LEPTIN AND TNF-ALPHA LEVELS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND THEIR RELATIONSHIP TO NUTRITIONAL PARAMETERS

KOAH HASTALARINDA LEPTİN VE TNF ALFA DÜZEYLERİ VE BUNLARIN NUTRİSYONEL PARAMETRELERELE İLİŞKİSİ

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SUMMARY

Aim: *This study aimed to detect serum leptin and TNF- α levels in COPD patients without weight loss during stable disease and acute exacerbation, and to investigate relationships between leptin, TNF-α and nutritional parameters at different stages of the disease.*

Material and Methods: *51 stable COPD patients, 14 COPD patients with acute exacerbation and 18 control subjects participated in this study. To eliminate the effects of sex differences, all patients and controls were male. BMI, triceps of skinfold tickness and serum albumin and serum leptin and TNF-α levels were measured in all participants.Leptin and TNF-α levels were measured by ELISA.*

Results: *Leptin levels were significantly higher in patients with exacerbation than in both stable-disease and control groups. Leptin levels were significantly correlated with the nutritional parameters in stable groups. However, in patients with acute exacerbation, a correlation between leptin and nutritional parameters was not found. There was no significant relationship between TNF-α and nutritional parameters in the three groups. While there was no correlation between serum TNF-α and leptin levels in COPD patients and*

ÖZET

Amaç: *Bu çalışmada kilo kaybı olmayan stabil ve akut atakta olan KOAH hastalarında serum leptin ve TNF-α düzeylerini saptamayı ve nutrisyonel parametrelerle serum leptin, TNF alfa düzeyleri arasındaki ilişki incelemeyi amaçladık.*

Yöntem ve Gereç: *51 stabil KOAH, 14 akut atakta olan KOAH ve 18 kontrol hastası çalışmaya dahil edildi. BMI, triceps deri kıvrım kalınlığı, serum albümin, leptin ve TNF-α düzeyleri hesaplandı. Leptin ve TNF-α düzeyi ELISA yöntemi ile ölçüldü.*

Bulgular: *Leptin düzeyleri alevlenmesi olan KOAH hastalarında stabil ve kontrol grubuna göre anlamlı olarak yüksek bulundu. Ancak, akut alevlenme olan hastalarda leptin ve beslenme parametreleri arasında bir ilişki bulunmadı. Bu üç grup arasında TNF-α ile beslenme parametreleri arasında anlamlı ilişki yoktu. KOAH hastalarında ve kontrol grunbunda serum leptin ve TNF-α düzeyleri arasında korelasyon saptanmazken, Serum TNF-α düzeyi ile beslenme parametreleri arasında da bu üç grup arasında ilişki bulunmadı.*

Sonuç: *TNF-α düzeyleri, yüksek serum leptin düzeyi ile ilişkili değildi; KOAH akut atak hastalarının sayısının az olması ve TNF- α değerlerinin sayısal olarak hesaplanamaması nedeniyle; TNF-α düzeyi*

control groups, Serum TNF-α levels did also not correlate with nutritional parameters in any of groups.

Conclusion *(1) circulating TNF-α levels were not associated with increased leptin levels and (2) because of the fewer number of the acute exacerbation of COPD patient and not calculated the value of TNF-alpha levels as a numeric value, we did not find elevated TNF-α levels in COPD patients with acute exacerbation (3) although leptin and nutritional parameters were correlated in the stable COPD patients, it was disappeared completely during an exacerbation and control groups. Therefore, there was no significant correlation between leptin and TNF-α during the regulation of the energy balance in COPD patients.*

ile yüksek serum leptin düzeyi arasında ilişki saptanmadı. Her ne kadar stabil KOAH hastalarında leptin ve nutrisyonel parametreler arasında korelasyon varken, akut atak olan KOAH hastalarında ve kontrol grupta yoktu. Bu nedenle, KOAH hastalarında enerji dengesinin düzenlenmesi sırasında leptin ve TNF-α arasında anlamlı bir korelasyon saptanmadı.

