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Interleukin 6 and Tumor Necrosis Factor-alpha Levels in Women with and without Glucose Metabolism Disorders

[Glukoz Metabolizma Bozukluğu Olan ve Olmayan Kadınlarda İnterlökin-6 ve Tümör Nekroz faktör-alfa Düzeyleri]

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ABSTRACT

Background: In this study, we aimed to investigate the concentrations of pro-inflammatory cytokines, such as interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) in women with normal glucose tolerance (NGT) and impaired glucose tolerance (IGT). The purpose of this study was to verify whether the pro-inflammatory cytokine- increase is a pathogenic indicator for glucose metabolism disorders.

Materials and Methods: A total of 118 subjects from women aged between 21 and 62 years (42.3± 11.7) were included in this study. Anthropometric and biochemical characteristics of them were assessed. IL-6 and TNF-alpha concentrations were measured by chemiluminescent immunometric assay.

Results: Amongst them, a total of 83 cases (70.3%) had NGT, 35 (29.7%) had IGT. By Pearson correlation coefficient, we found a significant relationship between serum IL-6 level and post-load 2 h glucose levels (r=0.252, P=0.006), BMI (r=0.274, P=0.003). IL-6 and TNF-α levels were significantly different between the groups (p<0.001, P =0.03, respectively).

Conclusion: This result shows that increased IL-6 and TNF-α level may be associated with impaired glucose homeostasis.

Key words: interleukin 6, tumor necrosis factor-alpha, impaired glucose tolerance, glucose homeostasis.

ÖZET

Amaç: Bu çalışmada, glukoz toleransı normal (NGT) ve bozulmuş (BGT) kadınlarda, interlökin 6 (IL-6) ve tümör nekroz faktörü alfa (TNF-α) gibi proinflamatuar sitokin konsantrasyonları araştırıldı. Bu çalışmanın amacı, proinflamatuar sitokin artışının glukoz metabolizma bozukluklarında patojenik bir belirteç olup olmadığını göstermektir.

Materyal ve Metot: Çalışmaya yaşları 21-62 (42.3± 11.7) arasında değişen toplam 118 kadın alındı. Bu kişilerin antropometrik ve biyokimyasal karakteristikleri araştırıldı. IL-6 ve TNF-α konsantrasyonları kemilüminesan immunassay ile ölçüldü.

Sonuç: Olguların 83'ünde (%70.3) glukoz toleransı normal, 35'inde (%29.7) bozulmuştu. Pearson korelasyon testinde, tüm olgularda serumda IL-6 düzeyi ile 2 saatlik glukoz seviyesi (r=0.252, P=0.006) ve BMI (r=0.274, P=0.003) arasında anlamlı ilişki saptandı. Gruplar arasında fark IL-6 ve TNF-alfa seviyesi için istatistiksel olarak anlamlı bulundu (sırasıyla P< 0.001 ve P=0.03).

Tartışma: Bu sonuçlar bozulmuş glukoz homeostazı ve artmış IL-6, TNF-alfa seviyesinin ilişkisini ortaya koymaktadır.

Anahtar kelimeler: İnterlökin 6, Tümör nekroz faktör-alfa, Bozulmuş glukoz toleransı, Glukoz homeostazisi

Introduction

Impaired glucose tolerance (IGT), is an intermediate stage in the progression toward type 2 diabetes (DM) and attributes to either insulin resistance (IR) or decreased insulin secretion or both. There is also evidence that IGT is related to obesity. It now appears that obesity is associated with a low- grade inflammation of adipose tissue, resulting from activation of innate immune system. Adipose tissue has been more recently recognized as an active participant in numerous immunologic processes (1). Especially in obesity, adipose tissue is characterized by an increased production and secretion of a wide range of pro- inflammatory molecules including interleukin-1ß (IL-1ß), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α). Recent data indicate that macrophages in obese adipose tissue are major source of these locally-produced pro-inflammatory cytokines (2). Therefore weight loss is associated with a reduction in the macrophage infiltration of adipose tissue and an improvement of the inflammatory profile (1- 6).

Chronic low-grade inflammation associated with the metabolic and immune systems involves a network of cellular and systemic responses that integrate many complex signaling pathways. Pro- inflammatory cytokines are also mediators of these pathways and they enter the circulation as a result of lipolysis. IL-6 is one of the first identified cytokines and it is implicated as a pathogenic marker of IR and cardiovascular disease. These cytokines have been demonstrated to involve in glucose metabolisms in mice (7). IL-1ß together with IL-6 concentration are suggested as predictors for type 2 diabetes in humans better than either cytokine alone. Similarly TNF-alpha is a pro- inflammatory cytokine, overproduced in adipose tissue of several rodent models of obesity and has an important role in the pathogenesis of IR in these species. However, its actual involvement in glucose metabolism disorders in humans remains controversial. It is suggested that all of these mediators may alter insulin sensitivity by triggering different key steps in the insulin signaling pathway and that overproduction of these mediators involved in the pathogenesis of IGT (8). It is also demonstrated that increases of these cytokines effect through intracellular signaling pathways involve the nuclear factor kappa B (NF- κB) and c- June N-terminal kinase (JNK) systems (7- 13).

