in the pathogenesis of non-insulin dependent diabetes (24). However, level of sE-selectin was found to be independently associated with diabetes (25, 26). In addition, the Women’s Health Initiative Observational Study proved that found E-selectin could be considered as a predictor of diabetes among U.S.A. women (27).

The underlying mechanisms for increased rate of cardiovascular morbidity and mortality among diabetic patients are not fully elucidated, however elevated markers of systemic inflammation and endothelial dysfunction are associated with excess visceral adiposity (28). Moreover, Goldberg stated that systemic inflammation affects platelet adhesion, oxidants production and platelet aggregation which induced cardiovascular disorders among diabetic patients(29).

In conclusion, there is a strong association between markers of endothelial dysfunction and systemic inflammation among type 2 diabetic patients. Novel therapeutic measures to ameliorate endothelial dysfunction and systemic inflammation may be essential in clinical management for type 2 diabetic populations.

Acknowledgment

Author thanks physicians of the outpatient clinic and all the laboratory staff members at King Abdulaziz University Hospital, Jeddah, Saudi Arabia, for their skillful assistance in the clamp procedures of this article and is grateful for the cooperation of all participants in this study.

REFERENCES