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Research Article

THE ASSOCIATION OF SERUM TNF-ALPHA AND HOMEOSTASIS MODEL ASSESSMENT INSULIN RESISTANCE INDEX (HOMA-R) IN DIABETES

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ABSTRACT
Previous observations have shown that tumour necrosis factor alpha (TNF-α) synthesis is increased in presence of obesity and type 2 diabetes, although the molecular mechanisms for this are less understood. To analyze whether serum TNF-α as inflammatory cytokine is associated with insulin resistance and glucose concentration in type II diabetes patients. Thirty three non-trained adult men with type II diabetes aged 36-49 years and body mass index 28-36 kg/m2 were recruited through an accessible sampling. Fasting blood samples was collected in order to measuring serum TNF-α, insulin and glucose concentration. Insulin resistance (IR) was calculated by the homoeostasis model assessment (HOMA-IR) index. Data were analysed by Pearson correlation coefficient test. A p value less than 0.05 was considered statistically signigcant. There were no significant correlation in serum TNF-α with insulin ($p = 0.07$, $R = 0.32$), Glucose ($p = 0.09$, $R = 0.29$) and insulin resistance ($p = 0.77$, $R = 0.05$). We observed that serum TNF-α is positively correlated with obesity markers such as body fat percentage ($p = 0.007$, $R = 0.46$). In conclusion, although serum levels of TNF-α as inflammatory cytokine is not associated with insulin resistance or glucose, but there is a strong correlation between that with obesity markers in type II diabetes. Based on these finding, it is likely that serum TNF-α affect insulin resistance or glucose concentration indirectly in this patients that future studies will be needed to address the relative importance.

Keywords: *Insulin Resistance, TNF-alpha, Diabetes*

INTRODUCTION
Insulin resistance syndrome has an important role in pathogenesis of type 2 diabetes and cardiovascular disease (Reaven, 1988; Saltiel, 2000). Multiple mechanisms are involved in these diseases. Increase in adipose tissue is one mechanism, which plays a major role in prevalence of those diseases. However, the major mechanisms involved in the relationship between increased adiposity and insulin resistance are not known yet (Kahn *et al.*, 2000; Flier, 2001). Adipose tissue is not only a great source of fat, but also a major regulator of metabolic mechanisms, which are regulated by several adipocytokine and cytokines, secreted by adipose tissue, as well as several other tissues such as leptin, adiponectin, resistin, TNF- α, CRP, Il-6. The latter tissues are involved in incidence of insulin resistance and type 2 diabetes (Saltiel, 2001; Shuldiner *et al.*, 2001; Goldfine *et al.*, 2003).
TNF-α is one important cytokine among peptides secreted from adipose tissue. TNF-α is mainly secreted by activated macrophages. However, several other tissues are also involved in secretion of systemic levels of this cytokine (Terlikowski *et al.*, 2001). Clinical studies suggested that TNF-α as an inflammatory marker is associated with hyperglycemia, insulin resistance and type 2 diabetes (Sandler *et al.*, 1990; Kanemaki *et al.*, 1998). This cytokine plays a major role in inflammatory processes. An increase in concentration of TNF-α accelerates synthesis of several inflammatory interleukins (Spranger *et al.*, 2006; Gerszten *et al.*, 1999). Plasma concentrations of TNF-α is positively correlated with TG, which is importsnt in incidence of metabolic abnormalities such as obesity, diabetes type 2, insulin resistance and cardiovascular diseases (Ye, 2008). This cytokine is produced 7.5 times more in obese individuals compared to lean subjects (Kern *et al.*, 1995). Several studies suggested that TNF-α increases production of vLDL, which justifies the positive association between TNF-α and TG (Qin *et al.*, 2008).

Several studies showed that TNF-α level is higher in diabetic patients compared to healthy subjects (Su *et al.*, 2010; Elmarakby *et al.*, 2010). In addition, insulin resistance is also associated with type 2 diabetes. As a result, higher levels of insulin resistance were observed in diabetic patients compared to healthy subjects. However, there are contradictory findings regarding the relationship between these two variables (TNF-α and insulin resistance) in diabetic patients. Then, it is not confirmed whether there is a relationship between these variables. However, several studies reported a positive correlation between these variables (Gustafson, 2010). On the other hand, several other studies reported that there is no relationship between these variables in diabetic patients (Yang, 2002). Based on this conflicting evidence, the present study aimed to assess the association between TNF-α as a proinflammatory cytokine with insulin resistance as well as other indicative markers of type 2 diabetes in adult men infected with this disease.