In this study, we assessed the relationship between some pro- inflammatory cytokines such as IL-6 and TNF-α with different degrees of glucose metabolism disorder.

Materials and Methods

A total of 118 women aged between 21 and 62 years (42.3± 11.7) who have applied to the department of clinical biochemistry, Izmir Tepecik Research Hospital, were included in this study. The Institutional Review Board approved procedures used in this study and informed consent of the patients was obtained. Individuals with diabetes were excluded from the study. The participants had no cardiovascular disease, hypertension, infections or any other serious medical problems, and were not taking long-term medication. As well as they did not smoke and intake alcohol. Body mass index (BMI) values were calculated as body weight divided by height squared and expressed in kg/m^2. Insulin resistance was calculated using the homeostasis model assessment of IR (HOMA-IR), using the formula: fasting insulin (μIU/mL) x fasting glucose (mg/dL) / 405 (13). All cases without a known clinical history of diabetes underwent an oral glucose tolerance test (OGTT).

We measured the serum glucose levels, 2 hours after we had given 75 g of glucose to each patient. Patients with 2 hour serum glucose levels <140 mg/dL were regarded as normal glucose tolerance (NGT; Group I), with 2 hour serum glucose levels between 140-200 mg/dL were evaluated as impaired glucose tolerance (IGT; Group II) and finally those with ≥ 200 mg/dL were evaluated as type 2 diabetes mellitus (DM) and these subjects were excluded from study. American Diabetes Association criteria were used to define normal and impaired glucose tolerance (14).

For biochemical analyses, all the serum samples were drawn into gel separator tubes (Vacutainer SST, Beckton Dickinson, France) between 08.00 and 08.30 a.m. after a 12- hour fasting. The blood samples were allowed to clot and then centrifuged at 3000 rpm for 10 min at room temperature. The serum were separated and stored at – 70°C until analysis. Serum levels of lipid parameters; total cholesterol, HDL cholesterol and triglyceride (TG) and glucose were determined by enzymatic methods with Olympus AU-2700 analyzer using reagents from Olympus Diagnostica (Hamburg, Germany). LDL-cholesterol was calculated by Friedewald's formula (13).

Fasting insulin levels were measured by chemiluminescent immunoassay with an Immulite2000 immunoassay analyzer (Siemens, Llanberis, United Kingdom). IL-6 and TNF-alpha levels were measured by solid-phase, chemiluminescent immunoassay with an Immulite1000 immunoassay analyzer (Siemens, Llanberis, United Kingdom). The detection limits of the assays were 2 pg/mL for IL-6 and 1.7 pg/mL for TNF- α. The intra-assay and inter-assay imprecisions were 6.2% and 7.5% for IL-6, 3.6% and 4.4% for TNF-α.

Statistical Analyses:

Due to the skewed distribution of IL-6 levels, 1/square root transformed values were used in all analyses. One-way ANOVA was performed for comparison of groups. A one-way analysis of covariance with age as covariates was performed for IL-6 and TNF-alpha. Relationships between variables were determined by Pearson's Correlation coefficient. P values less than 0.05 were considered as statistically significant. Analyses were performed using SPSS program (version 17.0) for Windows.

Results

The characteristics and descriptive statistics of study subjects according to glucose tolerance status are shown in Table 1. A total of 83 cases had a normal response to the OGTT (NGT group), and 35 had an impaired response to the OGTT (IGT group).

Serum concentrations of IL-6 and TNF-α were statistically and significantly higher in IGT group than NGT group (P <0.001 and P =0.03, respectively). Total cholesterol, LDL-cholesterol, HDL-cholesterol and TG levels were similar in the IGT group compared to NGT group (P >0.05). HOMA indexes in cases with IGT and NGT were also not different (P=0.11).

Serum IL-6 levels were positively correlated with postload 2 h glucose levels (r=0.252, P=0.006), BMI (r=0.274, P=0.003) and were not correlated with age, fasting glucose, serum fasting insulin levels, HOMA –IR and lipid parameters. No correlation between TNF- α and all measured parameters were determined.

Discussion

Cytokines act on the surrounding microenvironment by providing cell to cell signaling. The effects of cytokines on target cells may be inhibited or enhanced by other cytokines, hormones, and cytokine-receptor antagonists and circulating receptors. TNF-α and IL-6 are pro-inflammatory cytokines. On the other hand, inhibitory cytokines such as IL-4, IL-10 and TGF-β damp down the activation of inflammatory cells by inhibiting the release of pro-inflammatory cytokines. Chronic inflammation is considered to be involved in the pathogenesis of some age-related diseases such as DM, Alzheimer's disease, atherosclerosis and cancer. Because of those results, in this study, we excluded patients with DM, hypertension, acute infection and long term medication.

