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Differential Activity of Tumor Necrosis Factor-Alpha (TNF-α) in Diabetes Mellitus

Diabetes Mellitusta Tümör Nekroz Faktörü-Alfa (TNF-α) Aktivitesindeki Farklılık

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Abstract

Tumor necrosis factor-alpha (TNF-α) is a pro-inflammatory cytokine which influence insulin resistance of obesity and non-insulin-dependent (Type 2) diabetes mellitus (NIDDM), and accepted probably play a role in the pathogenesis of insulin-dependent (Type 1) diabetes mellitus (IDDM). To objective of the submitted investigation was to determine serum TNF-α levels in different types of diabetes and diabetic complications, and to evaluate the correlation between serum TNF-α and serum insulin, fasting glucose (GLU), triglyceride (TG), and high density lipoprotein cholesterol (HDL-C) and also body mass index (BMI). Serum levels of TNF-α in 11 patients with Type 1 and 74 patients with Type 2 diabetes mellitus, total 85 patients, were examined. In addition, the patients categorized according to their diabetic complications; nephropahthy (n=26), retinopathy (n=31), and without complications (n=28). In different pattern of diabetes, serum cytokine levels were compared to serum samples obtained 23 healthy controls. Serum TNF-α level was statistically significantly increased in diabetes as compared with controls ($p<0.0001$). Both, Type 1 and Type 2 diabetic patients serum TNF-α concentrations were found statistically significantly higher than in controls ($p<0.0001$). In nephropathy, although cytokine level was found higher than retinopathy this was not statistically important ($p=0.053$). Also, in nephropathy, serum TNF-α concentration was higher than diabetic patients without complications and this was statistically important ($p<0.05$). In retinopathy, serum TNF-α concentration was also higher than those diabetic groups without any complications but this was statistically not significant ($p=0.344$). We couldn't demonstrated any correlations between serum TNF-α and, serum insulin, fasting GLU, TG, HDL-C levels, and BMI. In conclusion, TNF-α may be involved in the pathogenesis of diabetes mellitus. However, the measurement of other cytokines (such as IL-1β, IL-6, and IFN-γ) together with TNF-α may provide and additional information in different types of diabetes and diabetic complications.

Key words: Cytokine, tumor necrosis factor-alpha, diabetes mellitus

Özet

Tümör nekroz faktörü-alfa (TNF-α) proinflamatuar bir sitokin olup, obesite ve insüline bağımlı olmayan (Tip 2) diabetes mellitusta (NIDDM) insüline direnci etkilemekte ve insüline bağımlı (Tip 1) diabette (IDDM) patogenezde muhtemelen rol oynadığı kabul edilmektedir. Bu çalışmanın amacı, diabetin tiplerinde ve komplikasyonlarda serum TNF-α düzeylerini saptamak ve bu düzeyler ile serum insülin, açlık glukozu (GLU), trigliserid (TG), yüksek dansiteli lipoprotein-kolesterol (HDL-C) ve aynı zamanda vücut ağırlık indeksi (BMI) arasındaki korelasyonu değerlendirmekti. Serum TNF-α düzeyleri, 11 Tip 1 diabetli ve 74 tip 2 diabetli toplam 85 hastada incelendi. Ayrıca, hastalar diabetik komplikasyonlarına göre, nefropatili (n=26), retinopatili (n=31) ve herhangi bir komplikasyonu olmayan (n=28) kişiler olarak sınıflandırıldı. Diabetin değişik tablolarından elde edilen serum sitokin düzeyleri, 23 sağlıklı kontrolden elde edilen serum örnekleri ile kar-

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şılaştırıldı. Diabetes mellitusta serum TNF-α düzeyleri kontrollere göre istatistiksel olarak anlamlı ölçüde yüksek bulundu (p<0.0001). Nefropatide, serum sitokin düzeyi retinopatiye göre yüksek bulunmasına rağmen, bu değer istatistiksel olarak anlamlı değildi (p=0.053). Aynı zamanda, nefropatideki serum TNF-α konsantrasyonu herhangi bir komplikasyonu olmayan gruba göre artmış bulundu ve bu istatistiksel olarak anlamlı idi (p<0.05). Retinopatide ise, serum TNF-α konsantrasyonu komplikasyonu olmayan diabetik gruba göre yüksek bulundu ise de, istatistiksel olarak anlamlı değildi (p=0.344). Serum TNF-α ile serum insülin, GLU, TG ve HDL-C düzeyleri ve BMI arasında herhangi bir korelasyon saptanamadı. Sonuç olarak, TNF-α diabetes mellitusun patogenezi ile ilgili olabilmektedir. Bununla beraber, TNF-α ile birlikte diğer sitokinlerinde (IL-1β, IL-6, ve IFN-γ) ölçülmesi diabetin tiplerinde ve diabetik komplikasyonlarda ek bilgi sağlayabilir.

