Insulin Resistance, Hypoadiponectinemia and Endothelial Dysfunction Biomarkers Among Type 2 Diabetic Patients

Osama H. Al-Jiffri, Fadwa M. Al-sharif and Essam H. Al-Jiffri

Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, Saudi Arabia

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Correspondence (Yazışma Adresi):
Dr. Osama H. Al-Jiffri
Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, King Abdulaziz University, P.O. Box 80324, Jeddah, 21589, Saudi Arabia.
E-mail: dr_jiffri1@outlook.com

ABSTRACT

Type 2 diabetes (T2DM) is a chronic and progressive disease that is strongly associated with all-cause and cardiovascular mortality. The present study aimed to detect the association between insulin resistance , adiponectin and endothelial dysfunction biomarkers among obese T2DM patients. One hundred obese Saudi patients with T2DM (58 males and 42 females). Their age was 44.61 ± 5.32 year, and a control group included one hundred healthy volunteers, who was gender and age matched. Obese T2DM patients showed significantly higher glucose, insulin, Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index, Inter-Cellular Adhesion Molecule (ICAM-1), Vascular Cell Adhesion Molecule (vCAM-1), E-selectin and significantly lower values of the quantitative insulin-sensitivity check index (QUICKI), and adiponectin levels in comparison to controls. Serum levels of adiponectin showed an association with insulin resistance and endothelial dysfunction biomarkers among type 2 diabetic patients. Within the limit of there is an association between insulin resistance and both hypoadiponectinemia and endothelial dysfunction.

Key Words: *Adiponectin, Insulin Resistance, Type 2 Diabetes, Obesity, Endothelial Dysfunction Biomarkers.*

Tip 2 Diyabetik Hastalarda İnsülin Direnci, Düşük Adiponektin Düzeyleri ve Endotelyal Disfonksiyon Biyobelirteçleri

ÖZET

Tip 2 diyabetes mellitus (T2DM) kardiyovasküler mortalite ile kuvvetle ilişkili kronik ve ilerleyici bir hastalıktır. Bu çalışmanın amacı, obez T2DM hastalarda insülin direnci, adiponektin ve endotel disfonksiyonu biyobelirteçlerini değerlendirmektir. T2DM olan 100 obez hasta (58 erkek ve 42 kadın, yaşları 44.61 ± 5.32 yıl) ve kontrol grubu olarak, 100 sağlıklı gönüllü bu çalışmaya katıldı. Obez T2DM'li hastalar kontrol grubu ile karşılaştırıldığında glukoz, insülin, Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) indeksi, Inter-Cellular Adhesion Molecule (ICAM-1), Vascular Cell Adhesion Molecule (vCAM-1), E-selectin seviyelerinin anlamlı olarak yüksek olduğu ve quantitative insulin-sensitivity check indeksi (QUICKI) ile adiponectin seviyelerinin ise anlamlı olarak düşük olduğu gözlendi. Tip 2 diyabeti olan hastalarda insülin direnci ve endotelyal disfonksiyon biyobelirteçleri ile serum adiponek-

tin düzeyleri arasında bir ilişki gözlendi. Tip 2 diyabeti olan hastalarda insülin direnci ile hem düşük adiponektin düzeyi, hemde endotel disfonksiyonu biyobelirteçleri arasında sınırlı bir ilişki vardır.

Anahtar kelimeler: *Adiponektin, İnsülin direnci, Type 2 diyabet, Obezite, Endotelyal disfonksiyon biyobelirteçleri.*

INTRODUCTION

The prevalence of diabetes around the world is alarmingly high and it is growing. The World Health Organization (WHO) estimated that in 2000 there were 171 million people with diabetes in the world and by 2030, that number is expected to rise to 366 million (1). Type 2 diabetes (T2DM) is an important cardiovascular (CV) risk factor (2). Obesity represents a state of increase in adipose tissue mass due to the increase in the number and size of adipocytes (3). Diabetes increases cardiovascular risk and reduces life expectancy, with most of excess mortality being attributable to cardiovascular causes (4). In addition, T2DM is typically associated with reduced HDL cholesterol (HDL-C) and impaired HDL function (5,6). The hypertension that typically accompanies T2DM seems to be the most significant contributor to this increased risk (7).