INTRODUCTION

Leptin is a protein which is mainly secreted by adipocytes. The first function described after the discovery of leptin was its role in body weight regulation [1]. Following interaction with specific receptors located in the central nervous system and peripheral tissues, leptin induces a complex response, including control of body weight and energy expenditure [2]. In humans, serum leptin levels strongly correlate with nutritional parameters such as body mass index (BMI) and fat mass (FM) [3–6].

In pathologic conditions such as chronic renal insufficiency (7) and bacterial endotoxemia (8), and with exposure to highdose glucocorticoids (9), inappropriately increased levels of leptin are thought to induce excessive metabolic effects underlying anorexia and loss of body weight.

Recently, cytokine-mediated metabolic derangements have begun to be considered as among the candidates responsible for cachexia in COPD patients (10).It has been suggested that tumor necrosis factor-α (TNF-α), a pleiotropic cytokine causing cachexia,(11) plays a part in metabolic changes associated with chronic wasting diseases such as cancer, cystic fibrosis, congestive heart failure (CHF), and COPD (10). In animal studies, administration of inflammatory cytokines that induce anorexia, such as tumor necrosis factor-α (TNF-α) or interleukin-1, resulted in the up-regulation of leptin mRNA in adipose tissues and an in increase of the serum leptin concentration (7,8,12). This finding suggests that leptin is not under a normal regulation mechanism and it is influenced by inflammatory mediators, and such a reaction may act as a mechanism of weight loss.

The aim of this study was to detect serum leptin and TNF-· levels in COPD patients without weight loss during stable disease and acute exacerbation, and to investigate relationships between leptin, TNF-α and nutritional parameters.

MATERIALS AND METHODS

Study Population

Fifty – one patients with stable COPD, 14 COPD patients with exacerbation and 18 healthy controls were entered into the study. Control subjects had no medical illness, had normal physical examinations, blood counts, chemistries and showed no symptoms or signs of infection at the time of study. A random group of patients with COPD, consecutively admitted to a pulmonary center, were included in the study when they fulfilled the following criteria: (1) COPD according to the guidelines of GOLD criteria[13] and chronic airway obstruction defined as a measured forced expiratory volume in 1 s (FEV1) < 70% of the reference value; (2) irreversible obstructive airway disease, i.e. < 12 % improvement in FEV1 expressed as percentage of the predicted value after inhalation of a short-acting ß2 agonist (200 Ìg), and (3) no

concomitant confounding disease such as malignancies, gastrointestinal or severe endocrine disorders, collagen vascular disease, cardiac failure, infections or recent surgery. An infectious exacerbation of COPD was defined when at least two of the following three criteria were fulfilled: (a) recent increase in dyspnea (b) increased sputum volume and (c) sputum purulence, provided that one of the two criteria is purulent sputum [13]. In order to increase homogeneity of the study population, only male subjects were included.

Body weight and height were measured and BMI was calculated for all subjects (body weight/body height2). Total skinfold thickness at four regions (biceps, triceps, subscapular, suprailiac) was evaluated by a caliper (Holstain). The patients were not receiving nutritional support therapy.

Pulmonary Function Tests

Pulmonary function tests were measured with a spirometry The highest value from at least three spirometric maneuvers was used. Arterial blood gas was analyzed in sitting patients breathing air.

Determination of Serum Leptin and TNF-α levels.

TNF-alpha and Leptin levels were measured quantitatively by using micro ELISA kits (Bender MedSystems, MedSystems Diagnostics GmbH, Wien, Austria and DRG Diagnostics GmbH, Marburg, Germany, respectively) on automated Alisei microplate analyzer (SEAC Radim S.R.L., Firenze, Italy) in accordance with the recommendations of the manufacturers. Standards, low and high level controls were used as supplied by the manufacturers. The most precise value for each sample was calculated by using an automated and computer-based program, quantitatively. All standards and controls were found within the acceptable validity ranges as stated by the manufacturers. TNF-alpha values higher than 15,6 pg/mL were evaluated over the normal reference interval (1.2-15.3 pg/mL) as described previously. Leptin values higher than 5 ng/mL were evaluated over normal reference intervals (normal population values; male: 3.84 ± 1.79, female: 7.36 ± 3.73).