Anahtar kelimeler: Sitokin, tümör nekroz faktörü-alfa, diabetes mellitus.

INTRODUCTION

Tumor necrosis factor-alpha (TNF-α) is identified as the uniting principle linking in the pathogenesis of insulin-dependent (Type 1) diabetes mellitus (IDDM) and non-insulin dependent (Type 2) diabetes mellitus (NIDDM) (1-6). Also, TNFa mRNA expression has been reported to be up-regulated in adipose tissue from several rodent models of obesity and diabetes and from obese humans. This elevated expression has been assumed to be associated with the development of insulin resistance (7). Insulin resistance, a smaller than expected response to a given dose of insulin, is associated with many common diseases including Type 2 diabetes mellitus (8-10). Elevated TNF-α initially increases, and then inhibits, the activity of a number of key enzymes involved in energy metabolism and major histocompatibility (MHC) class I molecule expression, These enzymes include: protein-tyrosine kinase (PTKase) and protein-tyrosine phosphatase (PTPase) which involved in energy metabolism, cell proliferation, and stimulation of the MHC class I molecule pathway. Of primary importance is the inhibiting effect of TNF-α on PTKase, since this induces insulin resistance in NIDDM and carcinoma, and PTPase, which inhibits MHC class I molecule expression. It is now clear that decreased signalling capacity of the insulin receptor is an important component of this disease (2,3).

Studies have shown that IDDM is associated with an increase in PTPase activity which leads to over-expression of MHC class I molecules and a concomitant destruction of pancreatic beta cells (1). It is know generally accepted that cytokines are implicated in the pathogenesis of autoimmune affecting the islet of Langerhans characterized by progressive loss of beta-cell function. Animal studies have shown that interleukin-1beta (IL-1β), TNF-α, and interferon-gamma (IFN-γ) affect Type 1 diabetes development profoundly (6,11). The autoimmune response against islet beta-cells is believed to result from a disorder of immunoregulation Type 1 cytokines to initiate a cascade of immune/inflammatory processes in the islet (insulitis) and culminating in beta-cell destruction. Type 1 cytokines activate; 1) cytotoxic T cells that interact specifically with beta-cells and destroy them, and 2) macrophages to produce proinflammatory cytokines (IL-1β, TNF-α, and IFN-γ) and oxygen and nitrogen free radicals that are highly toxic to islet beta-cell (12).

As shown *in vivo* in non-obese diabetic (NOD) mice, TNF-alpha-NOD mice express TNF-α solely in their islets from neonatal life onwards, and develop accelerated progression to diabetes. Further investigations in the TNF-alpha-NOD mice demonstrated that diabetes progression is dependent on CD8+T cells, with CD4+T cells playing a lesser role (13). It was shown that chronic exposure of adipocytes to low concentrations of TNF-α strongly inhibits insulin-stimulated glucose uptake. Thus, they suggested TNF-α directly interferes with the signaling of insulin through its receptor in adipose tissue and consequently blocks biological actions of insulen (10). On thish basis, wje investigated the serum level of TNF-α in patients with IDDM and NIDDM, complicated, and healthy controls, also to show that whether is there any relationship between their concentrations.

PATIENTS AND METHODS

Total 85 patients aged between 20-67 (mean age 55.4±10.8 years) were evaluated; 54 of them female (mean age 55.4±10.5 years) and 31 of them male (mean age 55.5±11.2 years). The mean disease duration was 10±9 years. 39 with hypertension (arterial blood pressure over 160/95 mmHg, disease duration 7.3±5.1 years). Eleven of them Type 1 and 74 of them Type 2 diabetes mellitus were examined. The patients subdivided according to diabetic symptoms: a) 26 patients with nephropathy, b) 31 patients with re-

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tinopathy, and c) 28 of them without these complications. All patients were examined in good glycometabolic control reached by oral hypoglycemiant (71 cases) or insulin (14 cases) treatment. Healthy controls were included 23 individuals (16 female, 7 male; mean age 31.1±9.5 years) without any healthy problem.