Adipose tissue is recognized as an active endocrine organ which secretes adipocytokines involved in the local and systemic regulation of numerous metabolic and inflammatory processes (8). Dysregulated endocrine function of the adipose tissue contributes to the development of obesity related metabolic disorders including insulin resistance, T2DM and atherosclerosis (9).

Adiponectin is an adipokine with insulin sensitizing and anti-inflammatory activities. Adiponectin and insulin resistance is an important link between visceral adiposity and atherosclerosis. Adiponectin improves systemic glucose tolerance and protects the vasculature from atherosclerosis (10). Adiponectin exerts both vasodilatory and insulin-sensitizing actions and its levels are decreased in insulin-resistant humans and animals (11). Circulating levels of adiponectin decrease both in obesity and in patients with T2DM. There is a close association between inflammatory marker, insulin resistance and incidence of T2DM (12). Additionally, hypoadiponectinemia is an independent risk factor for developing T2DM and cardiovascular disease [13).

Type 2 diabetic patients have abnormal levels of inflammatory markers which lead to endothelial cell dysfunction (14-15), that may be induced by hyperlipidemia, hyperinsulinemia and pancreatic ß-cell failure (15). Insulin has an essential role in regulation of 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, that is why endothelial dysfunction is associated with insulin resistance (16). Endothelial dysfunction is characterized by prothrombic properties, pro-inflammatory state and reduced vasodilation (17,18).

Lisowska et al. in their study on stable angina pectoris patients undergoing coronary artery bypass grafting found a significant correlation between adiponectin concentration and adhesion molecule CD146, a natural antithrombin glycoprotein – thrombomodulin (TM) concentration and the activity of Von Willebrand factor (VWF) as some researchers have confirmed a role of CD146 as an endothelial cell dysfunction marker (19,20). So, there is a belief that high level of adiponectin could play a protective role in the cardiovascular system. However, other studies showed no association between adiponectin and the development of CAD (21). Therefore, the present study aimed to detect the association between insulin resistance, adiponectin and endothelial dysfunction biomarkers among obese T2DM patients.

MATERIAL AND METHOD

Subjects

One hundred Saudi obese T2DM patients (58 males and 42 females) with body mass index (BMI) ranged from 30 to 34 Kg/m2, were selected from the out-patient diabetic clinic of the King Abdalziz Teaching Hospital and participated in this prospective study. 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% (22). 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. This study was approved by the Scientific Research Ethical Committee, Faculty of Applied Medical Sciences at King Abdulaziz University. All participants were free to withdraw from the study at any time.

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 ºC till further use.

A. Serum glucose, adiponectin, 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. Serum levels of adiponectin were determined using AviBion human adiponectin (Acrp 30) ELISA kit ref. no. ADIPO 25 (Orgenium Laboratories, Finland). 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 (mIU/ml)]/22.5 (23). However, insulin sensitivity was assessed by The quantitative insulin-sensitivity check index (QUICKI) using the formula: QUICKI=1/[log(insulin) + log(glucose)] (24). All serum samples were analyzed in duplicates.

B. Biomarkers of Endothelial function measurements:

Biomarkers of endothelial function included adhesion molecules (ICAM-1 and VCAM-1) and soluble E-selectin 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 , VCAM-1 and sE-selectin (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 T2DM patients was detected by Pearson's product moment correlation coefficients (r).

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

	Mean +SD		P-value
	Diabetic group	Control group	
Age (year)	44.61 ± 5.32	42.93 ± 6.87	P >0.05
Gender (M/F)	58/42	54/46	P >0.05
BMI (kg/m2)	30.26 ± 3.71	29.52 ± 3.44	P >0.05
Waist circumference (cm)	108.14 ± 9.83	105.24 ± 8.22	P >0.05
FBS (mg/dl)	186.27 ± 12.30*	91.55 ± 5.91	P <0.05
PPS (mg/dl)	248.36 ± 17.28*	118.76 ± 10.56	P <0.05

BMI= Body mass index; FBS = Fasting blood sugar ; PPS = Postprandial blood sugar;

(*) indicates a significant difference between the two groups, P < 0.05.