Statistical Analysis

Univariate analysis of variance was used to compare controls with COPD patients with respect to BMI and sum of skinfold thickness. In addition to BMI, and sum of skinfold thickness were used as covariate variables to compare leptin and TNF levels. In each group, Pearson's correlation coefficient was calculated. Data analyses and descriptive statistics were performed with the statistical package for social sciences (SPSS 9.05). $p \leq 0.05$ was regarded as statistically significant.

RESULTS

Clinical and demographic characteristics of the COPD patients and the healthy controls are shown in Table 1. The COPD patients had moderate/severe airway limitation, decreased arterial PO2, and normal/increased PCO2 values. The control subjects had normal forced vital capacity and FEV1 on spirograms. We analyzed serum leptin levels in the patients with COPD and the controls. Leptin levels were significantly higher in patients with exacerbation than in both stable-disease and control groups ($P< 0.05$) (Figure1).

Although serumTNF-α levels were higher in COPD patients (with excacerbation and stable disease) than in control groups; there was no statistically significant difference between the groups ($P< 0,005$) (Figure 2).

While there was no correlation between serum TNF-α and leptin levels in COPD patients and control groups. (Figure 3).Serum TNF-α levels did also not correlate with nutritional parameters in any of groups (Table 2).

We found a significant correlation between serum leptin levels and BMI ($r = 0.626$, $p = 0.000$), triceps thickness ($r = 0.407$, $p = 0.000$) as well as albumin level ($r = 0.250$, $p = 0.045$) in stable patients. These correlations were more pronounced in the stable patients than in the controls ($p < 0.05$). In the group with exacerbation, we also did not find any correlation

between leptin and nutritional parameters. Correlations between leptin levels and nutritional parameters are presented in Table 3.

Correlations between leptin and BMI; leptin and triceps thicknessin the patient and control groups are shown in figures 4–5, respectively.

Table 1. Clinical and demographic characteristics of the patients and controls.

	Exacerbation (n=14)	Stable disease (n=26)	Healthy controls (n=15)
Age	60,86 ± 20,80	58,14 ± 9,92	56,6 ± 7,80
BMI (kg/m2)	22,37 ± 5,65	26,90 ± 4,75	26,26 ± 2,66
Triceps skinfold thickness (cm)	23,07 ± 3,68	27,12 ± 3,95	28,58 ± 2,66
FEV1 (% of predicted)	36,29 ± 16,26[a]	55,29 ± 15,46[a]	91,50 ± 13,48
FVC (% of predicted)	55,07 ± 18,23[a]	71,67 ± 18,45[a]	87,00 ± 14,47
PaO2 (mmHg)	59,45 ± 2,20[b]	72,20 ± 2,02	N.D.
PaCO2 (mmHg)	56,96 ± 1,28[b]	46,40 ± 1,09	N.D.

N.D. = Not determined.

[a]p < 0,05 ; [b]p ≥ 0,05

Table 2. Correlation between Leptin levels and nutritional parameters in all groups

Correlation (r)	Control	Stable patients	Patients with exacerbation
Leptin – BMI	0,255 (p=0,306)	0,626 (p=0,000)	0,439 (p=0,073)
Leptin – Triceps thickness	0,088 (p=0,728)	0,407 (p=0,000)	0,321 (p=0,263)
Leptin – Albumin	0,096 (p=0,670)	0,250 (p=0,045)	0,296 (p=0,064)

Table 3. Correlations between TNF – α levels and nutritional parameters in patients and control.