After withdrawn the blood samples they were centrifuged at 4000 rpm for 10 minutes at 4°C. Then serum samples were divided aliquots and stored at -85°C for TNF-α insulin measurements. Cytokine and insulin measurements were done in each week after collected the samples using with the commercial BIODPC (Products Corporation, Los Angeles, CA, USA) kit (cat no: LKNF1 for TNF-α and cat no: LKIN1 for insulin) by IMMULATE hormona autoanalyzer via chemiluminescent enzyme immunometric assay. The reference range for TNF-α was nondetectable to 8.1 pg/ml for healthy controls and 6-27 μIU/ml for insulin in healthy controls in procedure. Serum glucose (GLU) (reference range 70-110 mg/dl), triglyceride (TG) (reference range 40-160 mg/dl), and high density lipoprotein cholesterol (HDL-C) (30-70 mg/dl) levels were determined IL-900 autoanalyzer via spectrophotometric colorimetric method.

Results were given as mean ± SD.I Nonparametric tests were used to analyze serum variables. Comparison of the data between different groups were performed using Students'-t test and the Mann-Whitney U test. The correlations between the variables in serum were determined via Spearman rank correlation coefficient test. SPSS program were used for statistical analysis.

RESULTS

In diabetes mellitus serum TNF-α was found (13.75±4.77 pg/ml) and this was statistically significantly higher than healthy controls (6.46±0.9 pg/ml) ($p<0.0001$). In Type 1 and Type 2 diabetic patients serum TNF-α levels were found (13.12±4.66 pg/ml) and (13.85±4.27 pg/ml), respectively and these were statistically significantly higher than in controls ($p<0.0001$). Although serum TNF-α was higher in Type 2 diabetic patients compares as Type 1, but the difference was statistically not important (Figure 1).

In nephropathy (17.81±5.93 pg/ml) the cytokine

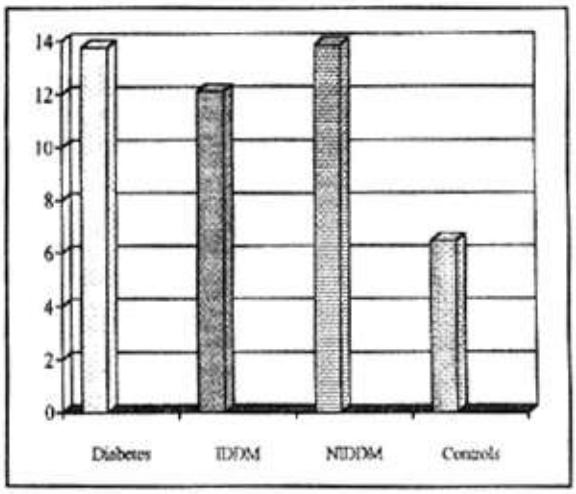

Figure 1. Serum TNF-α (pg/ml) in different types of diabetes mellitus.

level was found higher than retinopathy (13.25±4.02 pg/ml), but is was not statistically significant ($p=0.053$). Also, when compared with nephropathy and the groups withouth any complications, the mean values were (17.81±5.93 pg/ml) and (11.43±3.18 pg/ml), respectively, and the difference was statistically significant ($p=0.0446$; $p<0.05$). In retinopathy, serum TNF-α concentration (13.25±4.0 pg/ml) was also increased than those diabetic groups without any complications (11.43±3.18 pg/ml), but this was not statistically important ($p=0.344$) (Figure 2).

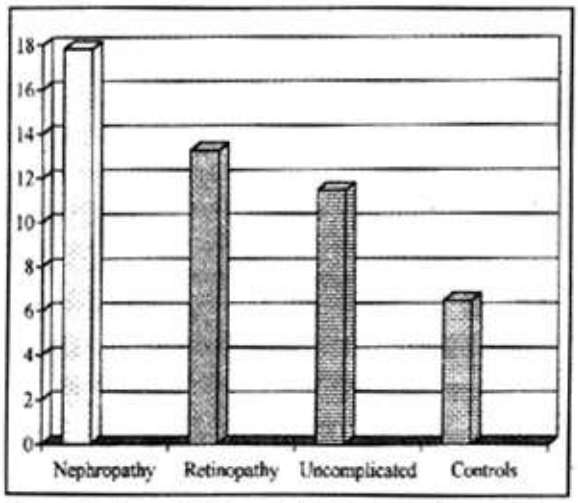

Figure 2. Serum TNF-α (pg/ml) in diabetic complications.

In nephropathy, retinopathy, and uncomplicated patients serum TNF-α levels were statistically significantly higher ($p<0.0001$). To note that, only in two of diabetic patients serum TNF-α levels were below 8.1 pg/ml, which is a reference value given for

healthy population. It is very important that 98 % of diabetic patients have higher levels that that of reference value.