MATERIALS AND METHODS

Method and Subjects

Thirty three sedentary, no athletes (BMI: 28-36 kg/m2) men, aged 36-49 years with type II diabetes were recruited for participate in this study through an accessible sampling. This study aimed to determine serum TNF-α in relation to insulin resistance in mentioned patients. Informed consent was obtained from all subjects before recruitment into the project.

Subjects were asked to complete questionnaires on anthropometric characteristics, general health, smoking, alcohol consumption and present medications. Participants were non-athletes, non-smokers and non-alcoholics. On the other hand, participants were included if they had not been involved in regular physical activity in the previous 6 months. Those patients unable to avoid taking hypoglycemic drugs or other therapeutic drugs within 12 hours before blood sampling were excluded. We also excluded those with other chronic diseases such as cardiovascular or kidney and liver diseases.

Anthropometry: Each subject's anthropometrical markers were measured. Height was measured without shoes on standing while the shoulders were tangent with the wall. Body weight was measured in duplicate in the morning following a 12-h fast. Obesity was measured by body mass index (BMI). Body mass index (BMI) was calculated by dividing body mass (kg) by height in metres squared (m2). Blood pressure was measured using the left arm after the subject had been sitting comfortably for 5 min, using an oscillometric device (Alpikado, japens).

Laboratory Analyses: The subjects arrived in the laboratory at 0800 after an overnight fast of at least 10 h. Fasting blood glucose (FBG), serum insulin and TNF-α concentration were measured in the patient after that blood samples were collected from the antecubital vein. ELISA method (Enzyme-linked Immunosorbent Assay for quantitative detection of human TNF-α) used for determine Serum TNF-α. Intra and inter-assay coefficients of variation were 6 and 7.4%, respectively. Glucose was determined by the oxidase method (Pars Azmoon kit, Tehran). Insulin was determined by ELISA method (Demeditec, Germany) and the intra- assay and inter-assay coefficient of variation of the method were 1.79% and 5.99 respectively.

Statistical Methods: Data were expressed as mean ± SD. Statistical analysis was performed with the SPSS software version 15.0. Normal distribution of data was analyzed by the Kolmogorov-Smirnov normality test. Significant associations between variables were determined by Pearson's correlation coefficients. An alpha-error below 5% was considered as statistically significant.

RESULTS AND DISCUSSION

Results

As previous mentioned, in this study, relation in serum TNF-α as inflammatory cytokine with determinative markers of type II diabetes were determined. We also determined the relation of this cytokine with some obesity markers. Table 1 show the descriptive anthropometric and biochemical features of the study patients.

There were no correlations between serum TNF-α concentrations and insulin concentration (p = 0.07, R = 0.32, Figure 1). No significant correlation was found in serum TNF-α with insulin resistance (p = 0.77, R = 0.05, Figure 2). We also observed no correlation between serum TNF-α and glucose concentration in studied patients (p = 0.09, R = 0.29, Figure 3).
The positive relationship observed between serum TNF-α with body weight (p = 0.000, R = 0.58) and BMI (p = 0.012, R = 0.43). Serum TNF-α was found to be positively associated with body fat percentage (p = 0.007, R = 0.46, Figure 4).

