Serum and Tear Leptin Levels in Patients with Allergic Conjunctivitis

Allergic Konjonktivitli Hastalarda Serum ve Gözyaşı Leptin Düzenleri

ABSTRACT

Objective: The production of leptin that acts as a pro-inflammatory mediator is increased in patients with various inflammatory and allergic diseases. Our aim was to investigate the serum and tear leptin levels in patients with perennial allergic conjunctivitis and to compare them with healthy controls. Material and Methods: Fifteen patients with perennial allergic conjunctivitis (8 females and 7 males, mean age: 22 years, mean body mass index: 24.17 ± 5.64 (±SD) kg/m²) were included in the study. Gender, body mass index and age-matched 15 healthy subjects (8 females, 7 males, mean age: 25, mean BMI: 22.47 ± 3.56 kg/m²) were selected as the control group. Serum and tear leptin levels were measured with enzyme-linked immunosorbent assay (ELISA) method. Results: Serum leptin levels showed no significant difference between the patients and controls (9.79 ± 7.73 ng/ml vs. 10.49 ± 7.26 ng/ml, p > 0.05). Tear leptin levels showed no significant difference between the patients and controls (8.90 ± 1.81 pg/ml vs. 8.68 ± 1.70 pg/ml, p > 0.05). Conclusion: Our data suggest that serum and tear leptin levels do not change in patients with perennial allergic conjunctivitis. Further studies are needed to investigate the levels of free and bound leptin and to determine leptin’s role in the pathogenesis of perennial allergic conjunctivitis.

Key Words: Leptin; conjunctivitis, allergic; inflammation

ÖZET

Amaç: Leptin, bir dizi allerjik ve enfamatuvar hastalıkta artış gösteren pro-enflamatuvar bir sitoaktır. Bu çalışmada perenial allerjik konjonktiviti hastalarda serum ve göz yaşlı leptin düzeylerini araştırmayı ve sağlıklı bireylerdeki düzeyleriyle karşılaştırmayı amaçladık. Gereç ve Yöntemler: Perenial allerjik konjonktivi 15 hasta (8 kadın ve 7 erkek) ile 15 sağlıklı birey (8 kadın ve 7 erkek) çalışmaya alındı. Ortalama yaş 24.9 ± 6.3 yaş, kontrol grubunda 24.8 ± 7.9 yaş idi. Vücut kitle endeksi 24.17 ± 5.64 kg/m², kontrol grubunda ise 22.47 ± 3.56 kg/m² idi. Serum ve göz yaşlı leptin düzeyleri enzimli-lnked immunosorbent assay (ELISA) yöntemile ölçüldü. Bulgular: Serum leptin düzeyleri hasta (9.79 ± 7.73 ng/ml) ve kontrol (10.49 ± 7.26 ng/ml) grupları arasında istatistiksel anlamlı bir fark göstermedi (p > 0.05). Gözyaşı leptin düzeylerinde de hasta (8.90 ± 1.81 pg/ml) ve kontrol (8.68 ± 1.70 pg/ml) grupları arasında anlamlı farklılık saptanmadı (p > 0.05). Sonuç: Perenial allerjik konjonktiviti hastalarda serum ve göz yaşlı leptin düzeylerinde farklılık olmadığı görüldü. Serbest ve bağlı leptin seviyelerinin artışını dikkate alındığında allerjik göz hastalıklarında leptin rolünü meilleurileceği kanaatindeyiz.

