Research Article

Inflammatory Biomarkers and Endothelial Dysfunction among Obese Patients with Type 2 Diabetes: A Correlational Study

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Abstract

Background: Systemic inflammation and endothelial dysfunction induce vascular complications of diabetes mellitus that impose a huge burden on diabetes mellitus complications management.

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

Materials and Methods: Forty obese patients with type 2 diabetes (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 participated in this randomized controlled trial. Their age ranged from 40 to 55 years were randomly 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 systemic inflammation and endothelial dysfunction among obese patients with type 2 diabetes.

Keywords: Endothelial dysfunction; Inflammatory cytokine; Obesity; Non-insulin dependent diabetes mellitus

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 β-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 systemic inflammation markers, endothelial dysfunction in obese Saudi patients with type 2 diabetes mellitus.

Materials and Methods

Subjects

Forty non-smokers obese type 2 diabetic patients visiting king Abdulaziz University Hospital, Jeddah, Saudi Arabia, were included in this randomized controlled study. The mean age was 47.73±6.87 year and mean body mass index was 33.11±2.25 kg/m² who

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

	Mean+SD		T-value	Significance
	Diabetic group	Control group		
Age (year)	47.73±6.87	35.40±6.05	5.42	P<0.05
BMI (kg/m²)	33.11±2.25	26.58±2.97	4.52	P<0.05
FBS (mg/dl)	180.32±14.45	87.63±6.75	9.87	P<0.05
PPS (mg/dl)	253.67±21.46	105.92±15.21	8.95	P<0.05

BMI: Body Mass Index; FBS: Fasting Blood Sugar; PPS: Postprandial Blood Sugar

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).

	Mean+SD		T-value	Significance
	Diabetic group	Control group		
TNF-α (pg/mL)	5.93±1.57	4.10±1.52	5.43	P<0.05
CRP(mg/dl)	16.34±3.22	10.16±2.61	6.25	P<0.05
IL-6 (pg/mL)	8.27±2.15	5.11±1.85	5.76	P<0.05
HBA1c (%)	9.13±2.15	6.05±0.87	6.13	P<0.05
ICAM-1 (ng/ml)	94.65±11.23	81.17±9.16	7.45	P<0.05
VCAM-1 (ng /ml)	832.31±46.11	724.16±38.22	9.27	P<0.05
E-selectin (ng/ml)	14.89±6.02	8.71±3.51	5.84	P<0.05

TNF-α: Tumor Necrosis Factor-Alpha; IL-6: Interleukin-6; CRP: C-Reactive Protein; HBA1c: Glycosylated Hemoglobin; ICAM-1: Inter-Cellular Adhesion Molecule; VCAM-1: Vascular Cell Adhesion Molecule

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

	ICAM-1 (ng/ml)	VCAM-1 (ng/ml)	E-selectin (ng/ml)
TNF-α (pg/mL)	0.875***	0.767***	0.618**
IL-6 (pg/mL)	0.635**	0.792***	0.753***
CRP(mg/dl)	0.914 ***	0.584**	0.881 ***
HBA1c (%)	0.473*	0.519**	0.476*

TNF-α: Tumor Necrosis Factor–Alpha; IL-6: Interleukin-6; CRP: C-Reactive Protein; HBA1c: Glycosylated Hemoglobin; ICAM-1: Inter-Cellular Adhesion Molecule; VCAM-1: Vascular Cell Adhesion Molecule; Spearman's correlation was used *: P<0.05 **: P<0.01 ***: P<0.001.

concentration, HbA1$_C$ 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 10h 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 2h. Plasma and serum were immediately separated by centrifugation and processed as before.

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 systemic inflammation, endothelial dysfunction in obese patients with type 2 diabetes 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.

Discussion

Currently, the steady rising medico-social burden of type 2 diabetes is positively correlated to the vascular system co-morbidities, 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

participated in the current study as group (A). Initially, a physician at King Abdulaziz University Hospital examined all participants; their medical history was taken to collect information about general condition, physical activity and current medications if any. All subjects with any pulmonary disorders, cardiovascular conditions as uncontrolled hypertension, congenital and rheumatic heart diseases were excluded from the study. In the other hand another forty age-matched non-diabetic healthy subjects were participated in the study as a control group (B). This study was approved by the Scientific Research Ethical Committee, Faculty of Applied Medical Sciences at King Abdulaziz University and all participants signed a written informed consent.

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).

Biochemical parameters: Biochemical parameters including serum Glucose, Glycosylated Hemoglobin (HbA1$_C$) and CRP all were measured at the same time after collection. Fasting serum glucose

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 TNF-α, IL-6 and CRP as an etiological 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]. This study has some limitations. First, it was not appropriate to carry out analyses by age group due to a reduction in a negligible sample size. Second, the subjects in this study were only Saudi subjects and capacity of insulin secretion and degree of obesity in Asians are known to be different from Western people, and thus our findings might not be applicable to Caucasians, therefore, a prospective future study is necessary to clarify this limitation.

Conclusion

There is a strong association between markers of endothelial dysfunction and systemic inflammation among obese patients with type 2 diabetes. Novel therapeutic measures to ameliorate endothelial dysfunction and systemic inflammation may be essential in clinical management for type 2 diabetic populations.

Acknowledgment

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