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Investigation of the possible relation of apelin, kynurenine and IL-4/IL-10/IL-12/ Tnf-α levels with clinical and metabolic parameters in obesity

Obezitede apelin, kinürenin ve IL-4/IL-10/IL-12/Tnf-α düzeylerinin klinik ve metabolik parametrelerle olası ilişkisinin araştırılması

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Abstract

Purpose: Obesity is a medical condition caused by excess body fat that accumulates at a level that can have a detrimental impact on health. Altered glucose and lipid metabolism, low-grade chronic inflammation contributes to the pathogenesis of obesity and obesity-related metabolic dysfunction. In our study, we purposed to specify the apelin, kynurenine, IL-4, IL-10, IL-12, TNF-α protein levels in obese individuals and healthy control groups, and to research the possible relationship between clinical parameters with the data to be obtained.

Materials and methods: The levels of apelin, kynurenine, IL-4, IL-10, IL-12, TNF-α in serum/plasma samples were determined with enzyme-linked immunosorbent assay. Absorbance of the samples were measured on a microplate reader spectrophotometrically at a wavelength of 450 nm.

Results: The levels of kynurenine, IL-4, and IL-12 in the serum were higher in control group than in obese patients (p=0.009, p=0.004, p=0.002, respectively). The levels of TNF- α, IL-10, and apelin did not differ substantially between the obese patients and the control group (p=0.277, p=0.711, p=0.472, respectively).

Conclusion: Inflammation and altered immune response are two important components of obesity. They play a significant part in the emergence of obesity-related metabolic disorders. Alterations in adipokine levels may lead to the occurence and maintenance of insulin resistance and systemic inflammation in obesity. The results demonstrate that kynurenine, IL-4, and IL-12 have a complex role in obesity and can be used as therapeutic targets.

Key words: Obesity, apelin, kynurenine, TNF-α, IL-4/IL-10/IL-12.

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Öz

Amaç: Obezite, sağlığı olumsuz etkileyebilecek düzeyde biriken aşırı vücut yağının neden olduğu tıbbi bir durumdur. Değişen glikoz ve lipid metabolizması, düşük dereceli kronik inflamasyon, obezite ve obezite ile ilişkili metabolik disfonksiyonun patogenezinde rol oynar. Çalışmamızda obez hastalarda ve sağlıklı kontrol gruplarında apelin, kinurenin, IL-4, IL-10, IL-12, TNF-α serum protein düzeylerini belirlemeyi ve klinik parametreler ile arasındaki olası ilişkiyi araştırmayı amaçladık.

Gereç ve yöntem: Serum/plazma örneklerindeki apelin, kinurenin, IL-4, IL-10, IL-12, TNF-α seviyeleri, enzim bağlantılı immunosorbent testi ile ölçüldü. Numunelerin absorbansı, 450 nm dalga boyunda spektrofotometrik olarak bir mikroplaka okuyucu ile belirlendi.

Bulgular: Kontrol grubunun serumlarındaki Kinurenin, IL-4 ve IL-12 düzeylerinin obez hastalara oranla anlamlı düzeyde daha yüksek olduğu belirlenmiştir (sırasıyla p=0,009, p=0,004, p=0,002). TNF-α, IL-10 ve apelin düzeyleri değerlendirildiğinde ise obez hastalar ve kontrol grubu arasında anlamlı bir fark olmadığı tespit edilmiştir (sırasıyla p=0,277, p=0,711, p=0,472).

Sonuç: Enflamasyon ve değişmiş bağışıklık yanıtı obezitenin iki önemli bileşenidir. Bu bileşenler obeziteye bağlı metabolik hastalıkların oluşumunda önemli rol oynamaktadırlar. Adipokin düzeylerindeki değişiklikler, obezitede sistemik inflamasyonun gelişimine ve insülin direncinin sürdülmesine yol açabilir. Elde edilen sonuçlar, kinurenin, IL-4 ve IL-12'nin obezitede karmaşık bir role sahip olduğunu ve terapötik hedefler olarak kullanılabileceğini göstermektedir.

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Anahtar kelimeler: Obezite, apelin, kinurenin, TNF-α, IL-4/IL-10/IL-12.

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Introduction

The World Health Organization (WHO) claims that, obesity is a condition that occurs as a result of aberrant and excessive fat accumulation that can impair human health. Obesity is a very serious problem today. Unfortunately, every year the number of deaths due to obesity is at least 2.8 million people in worldwide [1].