Table 1: The descriptive anthropometric and biochemical features of the studied patients

Variable	Mean	Standard deviation
Age *(year)*	42.6	3.7
Height *(cm)*	173	3
Weight *(kg)*	94	5.9
Systole (*mmHg)*	136	13
Diastole *(mmHg)*	90	8.7
Abdominal *(cm)*	106	5.7
Hip *(cm)*	103	4.8
WHO	1.02	.016
BMI *(kg/m2)*	31.41	1.75
Body fat *(%)*	31.1	1.11
TNF-α (*pg/ml)*	36.2	4.68
Insulin *(µIU/ml)*	8.32	1.06
Insulin resistance *(HOMA-IR)*	4.34	.75
Glucose *(mg/dl)*	213	41

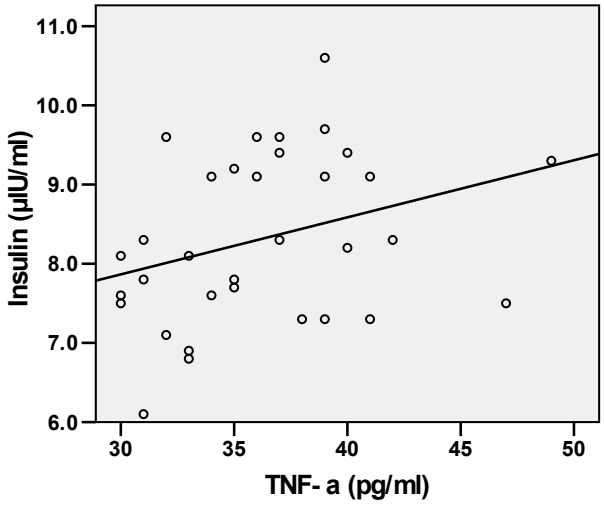

Figure 1: relationship between serum TNF-α and insulin in studied patient. No significant correlation

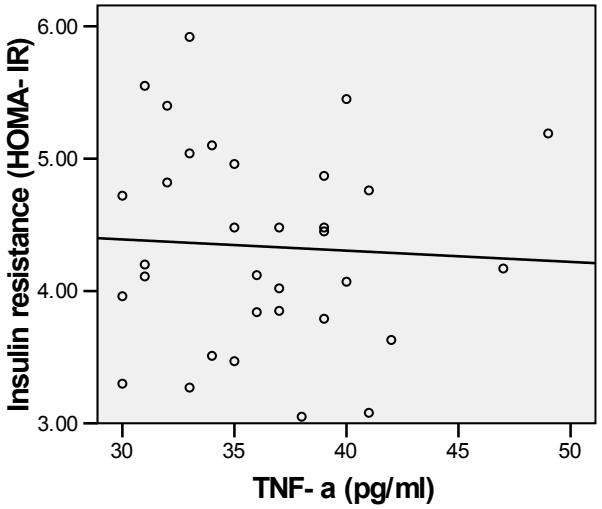

Figure 2: relationship between serum TNF-α and insulin resistance in studied patient. No significant correlation

Discussion

The close association between obesity and insulin resistance was reported repeatedly in scientific sources. In addition, inflammation plays an important role in obesity-induced insulin resistance. Nevertheless, the main molecular mechanisms involved in this association are not known yet (Hong *et al.*, 2009). It is known that circulating biomarkers such as TNF-α and fibrinogen are associated with development of

diabetes, particularly type 2 diabetes (Goldberg, 2009). TNF-α is a proinflammatory cytokine known as a mediator of insulin resistance, especially in obesity (Stefanyk *et al.*, 2010). The pathophysiologic mechanisms of insulin resistance and impaired insulin secretion are not yet fully known (Reaven, 1988; DeFronzo *et al.*, 1988). These mechanisms determine incidence of type 2 diabetes. However, clinical studies confirmed pivotal role of inflammatory markers and reactions in pathogenesis and type 2 diabetes (Catia *et al.*, 2007). It is also reported that fasting glucose levels are associated with TNF-α and visceral adipose tissue in type 2 diabetes. In addition, increased production of inflammatory molecules by visceral adipose tissue justifies the relationship between visceral obesity, insulin resistance and type 2 diabetes (Gustafson, 2010).