Anahtar Kelimeler: Leptin; allerjik konjonktivit; enfamasyon


Perennial allergic conjunctivitis (PAC) usually begins in second decade of life, and it is one of the most common diseases of young adults. Most of patients have personal or family history of other allergic conditions such as allergic rhinitis, atopic dermatitis and asthma. Perennial allergic conjunctivitis is an inflammatory ocular disease that affects the
quality of life of the patients for a long period of time. However it rarely causes vision loss. Perennial allergic conjunctivitis is usually diagnosed clinically. The signs and symptoms include itching, tearing, conjunctival edema, hyperemia, watery discharge, burning, photophobia and lid edema. The main target tissue is conjunctiva. In perennial allergic conjunctivitis, the allergen directly comes in contact with the conjunctiva and induces type I hypersensitivity reaction.\(^3\) It interacts with the IgE binding tissue mast cells and leads to release of chemical mediators.\(^4,5\) Cytology of tears and conjunctival scrapings demonstrated increased neutrophils and eosinophils. The definitive diagnosis of perennial allergic conjunctivitis is difficult and requires laboratory tests which do not always reflect the clinical diagnosis.\(^6\) However, in daily practice, disease can be easily diagnosed by the history of allergic disease, itchy symptoms and papilla formation of conjunctiva without the need to measure the serum IgE.\(^7\)

Leptin, a pleiotropic protein secreted by the adipose tissue, plays an important role in the regulation and modulation of body weight and, in metabolism, hematopoesis, angiogenesis and immunity.\(^8\) Body mass index (BMI) has been reported to be one of the important associated factors, and a positive association between BMI and serum leptin has been shown. Furthermore, leptin has structural similarities with some cytokines, including IL-(Interleukin)-6, IL-11, IL-12, and IL-15, as well as with granulocyte colony-stimulating factor.\(^9,10\) Leptin production is increased in patients with atopic asthma and other inflammatory and allergic diseases.\(^11,12\) Some evidence demonstrated that leptin acts as a pro-inflammatory mediator. Previous studies indicated that serum leptin concentration increases in allergic reactions of the respiratory tract, as well as IgE-associated atopic dermatitis, and it may play a role in the pathogenesis of allergic inflammation.\(^13,14\)

In our study, we assessed the serum and tear leptin levels in patients with allergic conjunctivitis and healthy controls. The aim of this study was to evaluate a possible relation between leptin level and perennial allergic conjunctivitis.

### MATERIAL AND METHODS

Fifteen cases with complaints of ocular allergy and 15 healthy controls with similar age, sex and BMI were enrolled in the study. Diagnosis of perennial allergic conjunctivitis was based on history and clinical examination. Inclusion criteria for the patients were diagnosis of allergic conjunctivitis, symptomatic period and patients that did not receive and medication for at least previous three months. Exclusion criteria were pregnancy or the possibility of pregnancy, acute systemic and local inflammation/infection, and systemic diseases such as hypertension, macro-albuminuria, depression, heart disease, renal failure, diabetes mellitus, and morbid obesity. Patients who were on systemic or topical antihistamincs, or anti allergic or anti-inflammatory agents in the previous three months were also excluded from the study. Control subjects were selected among patients with no allergy or atopy history, acute infection or inflammation, systemic disease, and obesity. Subjects in both groups declared that their weight had been stable for at least three months preceding their entry to the study. The BMI (defined as weight in kilograms divided by the square of the height in meters) was calculated for all of the subjects. The protocol for the study was approved by the Institutional Review Board of Firat University, Turkey. Sample collection and biochemical procedures were performed following the tenets of the Declaration of Helsinki and informed consent was obtained from all subjects.

### SERUM LEPTIN ANALYSIS

The design of the study rules out any interference by the diurnal variation in leptin levels, because all samples were collected at 08. 00 hours after overnight fasting in all subjects. Serum was separated (1500g, 10 min) and frozen at -80°C until the time of the assay. Serum leptin levels were measured with a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) using a human leptin kit (Biosource, Biosource Europe S.A. Belgium). The assay used monoclonal antibodies (M Abs) directed against distinct epitopes of human leptin. The intrassay coefficients of variation for the leptin ELISA were 3.4%, and the minimum detectable limit of the assay was 0.1 ng/ml.
TEAR LEPTIN ANALYSIS

Tear samples (volume 5-10 μl) were collected from the conjunctival sac with Whatmann 3 MM filter paper discs (6 mm in diameter) or with glass capillaries from the patients with PAC and with normal Schirmer I test without applying an irritant from healthy people. Tear samples were immediately frozen and stored at −70°C until analyzed. Leptin was measured in tear samples using Human Leptin ELISA kit (Ray Biotech, Inc. Norcross, GA, USA) according to the manufacturer’s protocol. The minimum detectable dose of Leptin was typically less than 6 pg/ml. The intra- and interassay coefficients of variation for leptin were < 10% and < 12%.