Group		TNF – α		p
		≤ 14,2	> 14,2	
COPD	BMI	26,08 ± 5,34	26,30 ± 5,04	0,636
	Triceps thickness	26,38 ± 4,38	25,73 ± 3,57	0,621
Control	BMI	26,19 ± 2,71	26,80 ± 4,24	0,779
	Triceps thickness	28,46 ± 2,81	29,50 ± 0,70	0,621

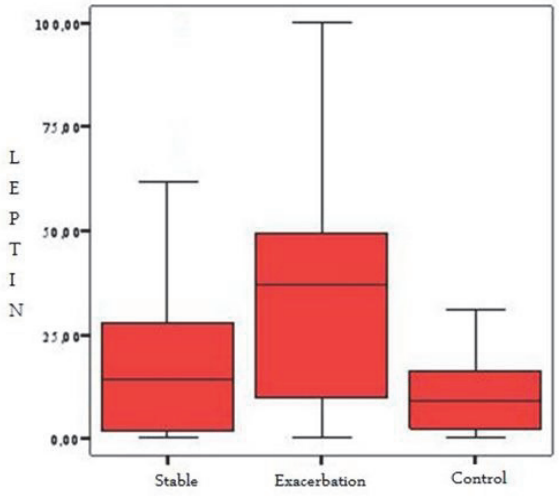

Figure 1. Serum Leptin levels COPD patients and control group.

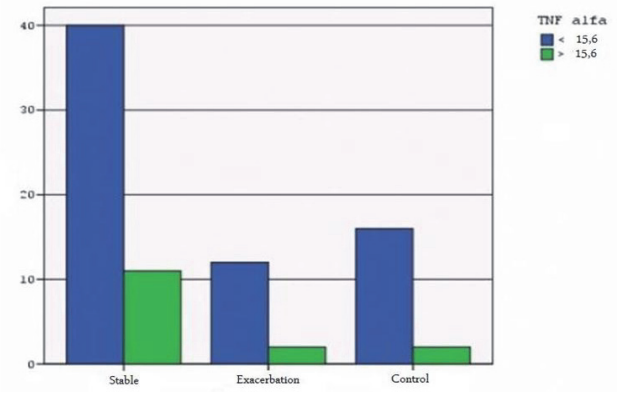

Figure 2. Serum TNF–α levels between stable exacerbation COPD patients and control groups

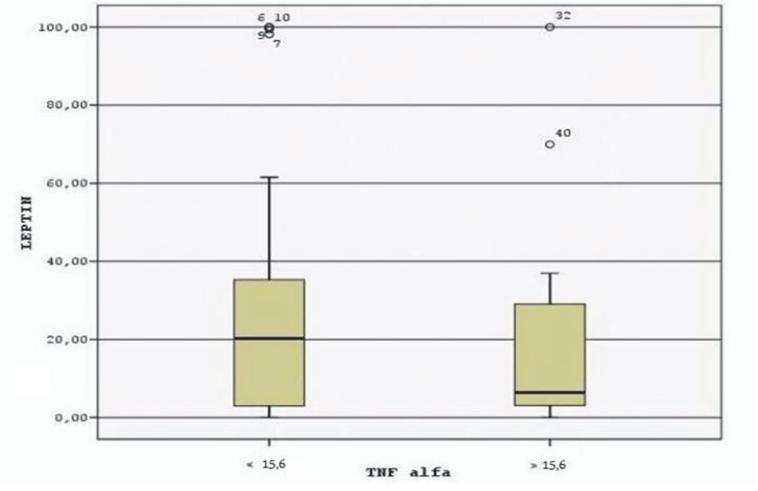

Figure 3. Correlation between the level Leptin and TNF – α.