The etiology of obesity involves altered glucose and lipid metabolism. Recent studies suggest evidence that tryptophan, an essential amino acid, is preferentially catabolized via the kynurenine pathway in obese patients, and therefore the level of circulating kynurenine increases (Kyn) [2]. Apelin is a regulatory peptide and the G-protein-coupled receptor's ligand (APJ). Apelin and APJ are largely expressed in many tissues and organs, including brain, heart, lung, liver, kidney, blood plasma, gastrointestinal tract, endothelial and adipose tissues [3]. Many studies have reported that increased plasma apelin is associated with metabolic pathologies. Recent studies show that apelin peptide may be a useful adipokine in metabolic disorders and it can be a potential therapeutic target for obesity and antidiabetic drugs [4, 5]. Apelin is upregulated in obesity. In clinical and experimental studies, serum apelin level or adipose tissue apelin expression increases in obesity and insulin resistance [6].

There are studies demonstrate that low-grade chronic inflammation plays a substantial role in the pathogenesis of obesity and obesity-related metabolic dysfunction. Chronic inflammatory alterations have been determined to be associated with the function of immune cells in many tissues such as hypothalamus, muscle, adipose tissue, liver, and pancreatic islet.

Regulatory T (Treg) cells, eosinophils, natural killer T cells (iNKT), and M2-like macrophages are commonly resided in adipose tissue. These cells also secrete T helper (Th) 2 cytokines and IL-10, which are anti-inflammatory cytokines that inhibit inflammation in adipose tissue. Chronic systemic inflammation brought on by altered cytokine activation and inflammatory signaling is linked to obesity. Numerous studies have attributed increased manufacture of inflammatory cytokines such as IL-6, TNF and some adipokines to obesity and increased insulin resistance during the inflammatory process. It is less clear how anti-inflammatory cytokines like IL-4 affect the emergence of insulin resistance or obesity. Insulin sensitivity and local immune response are controlled by IL-4 produced by adipocytes and hepatocytes. These findings suggest that IL-4 may attend in diet-induced obesity and metabolism processes. Additionally, IL-12 is crucial for the pathophysiology of type 1 diabetes [7].

In our study, we purposed to determine the apelin, kynurenine, TNF-α/IL-4/IL-10/IL-12 protein levels in obese individuals and healthy control groups, and to investigate the possible relationship between body fat parameters, glycolipid metabolism, insulin resistance and clinical parameters with the data to be obtained.

Materials and methods

Serum/plasma of blood samples taken from patients who applied to Internal Medicine Endocrinology outpatient clinic of Pamukkale University Hospital and diagnosed with obesity (n=31) and age-sex matched healthy control group (n=33) were studied. Demographic characteristics, history and examination findings of all cases included in the study were recorded. Since the demographic data of 2 people in the control group were not available, they could not be evaluated in the Pearson correlation analysis. Height and weight were measured using standard techniques in the patient group and healthy control group.

Body mass index was calculated as the ratio of weight to height squared (kg/m^2). Percentage of body fat distribution was measured by BIA (Bioelectrical impedance analysis) in PAU Endocrinology outpatient clinic. Concentrations of apelin, kynurenine, IL-4, IL-10, IL-12, TNF-α were measured in serum/plasma samples

with ELISA kits using the directions provided by the manufacturer (Bioassay Technology Laboratory). All samples and standards were run in duplicate. Absorbance of the samples were measured on a microplate reader spectrophotometrically at a wavelength of 450 nm.

Written informed consent was provided from all patients and the Pamukkale University ethics committee approved the study's design.

Enzyme-linked immunosorbent assay

Blood samples taken from the control and patient groups were centrifuged at 2500 rpm for 10 minutes to obtain serum. Apelin, kynurenine, TNF-α, IL-4, IL-10, IL-12 levels of serum samples were determined by applying ELISA kit protocols. In this context, the relevant protocol is as follows:

a. Standard preparation followed the kit protocol and to the appropriate wells, 50 µl of the standards were added.

b. The wells designated for the blank received 100 µL of Sample Diluent solution., and 50 µL of Sample Diluent solution for samples. Each sample was put to the appropriate wells in 50. Each well received 50 µL of Biotin-Conjugate solution. The plate was sealed, and it was allowed to rest for two hours at room temperature.

c. The sealer was removed, the wells were decanted and washed with wash buffer.

d. Each well received 100 µL of the streptavidin-HRP solution. After sealing the plate, it was left at room temperature for an hour. The wells were decanted and cleansed with wash buffer once the sealant was removed. The substrate solution was put into each well at a volume of 100 µL. At room temperature, the plate was incubated for 10 minutes.

e. To each well, 100 µL of Stop Solution were added.

f. The samples' absorbance was determined spectrophotometrically on a microplate reader at a wavelength of 450 nm.

