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Increased Adipose Tissue Expression of Tumor Necrosis Factor-Alpha and Insulin Resistance in Obese Subjects with Type II Diabetes

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Abstract: The aim of this study was to compare the changes in TNF-α, HbA1c, blood lipids profile, renal profile and blood parameters in non insulin dependent diabetic obese patients and non diabetic subjects. Forty male NIDDM patients and forty age-matched non-diabetic subjects ranging in age from (40–60) years were collected from diabetic clinic at King Abdulaziz University Hospital. All patients with type II diabetes (NIDDM) were without diabetic complications, such as diabetic nephropathy, neuropathy, retinopathy, hypertension and heart disease. There were no significant differences between the diabetic patients and non-diabetic subjects of prevalence of Male gender, Age, Cholesterol, Triglycerides, LDL, HDL, Hgb, RBCs, WBCs, HCT, platelets count, Albumin, Urea, Creatnine and Total protein. However, compared with non-diabetic subjects, diabetics patients had higher Fasting Serum Glucose (FSG) ($p < 0.0001$), Tumor necrosis factor-α (TNF-α), ($p < 0.0001$), Hemoglobin A_{1c} (HbA_{1c}) ($p < 0.0001$) and Body Mass Index ($p<0.0001$). Current observations add further support to the evidence that TNF-α plays an important role in insulin resistance associated with obesity and/or diabetes mellitus. Further investigations in larger cohort are recommended to support the obtained outcome.

Key words: Tumor necrosis factor-α · Type II diabetes mellitus · Insulin resistance · Obesity

INTRODUCTION

Type II diabetes is characterized in part by elevated plasma levels of free fatty acids (FFAs) and glucose and is associated with a cluster of abnormalities, such as central obesity, dyslipidemia, hyperinsulinemia, elevated plasma inflammatory markers, vascular abnormalities and hypertension. This cluster of abnormalities, referred to as the metabolic or insulin resistance syndrome, is associated with increased risk for cardiovascular and cerebrovascular diseases [1] and [2].

Type II diabetes is a heterogonous syndrome characterized by insulin resistance and/or defective insulin secretion. There seems to be racial difference in insulin resistance in type II diabetes. The prevalence of white type II diabetic patients and found that 92% of type II diabetic patients were insulin-resistant. Recently it was reported that 60% of type II diabetic patients with BMI less than 30 kg/m^2 were insulin-resistant in African-American populations [3].

Insulin resistance is a fundamental defect that precedes the development of the full insulin resistance syndrome as well as β cell failure and type II diabetes. Tumor necrosis factor-α (TNF-α), a paracrine/autocrine factor highly expressed in adipose tissues of obese animals and human subjects, are implicated in the induction of insulin resistance seen in obesity and type II diabetes [4].

Tumor necrosis factor-alpha (TNF-α) can be produced by adipose tissues [5], kidneys [6] and atherosclerotic lesions in the arterial walls [7]. In the kidneys, advanced glycation end-products (AGEs) [8], angiotensin II [9] and oxidized LDL [10] can stimulate TNF-α synthesis from the renal cells and initiate local effects of renal damage. The activities of this cytokine are not limited to the renal injury; intra-arterial TNF-α administration can cause vascular inflammation and impair endothelial function [11]. Circulating levels of TNF-α were elevated in patients with metabolic syndrome [12], regarded as a pre-diabetic state and in obese [13] or nephropathic [14] patients with type II diabetes. Such patients are therefore considered at high risk of CVD events.

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Tumor necrosis factor-α (TNF-α) is thought to participate importantly in insulin resistance in obese subjects by inhibiting tyrosine kinase activity at the insulin receptor. Phosphorylation of the insulin receptor by tyrosine kinase is critical to the binding of insulin to its receptor. Increased adipose and muscle expression of TNF-α has been demonstrated in human obesity [15] and [16].

According to the International Diabetes Federation (IDF), 246 million people worldwide have diabetes and non insulin dependent diabetic patients (NIDDM) accounts for 90% to 95% of all diagnosed cases associated with obesity [17]. The long-term complications associated with NIDDM are both microvascular and macrovascular in nature and include the following: retinopathy, peripheral and autonomic neuropathy, nephropathy, peripheral vascular disease, atherosclerotic cardiovascular and cerebrovascular disease, hypertension and susceptibility to infections and periodontal disease [18].