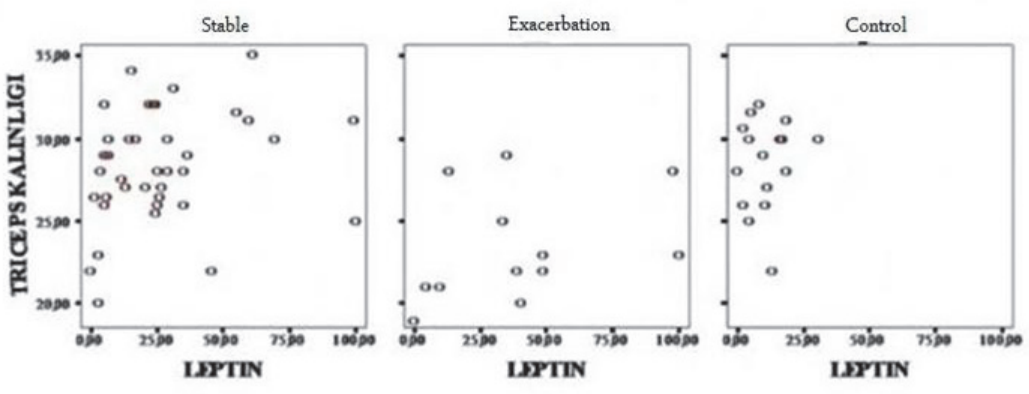

Figure 4. Correlations between Leptin and BMI in patient and control groups.

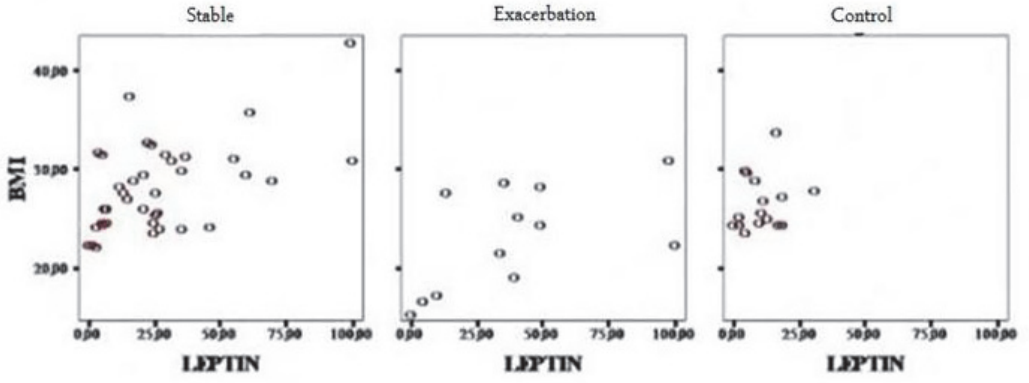

Figure 5. Correlations between Leptin and Triceps thickness in patients and control groups.

DISCUSSION

This study demonstrated that serum leptin levels were significantly higher in patients with exacerbation than in both stable-disease and control groups .Similar results have been reported by Yuan et al. [14], Takabatake et al. (15) and Çalıkoğlu et al(16). A significant increase in leptin levels was found in the patients with acute exacerbation. Increased leptin levels during acute exacerbation of the disease were demonstrated by Creutzberg et al. [17], and increased leptin levels returned to baseline after 7 days of exacerbation in treated patients. Higher leptin levels in the acute exacerbation of the disease may be considered as a systemic inflammatory response. Also Prokopis et al. found that COPD exacerbations are characterized by increased levels of leptin and the proinflammatory cytokines TNF-α, IL-1β, IL-6 and IL-8 and decreased levels of IGF-I on D1. Although circulating plasma leptin decreases and plasma IGF-I increases, they still remained different compared to healthy controls on D15 (18).

Except for the exacerbation group, a significant correlation between leptin levels and nutritional parameters (BMI, triceps thickness and albumin) was observed in the patients with stable COPD compared to the healthy subjects. These findings were in accordance with previous studies [14, 15, 19). Creutzberg et al. [17] also reported that while there was no correlation on the 1st day of acute exacerbation, a significant correlation was observed

between leptin levels and nutritional parameters on the 7th day of exacerbation. In another study, there was no difference in the concentrations of plasma leptin between the COPD patients without weight loss and who had clinically stable disease and those COPD patients with acute exacerbation (20,21). In this study the positive correlation between plasma leptin and BMI or triceps skinfold thickness in chronic stable COPD patients demonstrates that the plasma leptin concentrations remain controlled in stable COPD patients.