In patients using oral hypoglycemiant the mean insulin value was found 14.43±11.94 µIU/ml and in healthy group insulin level was 6.8±0.75 µIU/ml, and the difference between the two group was statistically not important (p=0.185). In diabetic group, the mean values of fasting GLU, TG, and HDL-C were found (166.0±63.89 mg/dl), (210.63±126.29 mg/dl), and (53.1±16.24 mg/dl), respectively. In controls GLU, TG, and HDL-C concentrations were (94.11±10.11 mg/d.l), (93.25±30.0 mg/dl), and (62.28±9.0 mg/dl), respectively. As compared the mean values, in diabetes GLU and TG leves were statistically significantly higher than controls (p=0.0001; p<0.001) and (p=0.0007; p<0.001), respectively. The contrary, HDL-C levels in diabetic was statistically significantly lower than controls (p=0.025; p<0.05). We couldn't demonstrated any correlations between TNF-α and insulin, GLU, TG, and HDL-C levels and BMI. In diabetic and healthy subjects BMI was found (28.11±3.87 kg m^{-2}) and (23.69±1.56 kg m^{-2}). The difference was statistically significant (p=0.006; p<0.01). No correlation was found serum TNF-α and BMI. Actually, to remember that we didn't include our diabetic subject in obes group, because of their mean BMI value was below 30 kg m^{-2}.

DISCUSSION

Recent studies examining the link between insulin resistance and the development of obesity and NIDDM are consistent with the involvement of TNF-α as a central role (3,4,9). Also, TNF-α is known to play an important role in autoimunity and pathogenesis of IDDM (6). Via immunohistochemical technique, Limb et al (5) demonstrated that platelets from IDDM patients with active proliferative diabetic retinopathy, proportion of staining for TNF-α was significantly higher than in patients without microvascular complications, quiscent proliferative diabetic retinopathy or healthy subjects. They suggest that increased platelet expression of TNF-α in IDDM may constitute important markers of thrombocyte abnormalities during the development of microvascular complications of diabetes mellitus. In retinopathy, we also found higher level of serum TNF-α as compared to controls. But, to note that, we didn't discriminate the patients had nephropathy in IDDM or NIDDM.

Nilsson et al (4) showed that plasma TNF-α concentration was found higher in patients with moderately and severely insulin resistant than that of healthy controls and also, in insulin resistant patients, the level of TNF-α was little higher as compared with moderately insulin resistant subject, but this was found not important. They suggested that TNF-α may be involved in the pathogenesis of NIDDM. In addition, they found a positive correlation between TNF-α and BMI, fasting GLU, TG and a negative correlation with HDL-C. To contrary, Chabova et al (14) reported that there was no correlation between plasma TNF-α and BMI and fasting GLU. Furthermore, they showed showed no correlation between plasma TNF-α with serum creatinine and glycated haemoglobin. So, they claimed that TNF-α levels didn't correlate with age, renal function, and compensation of diabetes. Like Chabova, we couldn't demonstrate any correlation between serum TNF-α and serum fasting GLU and BMI. In our study, the lack of correlation between serum TNF-α and BMI may be due to the selection of our patients. Because we didn't do any discrimination in our patients related with their BMI, so the mean value of BMI was below obesity border. Foss et al (15), demonstrated higher serum TNF-α in Type 1 diabetic patients than that of healthy controls. Also, they reported that serum TNF-α levels progressively increased from the well to the poorly controlled diabetic groups, which parallel levels of HBA1c and they suggested that this situation showing a relationship between levels of this cytokine and protein glycosylation. Cavallo et al (6) found that raised levels of serum TNF-α were detectable using an immunoenzymatic assay whereas TNF-α bioactivity (determined bioassay) in these samples was negligible. Upon these data they concluded that the presence of immunoreactive TNF-α in the patient's serum may reflect an increased localized production of this cytokine at pancreatic level.

We demonstrated higher serum TNF-α concentration in diabetic nephropathy as compared with retinopathy, uncomplicated patients, and healthy controls. Thus, TNF-α may be predicate or distinctive marker for this condition alone or together with N-acetyl-β-D-glucosaminidase (NAG) which is a marker for nephropathy. As seen in above explanations, there was a lot of controversial data have been found related to TNF-α and the pathogenesis of diabetes mellitus. These findings together with pre-

vious data suggest TNF-α may have multiple and diverse roles in different types of diabetes and diabetic complications.

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