Statistical analysis

Using SPSS 23.0 version, the study's data were statistically analyzed. All data were presented as mean ± standard deviation. To examine differences between independent group, a one-way ANOVA technique was utilized and for the correlation between continuous variables, Pearson correlation analysis was employed. $P<0.05$ was considered statistically significant.

Results

The levels of Kynurenine, IL-4, and IL-12 in the serum were higher in control group than in obese patients ($p=0.009$, $p=0.004$, $p=0.002$, respectively) (Figure 1). The levels of TNF-α, IL-10, and Apelin did not differ substantially between the obese patients and the control group ($p=0.277$, $p=0.711$, $p=0.472$, respectively).

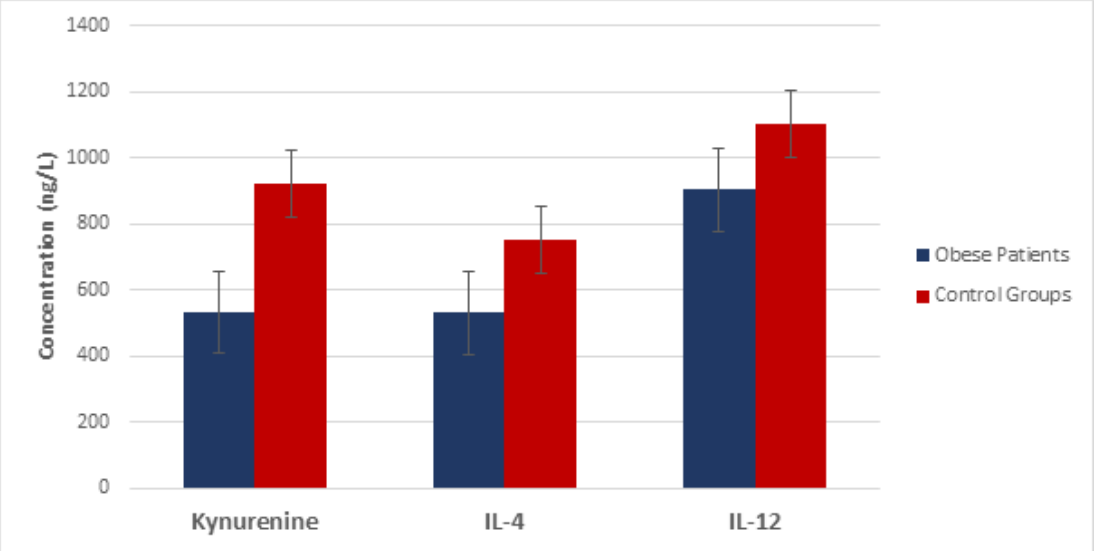

Figure 1. Kinurenin, IL-4, and IL-12 levels in serum samples of obese patients and control group

These serum protein levels were also compared with age, gender, body mass index (BMI), fat ratio, basal metabolic rate (BMR), (Table 1). When the correlation between kynurenine and gender, body mass index, age, basal metabolic rate and fat ratio was evaluated, it was specified that there was a negative correlation between kynurenine and age, BMI and fat ratio (p=0.002, p=0.033, p=0.014, respectively). Even though that there was no correlation between the levels of TNF-α and IL-10 in the obese patients and the control group there was a negative correlation between TNF-α and IL-10 with age (p=0.010, p=0.006). It was found that there was a negative correlation between IL-4 and age, BMI and fat ratio (p=0.002, p=0.010, p=0.006, respectively). In addition, it was specified that there was a negative correlation between IL-12 and age, BMI and fat ratio (p=0.019, p=0.014, p=0.018, respectively).