It has been reported that TNF-α levels were higher in bronchial biopsies and induced sputum of COPD patients [22, 23]. In addition, it has been shown that both circulating levels of TNF-α [15] and TNF-α production by peripheral blood monocytes [24] were increased in weight-losing stable COPD patients, suggesting that activation of the TNF-α system was associated with the cachexia observed in COPD patients. Yamamoto et al. [25] showed that TNF-·α levels were increased in stable COPD patients with weight loss and also demonstrated that TNF-α levels were negatively correlated with FM. In a recent study by Calikoglu et al serum leptin and TNF-α levels were increased in COPD patients with exacerbation in comparison to COPD patients with stable disease and healthy controls [16]. Moreover, and in accordance with our findings, a strong correlation between TNF-α and leptin was nobserved only in COPD patients with exacerbation but not in stable COPD patients and healthy individuals [16]. In a study Prokopis et al the positive correlation between leptin and TNF-α which was seen in our COPD patients on D1 of the exacerbation, supports an inflammatory-related disturbance in leptin metabolism in COPD. In another study, no differences were seen in the concentrations of soluble TNF-α R55 and R75 between patients with exacerbated COPD and healthy subjects, and these concentrations did not change with exacerbation therapy [17].

In our study, TNF-α levels were not found to be significantly elevated in patients with exacerbation. Furthermore, we did not find any relationship between leptin and TNF-α levels in patients with exacerbation, stabil diseas and control groups. Also there was no significant correlation between TNF-α and nutritional parameters in any group. These observations suggest that TNF-α seems not to play an important role in malnutrition and exacerbation in patients with COPD. Similar results have been reported by Yuan et al (14), Tabatake et al (15) and Yang et al (24).These findings suggested that leptin is not primarily under the control of the TNF-α system.

A reason of no relationship between TNF-alpha and leptin levels might have been the value of TNF –alfa and that presents a limitation of our body. The level of TNF -α were not calculated as a numeric, because of the device. For this reason we diveded the patients into two groups according to TNF-alfa (TNF- alfa < 14,2 and TNF- alfa ≥ 14,2) and therefore we could not give a numeric value. And also the second limitation of the present study is the number of the patients in our groups.

Experimental animal (8) and human (26) studies have provided evidence for a link between leptin and proinflammatory cytokines. TNF-α and IL-1 treatment of fasted hamsters increased the concentration of leptin in circulation and the leptin messenger RNA in adipose tissue (8). Zumbach et al. (26) reported that administration of endotoxin or cytokines such as TNF-α or IL-1 produced a prompt and dose-dependent increase in the serum leptin levels in humans. In this study, we could not find any relationship between the plasma leptin concentration and the activity of the TNF-α system. However, there was positive correlation between the plasma leptin concentration and the BMI, triceps skinfold thicness and albumin. These findings

suggest that the plasma leptin concentration was primarily affected by the body composition, the BMI or the albumin rather than by the activity of the TNF-α system.

In summary, we found that the plasma leptin levels were higher in COPD patients with acute

exacerbation and also they were correlated with body composition parameters such as the body mass index and triceps skinfold thicness in stable COPD patients. There was no difference in the activity of the TNF-α system between nutrional parameters such as BMI, albumin and triceps skinfold thickness. Moreover, there was no correlation between the plasma leptin levels and the TNF-α system.

In conclusion, (1) circulating TNF-α levels were not associated with increased leptin levels and (2) because of the fewer number of the acute exacerbation of COPD patient and not calculated the value of TNF-alpha levels as a numeric value, we did not find elevated TNF-α levels in COPD patients with acute exacerbation (3) we although leptin and nutritional parameters were correlated in the stable COPD patients, it was disappeared completely during an exacerbation and control groups. Therefore, there was no significant correlation between leptin and TNF-α during the regulation of the energy balance in COPD patients.

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