Accumulation of adipose tissue in organs such as the liver and/or skeletal muscle underlies insulin resistance in obese subjects. Counter regulatory hormones e.g. TNF-alpha and resistin secreted from adipose tissue could also hypothetically cause insulin resistance via direct actions in insulin sensitive tissues such as skeletal muscle [19].

Tumor necrosis factor alpha (TNF-α) is a cytokine with a wide range of proinflammatory activities [20]. It is primarily produced by monocytes/macrophages [21], although significant amounts are also secreted by several other cell types. Disturbances in the TNF-α metabolism have been implicated in metabolic disorders, such as obesity and insulin resistance [22], indicating that perturbations of TNF-α metabolism may affect the onset of non-insulin-dependent diabetes mellitus and play a role in the development of cardiovascular disorders. Indeed, increased plasma concentrations of TNF-α have been found in patients with premature coronary artery disease [23]. However, it remains unclear whether elevated serum TNF-α in patient with manifest atherosclerosis derives from atherosclerotic plaques or from nonvascular sources [24].

The mechanisms via which obesity causes insulin resistance of glucose or free fatty acid (FFA) metabolism are still incompletely understood. It has been suggested that in obese subjects the excess release of FFA from adipose tissue inhibits glucose uptake in peripheral tissues and stimulates hepatic glucose production [25]. Another possibility is that accumulation of adipose tissue in organs such as the liver and/or skeletal muscle underlies insulin resistance in obese subjects. Counter regulatory hormones e.g. TNF- alpha and resistin secreted from adipose tissue could also hypothetically cause insulin resistance via direct actions in insulin sensitive tissues such as skeletal muscle. Regarding the cellular mechanisms underlying insulin resistance in obesity, obese subjects have a decrease in insulin-stimulated tyrosine kinase activity of the insulin receptor in skeletal muscle [26].

The aim of this study was to compare the changes in TNF-α, HbA1c, blood lipids profile, renal profile and blood parameters in non insulin dependent diabetic obese patients and non diabetic subjects.

MATERIALS AND METHODS

Inclusion Criteria: Forty NIDDM and forty age-matched non-diabetic male subjects ranging in age from 40–60 years were collected from diabetic clinic at King Abdulaziz University Hospital.

Exclusion Criteria: Type II diabetes (NIDDM) with diabetic complications, such as diabetic nephropathy, neuropathy, retinopathy, hypertension and heart disease based on clinical and laboratory investigations.

Methods

Sample Processing: Samples were collected after 12 hours fasting, hemolyzed and lipemic samples were excluded. Samples were centrifuged (at 3500 rpm for 5 minutes), to separate the plasma. All samples stored after centrifugation at -70°C until time of processing. These samples were collected in three different vacutainer tubes. One tube for measuring the Lipid Profile (Total Cholesterol, Triglycerides, LDL and HDL) and Renal Profile (Total Albumin, Creatinine, Urea and Total Protein), the second tube contains Lithium Heparin to measure TNF-α, where the third tube contains EDTA K_2, EDTA K_3 for measuring complete blood count and Glycosylated Hemoglobin (HbA_{1C}).

Methods And Technique

Evaluation of Anthropometric Parameters (Body Mass Index): All measurements were performed before treatment and after two months at the end of the study. The participants were measured whilst wearing their undergarments and hospital gowns. Height was measured with a digital stadiometer to the nearest 0.1 cm (JENIX DS 102, Dong-sang, South Korea). Body weight was measured on a calibrated balance scale to the nearest 0.1 kg (HC4211, Cas-Korea, South Korea) and BMI was calculated as Body weight/$Height^2$.

Biochemical Parameters: Biochemical parameters including serum Glucose, Glycosylated Hemoglobin (HbA_{1C}), Urea, Creatinine, Albumin, Total protein, Total cholesterol and Triglycerides all were measured at the same time. Fasting serum glucose concentration was measured using an enzymatic reaction and also used to measured triglycerides (TG), total cholesterol and its fractions; high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL). Total serum Albumin, Creatinine, Urea and Total protein were measured. All of the biochemical tests were done using the reagents from Dimenition RxL Diagnostics machine at king Abdul-Aziz University Hospital.