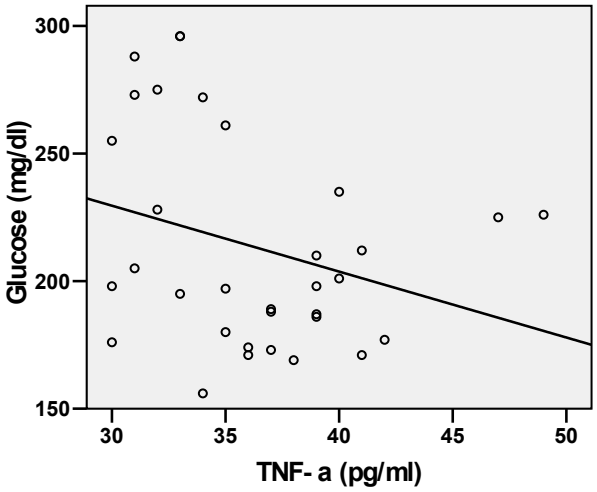

Figure 3: Relationship between serum TNF-α and glucose concentration in studied patient. No significant correlation

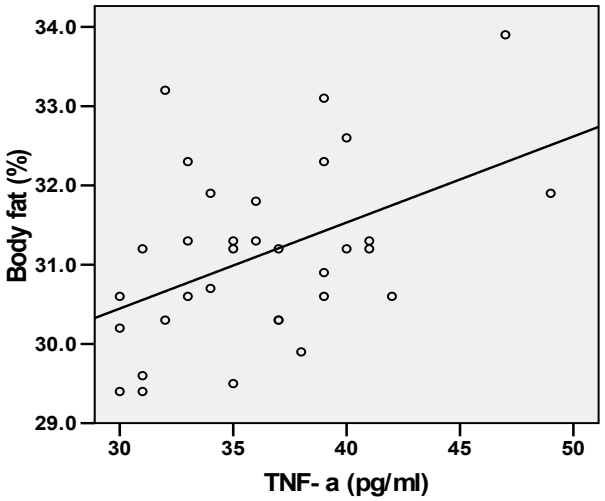

Figure 4: Relationship between serum TNF-α and glucose concentration in studied patient. A significant positive correlation

However, several studies showed that there is no association between TNF-α and indicative markers of type 2 diabetes (Yang *et al.*, 2002; Nilsson-Ohman *et al.*, 2009). In support of these results, findings obtained from this study also indicated that there is no association between TNF-α and indicative markers of type 2 diabetes. In other words, in the present study, no significant correlation was found between serum levels of TNF-α and insulin resistance, blood glucose and serum insulin levels in diabetic patients. In support of these results, no correlation was found between insulin resistance and TNF-α and other inflammatory cytokines such as IL-6 and CRP in men with type 2 diabetes in a recent study (Nilsson-Ohman *et al.*, 2009). In another study, the researchers addressed that such cytokine as TNF-α and resistin do not predict insulin resistance in obese individuals (Yang *et al.*, 2002).

It is identified that inflammatory cells and pro-inflammatory cytokines such as TNF-α and IL-1ß are effective in diabetes due to vascular inflammation (Elmarakby *et al.*, 2010). However, another study findings showed that TNF-α determines vascular inflammation in diabetic rats (Nilsson-Ohman *et al.*, 2009). In another study, researchers found out that apart from leptin and to some extent adiponectin, other adipocytokine such as TNF-α do not independently predict insulin resistance in obese and healthy subjects (Hong *et al.*, 2009). In another study, a poor correlation was observed between insulin levels and IL-6 and TNF-α in obese subjects (Martos-Moreno *et al.*, 2010).

Several studies also addressed that insulin resistance levels, apart from TNF-α, are considered as inflammatory cytokines (Peti *et al.*, 2010). Despite these contradictory findings, most scientific resources reported that higher levels of TNF-α is an inflammatory cytokine in type 2 diabetic patients compared with healthy subjects (Goldberg, 2009). However, small sample size was one limitation in the present study. Hence, lack of association between TNF-α and indicative markers of type 2 diabetes in the present

study may be attributed to small sample size. TNF-α may neither directly nor indirectly affects blood glucose levels and insulin resistance by affecting other inflammatory markers and peptide mediators in these patients. However, despite the lack of significant association between TNF-α with determinant markers of diabetes, but the findings suggest that this inflammatory cytokine is positively correlated with obesity markers such as body weight, BMI and body fat percentage. Thus, in our study, increased levels of body fat are accompanied with high levels of TNF-α as an inflammatory cytokine.

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