Table 1. Correlations between the apelin, kynurenine, IL-4/IL-10/IL-12/TNF-α levels and clinical parameters in total groups

	Gender	Age	BMR	BMI	%Fat
Kynurenine					
Pearson Correlation	-.126	-.392**	-.078	-.271*	-.310*
Sig (2-tailed)	.321	.002	.547	.033	.014
N	64	62	62	62	62
TNF-α					
Pearson Correlation	-.048	-.326**	-.031	-.136	-.157
Sig (2-tailed)	.707	.010	.809	.293	.222
N	64	62	62	62	62
IL-4					
Pearson Correlation	-.115	-.381**	-.114	-.323*	-.345**
Sig (2-tailed)	.368	.002	.378	.010	.006
N	64	62	62	62	62
IL-12					
Pearson Correlation	-.039	-.298*	-.187	-.309*	-.299*
Sig (2-tailed)	.759	.019	.146	.014	.018
N	64	62	62	62	62
IL-10					
Pearson Correlation	-.154	-.347**	.080	-.075	-.110
Sig (2-tailed)	.223	.006	.538	.563	.395
N	64	62	62	62	62

*/** Statistically significant against other groups (**p<0.01 *p<0.05)

Discussion

Obesity is a chronic condition characterized by an increase in body fat mass relative to lean body mass and is brought on by the body consuming more energy than it expends through meals. Obesity, a public health issue whose prevalence has been increasing in recent years. It is responsible for various factors such as different dietary habits, gender, smoking, age, marital status, education level, and alcohol consumption, and lack of physical activity. While the World Health Organization (WHO) accepts obesity as one of the 10 riskiest diseases, it has been determined that obesity is closely related to cancer in the latest studies conducted by the same organization.

Obesity is associated with a chronic systemic inflammation and immune activation. Therefore, it can be classified as an inflammatory disease. If the daily energy intake is more than the consumed energy, the energy that cannot be consumed is stored as fat in the body and causes obesity [8]. In general, obesity is accompanied by abnormal metabolism of amino acids [9].

Previous studies suggest evidence that tryptophan, an essential amino acid, is preferentially catabolized via the kynurenine pathway (KP) in obese patients, and therefore increases in circulating Kynurenine (Kyn) [2]. In our findings, we observed that kynurenine levels in serum samples of the control group were pointedly higher than those of obese patients (p=0.009). Mangge et al. [10] determined that the ratio of Kyn and Kyn/Trp in serum samples of obese adult patients was significantly higher than the healthy group, but the ratio of Kyn and kyn/Trp was significantly lower in individuals

under the age of 18 compared to the control group. Kyn is generally considered to be an immunosuppressive factor, and obesity is always accompanied by low-grade chronic inflammation. Therefore, the increase in circulating Kyn is thought to be a compensatory effect [11, 12]. However, it also appears that this increase in Kyn does not improve the inflammatory microenvironment in obese individuals [13, 14]. This suggests that Kyn has a complex role in obese individuals. The level of serum Trp and its metabolites, such as Kyn, Kynurenic acid (Kyn A), 3-hydroxykynurenine (3HKyn), xanthurenic acid (XA), quinolinic acid(QA) and KP enzymes were determined to be associated with type 2 diabetes [15-17]. Alteration in the levels of circulating KP metabolites has also been described in obesity and obesity-related metabolic disorders [18, 19]. However, according to studies, the relationship of these metabolites with obesity is controversial [20, 21]. The regulation of KP is affected by various factors such as age, gender, body mass index, inflammatory state. These different factors can affect the results of human studies, particularly in those with small sample size. Therefore, kyn levels should be evaluated in a larger sample group among the groups in which variables such as age and gender are classified. In our findings, it was specified that there was a negative correlation between kynurenine and age, BMI and fat ratio (p=0.002, p=0.033, p=0.014, respectively).

Apelin is a regulatory peptide and the G-protein-coupled receptor's ligand (APJ). Apelin and APJ are largely expressed in many tissues and organs, including brain, heart, lung, liver, kidney, blood plasma, gastrointestinal tract, endothelial and adipose tissues [3]. A significant number of clinical studies investigating apelin levels in body fluids, both in healthy controls and in patients with different pathologies, have been reported. Recent research shows that apelin peptide may be a useful adipokine in metabolic disorders and be used as a promising therapeutic target for obesity and antidiabetic drugs [4, 5]. Apelin is upregulated in obesity. In clinical and experimental studies, serum apelin level or adipose tissue apelin expression increases in obesity and insulin resistance [6]. Apelin supplementation is reported to improve in vitro insulinotropic activity, glucose uptake by adipocyte, glucose elimination, and insulin release in obese mice [22]. After 28 days of apelin administration, obese and insulin-resistant rats showed a significant improvement in insulin sensitivity as well as a decrease in body fat [23]. In our findings, we could not reach a significant result when the apelin levels of the obese patients were compared with the apelin levels of the control group.