Complete Blood Count and HbA_{1c}: All blood samples were analyzed by complete blood count (CBC) using the Beckman Coulter machine, at king Abdul-Aziz University Hospital, this process include the measurement of all blood component (RBCs, WBCs and platelets), hemoglobin (Hgb) and Hematocrit (Hct). HbA_{1c} was quantitatively determined by COBAS Integra 400 plus.

Tumor Necrosis Factor-Alpha: Plasma TNF-α concentration was measured by enzymatic immunoassay kit (GE Healthcare Amersham TNF-α Human, Biotrak Easy ELISA) which totally depends on ELISA Sandwich Technique. An anti-TNFα monoclonal coating antibody is adsorbed in the sample or standard binds to antibodies adsorbed to the micro wells. A biotin-conjugated polyclonal anti-TNF-α antibody binds to TNF-α captured by the first antibody. Streptavidin-HRP binds to the biotin conjugated anti-TNF-α. Following incubation unbound biotin conjugated anti TNF-α and Streptavidin-HRP is removed during a wash step. Substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of TNF-α present in the sample. The reaction is terminated by addition of acid. Absorbance is measured at 450 nm. A standard curve is prepared and TNF-α sample concentration determined.

Statistical Analysis: Data analyses were performed with the Stat soft wear program. The results or continuous variables are given as means (±SD). *T*-test is used to check the significant difference between diabetic patients and non-diabetic subjects in many parameters such as (Glucose, HbA_{1c}, Body Mass Index, Lipid Profile (Total Cholesterol, Triglycerides, LDL and HDL), Renal Profile (Total Albumin, Creatinine, Urea and Total Protein) and Complete Blood Count).

RESULTS

Physical parameters of diabetic and non-diabetic subjects (number of participants, Age, BMI and duration of diabetes) were summarized in Table (1). The mean (X) and the SD were calculated for each parameter and *P*-value was calculated for the assessment of the clinical significant difference. Body Mass Index (BMI) in diabetic patients was higher than non-diabetic subjects and showed significant difference between the two groups. *P*-value was (<0.0001) as demonstrated in Table (1).

The Comparison between diabetic and non-diabetic groups showed that fasting serum glucose levels was higher in diabetic subjects than non-diabetic ones. The *P*-value was (<0.0001) as seen in Table (2). HbA_{1c} of diabetic patients was elevated and higher than non-diabetic group (*P*-<0.0001) which considered a clinically significant Table (2). For the TNF-α calculation, there was a significant difference among diabetic and non-diabetic subjects with a *P*-value (<0.0001) as illustrated in Table (2).

Table 1: Physical parameters of diabetic and non-diabetic subjects

Physical Parameters	Diabetic patients n=40	Non-diabetic subjects n=40	*p*-Value
Age (year)	54.5±8.1	47.8±5.8	_
BMI (kg/m^2)	27.1±1.4	23.6±2.6	< 0.0001
Duration of diabetes (year)	13.7±7.2	_	_

Table 2: Comparison between diabetic and non-diabetic subjects for biochemical tests

Blood Tests	Diabetic patients n=40	Non-diabetic subjects n=40	*p*-Value
Glucose (mmol/l)	10.3±4.03	4.8±1.3	<0.0001
HbA1c (%)	8.5±2.5	3.6±0.5	<0.0001
TNF-α	1.9±0.5	1.07±0.2	< 0.0001

Table 3: Relationship between subjects for risk factors for Coronary Heart Disease

Lipid	Diabetic patients	Nondiabetic subjects	*p*-Value
Cholesterol (mmol/l)	4.6±1.2	4.01±0.59	0.0068
TGs (mmol/l)	1.7±1.6	1.5±0.93	0.4963
LDL (mmol/l)	3.4±0.97	1.9±0.97	<0.0001
HDL (mmol/l)	0.98±0.21	1.7±0.34	< 0.0001

Table 4: Relationship between different blood components and the clinical characteristics of the subjects

Blood components	Diabetic patients	Nondiabetic subjects	*p*-Value
Hgb (g/dl)	13.4±1.6	13.4±1.2	1.0000
RBCs	4.7±0.62	4.6±0.52	0.4368
WBCs	7.6±2.9	7.2±2.07	0.4798
HCT (%)	39.7±5.03	39.3±4.05	0.6963
PLT	283.7±102.6	274.4±53.3	0.6124