***Table 2**. Mean value and significance of biochemical parameters of type 2 diabetic patients and control subjects.*

	Mean +SD		T-value	P-value
	Diabetic group	Control group		
Insulin (mU/l)	15.76 ± 3.52*	8.22 ± 2.61	8.32	P <0.05
QUICKI	0.119 ± 0.018*	0.178 ± 0.027	7.16	P <0.05
HOMA-IR	5.85 ± 1.71*	2.93 ± 1.06	6.44	P <0.05
HBA1c (%)	9.21 ± 2.83*	6.11 ± 0.95	8.25	P <0.05
Adiponectin (µg/ml)	4.36 ± 1.62*	8.25 ± 2.14	7.41	P <0.05
ICAM-1 (ng/ml)	95.11 ± 10.35*	80.23 ± 7.52	6.52	P <0.05
VCAM-1 (ng /ml)	823.42 ± 35.19*	718.63 ± 27.41	8.73	P <0.05
E-selectin (ng/ml)	15.13 ± 4.61*	8.55 ± 3.72	6.48	P <0.05

HOMA-IR: Homeostasis Model Assessment-Insulin Resistance index; QUICKI: The quantitative insulin-sensitivity check index; HBA1c: 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.

RESULTS

One hundred obese Saudi T2DM patients were enrolled including 52 men and 48 women, had a mean age of 44.61 ± 5.32 year and one hundred healthy subjects, had a mean age of 42.93 ± 6.87 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. T2DM patients were more insulin resistant as indicated by higher values of insulin and HOMA-IR, and lower values of QUICKI. Also, T2DM patients showed significantly higher glucose, insulin, Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index, ICAM-1, VCAM-1 and E-selectin in addition to significantly lower values of the quantitative insulin-sensitivity check index (QUICKI) and adiponectin levels in comparison to controls (Table 2).

Table 3 summarizes the relationship between parameters of glucose control in NAFLD patients. Serum levels of leptin, resistin, TNF- α and IL-6 showed an inverse relationship with QUICKI and a direct relationship with serum insulin, HOM-IR. However, levels of glucose control and adiponectin showed a direct relationship with QUICKI and an inverse relationship with serum insulin, HOM-IR (Table 3).

DISCUSSION

Type 2 diabetes mellitus is characterized by hyperglycemia due to insulin resistance, which over time leads to a myriad of micro- and macrovascular complications. Individuals with T2DM are at much higher risk (two to four times that of the background population) of developing coronary artery disease (25), peripheral vascular disease (26), and cerebrovascular disease (27). Mortality from cardiovascular disease may be up to four times higher in patients with T2DM (28). However, adiponectin is a circulating adipose tissue-derived a hormone that is down regulated in obese individuals (29). Experimental studies show that adiponectin plays a protective role in the development of insulin resistance, atherosclerosis, and inflammation (30). Insulin resistance and its manifestations predict and precede T2DM and its cardiovascular complications (31). Insulin resistance characterizing obese subjects has also been shown to be associated with endothelial dysfunction (32,33).

Our study underscores that patients with type 2 diabetes had insulin resistance and adiponectin reduction and endothelial function biomarkers alterations. Though type 2 diabetes patients showed significantly higher glucose, insulin, HOMA-IR , ICAM-1 , vCAM-1, E-selectin and significantly lower values of QUICKI and adiponectin levels in comparison to controls.

In the present study VCAM-1 , ICAM-1 and E-selectin level were significantly higher in T2DM patients than the healthy

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

	QUICKI (%)	HOMA-IR (%)	HBA1c (%)
Adiponectin (µg/ml)	0.531*	0.628*	0.519*
ICAM-1 (ng/ml)	0.642*	0.631*	0.714*
VCAM-1 (ng/ml)	0.611*	0.542*	0.593*
E-selectin (ng/ml)	0.582*	0.618*	0.552*

HOMA-IR : Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index, QUICKI : The quantitative insulin-sensitivity check index ; HBA1c = glycosylated hemoglobin; ICAM-1 – Inter-Cellular Adhesion Molecule ; VCAM-1 – Vascular Cell Adhesion Molecule; Spearman's correlation was used *: P < 0.05.