Low-grade increases in levels of circulating TNF-α and IL-6 also act as strong predictors of all-cause mortality risk in several studies of elderly cohorts. The effects of inflammatory mediators upon survival are independent of pre-existing morbidity and other traditional risk factors for death such as smoking, high blood pressure and BMI. Currently it is suggested that cytokines may induce exaggerated pathological processes and they may be sensitive markers of pre-clinical disorders in elderly populations. Despite both groups were not homogenous regarding to average age, all patients were under 60 years old. There was not any significant correlation between age and IL-6 levels in our study as well. Besides, TNF-α levels were not correlated with age, either. (4, 5, 12, 15-22).

The influence of endogenous lipids on immune function has been widely investigated during the past three decades. It is clear from whole- animal studies that obesity and consumption of high fat-diets, particularly saturated fat, depress both innate and adaptive immune competences. The relationship between lipids and immune response is complex, multifactorial, and still poorly understood. The deleterious effect of lipids depends on some mediators. Classically, cytokines such as TNF-α, has been proposed as regulator of lipid metabolism, however, data about impact of obesity on the relationships between TNF-α and plasma lipids remain controversial. TNF-α injection induces an increase in the concentration of plasma triglyceride and very low density lipopro-

Table 1. Clinical characteristics of the study population according to glucose tolerance status

	NGT group (n:83)	IGT group (n:35)	P
Age (years)	40.6 ± 10.9	46.3 ± 12.7	.014
BMI (kg/m^2)	30,8 ± 5.9	33.2 ± 7.4	.06
Fasting glucose (mg/dL)	100.3 ± 13.9	111.0 ± 13.1	<.001
Glucose 2-h (mg/dL)	101.1 ± 22.2	167.5 ± 19.7	<.001
Fasting insulin (mU/L)	11.6 ± 8.1	12.7 ±7.0	.48
HOMA (present= ≥2.7)	2.8 ± 1.9	3.5 ± 1.8	.11
Triglycerides (mg/dL)	139 ± 85	152 ± 92	.49
Cholesterol (mg/dL)	200 ± 39	201 ± 34	.94
HDL cholesterol (mg/dL)	50 ± 12	50 ± 12	.97
LDL cholesterol (mg/dL)	122 ± 30	121 ± 29	.70
TNF-α (pg/mL)	8.8 ±2.67	10.2 ± 3.2	.03
IL-6 (pg/mL)	3.2 (2.0 -7.7)	5.6 (2.0-37.8)	<.001

Data are means ± standart deviation
NGT: Normal glucose tolerance
IGT:Impaired glucose tolerance

teins. The pro-inflammatory cytokine production is elevated in diabetes and in cases of elevated lipids. It has been suggested that TNF-α alters insulin secretion through direct or through stimulation of free fatty acid production and also altered glucose homeostasis consequently. In our study there was not any statistically significant difference between lipid levels in both groups. Pearson correlation test also revealed that IL-6 levels and lipid parameters were not correlated. These results were confirmed previous literature in terms of lipid parameters and cytokine levels (23- 25).

Recent data suggest that obesity may cause the low-grade inflammation and that over nutrition is associated with the infiltration of adipose tissue by inflammatory cells. Although the precise cell types involved in this process remain uncertain, evidence is mounting that implicates adipose tissue as a significant contributor to inflammation in obesity and a mediator of insulin resistance. It is becoming clear that the increased adipose tissue is not a simple reservoir for excess nutrients, but rather an active and dynamic organ capable of expressing several cytokines. A positive correlation was determined between the body mass index and the percentage of resident macrophages on the fat tissue which are responsible for cytokine production. Marked impairment of the glucose tolerance and the significant elevation of some pro-inflammatory cytokines in obesity have also been reported in several animal models. In our study; we have also determined positive correlation between serum IL-6 levels and BMI. However, there are no significant differences between BMI of two groups and serum IL-6 levels were significantly higher in only IGT group. This result appears that, obesity is associated with overproduction of IL-6 (2-5, 12, 15- 17).

A combination of independent and complementary studies has provided molecular insights into the regulation of energy metabolism involving the coordination by signal transduction pathways which act directly onto the modulation of nutrient uptake and metabolism. Both the mediator proteins and the insulin receptors were shown to be expressed on immune cells such as macrophages, neutrophils, and B- and T-lymphocytes. They are responsive to both immune stimulation and insulin secretion by the several signaling pathways that modulate the glucose uptake and metabolism of immune cells. The precise signaling mechanisms in immune cells still remain uncertain. But pro-inflammatory cytokines were shown to impair the glucose homeostasis in vivo. In this study, IL-6 and TNF-α concentration were higher in subjects with IGT than with NGT. This results implicate that IL-6 and TNF-α have an important role in the regulation of glucose homeostasis, without considering hormonal status (6, 12, 13, 15, 18- 21).

This study has some limitations. First, subjects in the NGT group were significantly younger than those in the IGT group, which could explain some of the differences between the groups, although adjustment for age was done in statistical analyses of the data. Second, the waist circumferences and the menopausal status of the cases were not considered. Similarly CRP level of cases were not calculated in this study.

In conclusion, this study shows systemic up-regulation of IL-6 and TNF-α in woman with IGT. Therefore measurement of IL-6 and TNF-α level are probably important in determining glucose regulation disorders. We have also found that there is no association between these two cytokines and other parameters such as obesity, high cholesterol levels and old age.

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