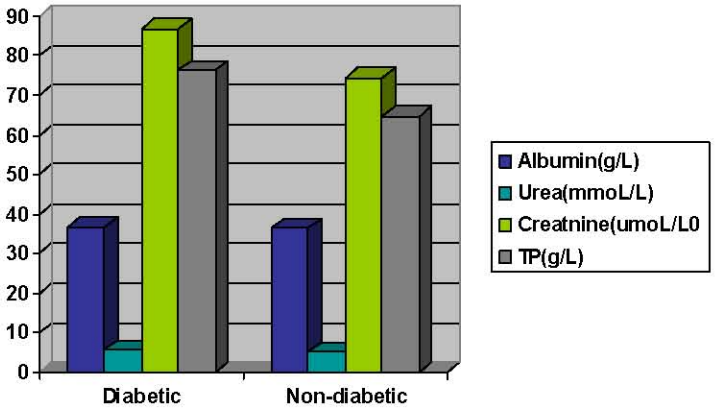

Fig. 1: The laboratory measurements of the subjects according to risk factors for renal insufficiency

Lipid profile was done to represent the relationship between the subjects for higher risk of coronary heart disease (CHD). Serum cholesterol was elevated in diabetic patients and showed significant difference among the two groups (*P*-0.0068)). The result of Triglycerides (TG) of diabetic patients was as the same as non-diabetic subjects with no significant difference, on the other hand LDL and HDL levels showed significant differences between the two subjects (*P*<0.0001) as seen in Table (3).

In addition, there were no significant differences between the diabetic patients and non-diabetic subjects for the occurrence of complete blood count (CBC) as well as renal function tests (Albumin, Urea, Creatinine and Total protein) (Table 4 and Figure 1).

DISCUSSION

Previous studies have demonstrated that obesity is highly associated with insulin resistance and obesity is considered the most important risk factor for Type II diabetes mellitus apart from the well-documented genetic predisposition. Although the molecular basis of the relationship between obesity and Type II diabetes remains poorly understood, TNF-α produced by adipocytes has been suggested to play a key role in the insulin resistance of obesity and may contribute to the development of Type II diabetes mellitus. Several studies have documented increased adipose expression of TNF-α mRNA in non-diabetic subjects with obesity-dependent insulin resistance, in normoglycemic subjects with increased insulin resistance and in Type II diabetic subjects [5].

The direct effects of TNF-α on the functions of adipose tissue including induction of lipolysis, inhibition of insulin signaling and alterations in expression of adipocyte important genes through activation of NF-êB, as well as their pertinence to insulin sensitivity of adipocytes. Also, TNF-α to inhibit synthesis of several adipocyte-specific proteins including Acrp30 (adiponectin) and enhance release of free fatty acids (FFAs) from adipose tissue and discuss how these factors may act as systemic mediators of TNF-α and affect whole body energy homeostasis and overall insulin sensitivity [17].

In this study it has been found that there were a significant increased in the HbA_{1c} percentage compared to normal subjects. The elevated HbA_{1c} percentage in the diabetic patients is indicated of a poor glycemic control which is thought to be implicated in the formation of micro- macrovascular complication and increased risk of chronic heart diseases (CHD), the worldwide incidence of obesity and type-II diabetes has grown dramatically in recent years, in what has been described as a pandemic. According to BMI results patients with type-II diabetes were overweight could be due to age, lack of physical activity and habit of eating.

Results were collected from lipid profile had not shown any significant abnormlaities compared to control subjects which may be due to restrection of food intake and also due to the duration of the disease. Also, results were collected from blood parameters shows the same results in both groups which may be due to that patients were not suffering from any other abnormalities.

Results were collected from renal profile had not shown significant abnormlaities indicating that those patients did not reach the complication of diabtes yet may be because of the duration of being diabetics (less than 13 years). The best index of nutritional status is serum albumin which makes up to 60% of the circulating proteins. Low albumin level may predict poor survival in end-stage renal disease. However, due to restriction of dietary protein which retard the progression of chronic kidney disease. However, circulating level of TNF-α is elevated in patients with metabolic syndromes such as obesity or patients with type II diabetes. The elevation of this cytokines may be due to disturbances of TNF-α.

CONCLUSION

Our current observations add further support to the evidence that TNF-α plays an important role in insulin resistance associated with obesity and/or diabetes mellitus in humans

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