Increased production of inflammatory cytokines including IL-6 and TNF has been related in numerous studies and some adipokines have been linked to insulin resistance development and obesity throughout the inflammatory process. It is less clear how anti-inflammatory cytokines like IL-4 affect the emergence of insulin resistance or obesity. In a study, it was observed that IL-4 production by splenic lymphocytes from diet-induced obese mice increased and serum IL-4 amount decreased in Sprague-Dawley rats after visceral fat removal surgery [24]. Insulin sensitivity and local immune response are controlled by IL-4 produced by adipocytes and hepatocytes. In our findings, we specified that the IL-4 level in the serum was pointedly lower in obese individuals compared to the healthy control group (p=0.004). Additionally, it was specified that there was a negative correlation between IL-4 and age, BMI and fat ratio (p=0.002, p=0.010, p=0.006, respectively). These results imply that IL-4 may involve in diet-induced obesity and metabolism processes.

Chronic inflammatory changes; It has been showed that it is associated with the function of immune cells in many tissues such as hypothalamus, adipose tissue, muscle, liver and pancreatic islet. Regulatory T (Treg) cells, eosinophils, invariant natural killer T cells (iNKT), and M2-like resident macrophages are commonly found in adipose tissue. These cells also secrete T helper (Th) 2 cytokines and IL-10, which are anti-inflammatory cytokines that inhibit inflammation in adipose tissue. In a study conducted by Calcaterra et al. [25] on obese children and adolescents, they found that IL-10 protein levels were high in obese individuals and stated that metabolic syndrome was not associated with low levels of IL-10. In a study by Pereira et al. [26], protein levels of IL-10 were also determined to be higher in obese individuals. In a study by Schmidt et al. [27] conducted with 117 obese and 83 healthy controls, the serum level of IL-10 was determined to be pointedly

higher in obese patients. In a study conducted by Arismendi et al. [28] with 129 morbidly obese individuals, the serum levels of IL-10 in the obese and control groups were examined and they showed that the serum level of IL-10 was higher in obese individuals than in controls. They observed that IL-10 levels decreased in the same patients when bariatric surgery was subsequently performed. Esposito et al. [29] also discovered that the IL-10 level in obese women was higher than in non-obese women, but the IL-10 protein level was lower in both obese and non-obese women with metabolic syndrome. In another study, the effect of IL-10 on the metabolic syndrome was investigated and it was revealed that the IL-10 protein level was significantly reduced in both men and women with metabolic syndrome [30]. In a study on childhood obesity, it was determined that the IL-10 level in obese individuals was lower than in controls. It has also been reported to have a role in increased inflammation, tissue damage and obesity [31]. These results show that IL-10 has a protective role in inflammation. In our study, we showed that the serum IL-10 level of the obese patient group was lower than the serum IL-10 level of the healthy control group. But the difference was insignificant. Although there was no meaningful correlation between IL-10 levels and obese patients and control group, there was a negative correlation between IL-10 with age (p=0.006).

Additionally, IL-12 is crucial for the pathophysiology of type 1 diabetes [32]. According to studies, IL-12 may contribute to the emergence of insulin resistance in obese mice. Suárez Álvarez et al. [33] found that IL-12 levels were pointedly higher in overweight and obese individuals compared to the control group. Nikolaju et al. [34] indicated that there was a statistically significant positive correlation between IL-12 levels and total cholesterol levels in overweight and obese women. In our study, we specified that the serum IL-12 level of the healthy control group was pointedly higher than the serum IL-12 level of the patients with obesity (p=0.002). Additionally, it was signified that there was a negative correlation between IL-12 and age, BMI and fat ratio (p=0.019, p=0.014, p=0.018, respectively).

In conclusion, inflammation and altered immune response are two important components of obesity. They play a significant role in the development of metabolic diseases associated with obesity. Changes in adipokine levels may lead to the development and maintenance of insulin resistance and systemic inflammation in obesity. The results demonstrate that kynurenine, IL-4, and IL-12 have a complex role in obesity and can be used as therapeutic targets.

Conflict of interest: No conflict of interest was declared by the authors.

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Contributions of the authors to the article

I.C.B.M. and Y.D. constructed the main idea and hypothesis of the study. I.C.B.M., Y.D., G.F.Y., E.P. and Z.A. developed the theory and organized the material and method section. Data collection was done by G.F.Y. and E.P., and data analysis was done by I.C.B.M. and Y.D. evaluated the data in the results section. The discussion section of the article was written by I.C.B.M. and Y.D. reviewed the article and made the necessary corrections and approved it. In addition, all authors discussed the entire study and approved the final version.