control subjects. Therefore, the results in this study are consistent with Meigs et al., who reported that endothelial dysfunction predicts T2DM among women (34). Also, Thorand et al., supported the role of endothelial dysfunction in the pathogenesis of T2DM (35). However, level of sE-selectin was found to be independently associated with diabetes (36,37). 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 (38). Also, results of the present study proved that adiponectin was decreased among obese T2DM patients; this could be because in the states of increased adiposity, the enlarged adipocytes produce less adiponectin as it is secreted predominantly by the pre-adipocytes (39). One of the mechanisms for the negative correlation between adiposity and adiponectin levels might be the increased secretion of TNF-alpha (TNF-α) from accumulated visceral fat which potentially inhibits adiponectin secretion (40-42).

In our study, there was significant correlation between insulin resistance and adiponectin alteration in T2DM patients. A study conducted by Vaverkova et al. found an independent positive association of sVCAM-1 with adiponectin and also in another previous study of high risk dyslipidemic patients (43,44) represents another effect of adiponectin, which might be detrimental in high risk populations. The positive association of sVCAM-1 with adiponectin was also described recently in T2DM patients with diabetic nephropathy and was associated with endothelial dysfunction measured by flow mediated dilatation (45).

The exact mechanism by which insulin resistance is associated with endothelial dysfunction can be explained by diminish dihydropterin reductase activity that induced by insulin resistance with resultant depletion of Tetrahydrobiopterin (BH4) which is an essential cofactor for the catalytic activity of NOS (Nitrous Oxides) (46). This depletion leads to increased oxidative stress levels and endothelial dysfunction. As insulin is a vasodilator and stimulates endothelial NO production (47,48). Several studies have demonstrated that NO-mediated vasodilation is abnormal in patients with type 2 diabetes (49). In the same way insulin resistance may contribute to endothelial dysfunction, defects in NO-mediated vasodilation may contribute to insulin resistance (50). Moreover, another possible mechanism for endothelial dysfunction induced by insulin resistance which is accompanied by an impaired ability of insulin to inhibit very low density lipoprotein (VLDL) production in the liver in patients with type 2 diabetes (51). An increase in serum triglycerides is accompanied by generation of small dense LDL particles, contribute to endothelial dysfunction in patients with type 2 diabetes (52).

The exact mechanism by which insulin resistance is associated with hypoadiponectinemia can be explained by high blood glucose and high blood fat induced by hypoadiponectinemia will result in high blood glucose and high blood fat. High blood fat is the direct cause of insulin resistance. According to studies by Dresner et al. (1999) and Griffin et al. (1999), an increase in the delivery of fatty acids to muscle or a decrease in intracellular metabolism of fatty acids leads to an accumulation of intracellular

fatty acid metabolites, such as diacylglycerol, fatty acyl CoA, and ceramides. These metabolic activities activate a serine/threonine kinase cascade leading to phosphorylation of serine/threonine sites on insulin receptor substrate 1 (IRS1) and insulin receptor substrate 2 (IRS2). This, in turn, reduces the ability of the insulin receptor substrates to activate phosphatidylinositol 3 kinase (PI 3 kinase) and thus reduces the activity of glucose transporter 4 (GLUT4). As a consequence, the glucose transport activity of insulin receptors is diminished, which reduces glucose uptake in the skeletal muscle cells. On the basis of the statements given earlier, the cascade of mechanism from hypoadiponectinemia to T2DM. The decrease of plasma adiponectin can cause decreased glucose uptake, increased gluconeogenesis, and decreased fatty acid oxidation in the skeletal muscles and the liver. The decrease of fatty acid oxidation causes the increase of free fatty acids, following increase of insulin resistance, and finally a decrease in glucose uptake (53,54). The decrease in glucose uptake and the increase of gluconeogenesis ultimately result in the increase of plasma glucose and T2DM (55).

Conclusion

Within the limit of there is an association between insulin resistance and both hypoadiponectinemia and endothelial dysfunction.

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