STATISTICAL ANALYSIS

Statistical analysis was performed using the Statistical Package for the Social Sciences version 11.0 (SPSS, Chicago, IL, USA). Numeric variables were compared by using the Mann-Whitney U test, categorical variables were compared by Chi-square test. Correlations were assessed using the Pearson product moment test. Results were given as means ± standard deviations. Leptin levels of the groups were also analyzed after adjusting for the BMI using analysis of covariance (ANCOVA). P value less than 0.05 was considered significant.

RESULTS

Demographic features and serum and tear leptin levels in perennial allergic conjunctivitis and controls are given in Table 1. Both the patient and control groups included 7 males and 8 females. Mean BMI was 22.47 ± 3.56 in the control group, and 24.17 ± 5.64 in the patient group. The two groups were matched in terms of age and BMI, and there was no significant difference between the groups (p= 0.281 and p= 0.336, respectively). The mean age was 25 years (range: 16-28) in the controls and 22 years (range: 14-25) in the study group, respectively. The difference between the groups was not statistically significant (p= 0.281).

Serum leptin levels were 10.49 ± 7.26 ng/ml in the control group and 9.79 ± 7.73 ng/ml in the study group (Table 1). The difference between the groups was no statistically significant (p= 0.833).

Tear leptin levels were 8.90 ± 1.81 pg/ml in the control group and 8.68 ± 1.70 pg/ml in the study group (Table 1). The difference between the groups was not statistically significant (p= 0.805).

Tear and serum leptin levels positively correlated in the study group (r= 0.552, p= 0.041, Figure 1). However this correlation was not observed in the healthy control subjects (r= 0.032, p= 0.934).

![FIGURE 1: Tear and serum leptin levels were positively correlated in the study group with perennial allergic conjunctivitis but this correlation was not seen in the healthy control subjects.](image-url)
Insignificant differences for age and BMI at the time of admission were observed between groups, and therefore ANCOVA was performed to make correction the serum and tears leptin levels for the age and BMI. Serum and tear leptin levels were still found to be insignificantly different between the patient and control group after adjusting (p=0.147 and p=0.791, respectively).

**DISCUSSION**

Cytokines have been shown to play important pathogenetic roles in allergic inflammation. Leptin plays an important role in inflammation as it activates monocyte/macrophages and potentiates production of the proinflammatory cytokines, tumor necrosis factor-α, IL-6, IL-9, IL-12, and directs T cell differentiation to Th1 phenotype it expresses the adhesion molecules on endothelial cells, IL-2 and interferon-γ as a strong pro-inflammatory cytokine and it has also been reported to modulate T-helper cell activity in the cellular immune response. The elevation of serum leptin levels that takes place during infection and inflammation strongly suggests that leptin is a part of the cytokine network that manages the inflammatory-immune response. Additionally, leptin has been shown to be an eosinophil survival factor and to mediate proliferative and antiapoptotic activities in different cell types including T cells and eosinophils.

The pro-inflammatory mediators released by mast cells include histamine, leukotriene 4, prostaglandin D2, tryptase, chymase, carboxypeptidase A, cathepsin G, platelet activating factor, and other eosinophil and neutrophil chemo attractants.

In experimental studies, it has been determined that leptin level acutely increases by inflammatory stimuli like endotoxins, and by the administration of pro-inflammatory cytokines like as mentioned above.

However, data reported from human studies do not always agree with results obtained from animals. Results from studies conducted in patients with sepsis demonstrated either the elevation of leptin level or no alteration.

However, no correlation between leptin levels and disease activity and no increase in serum leptin levels have been reported in patients with rheumatoid arthritis or inflammatory bowel disease. In chronic obstructive pulmonary disease, circulating leptin levels have been reported to be either physiologically regulated or related to the inflammatory status.

Güler et al investigated the serum leptin levels in asthmatic children and healthy controls, and found significantly higher levels in children with atopic asthma.

Mai et al investigated the serum leptin and IFN-γ in asthmatic children, and they did not find a statistically significant difference between the non overweight asthmatic children and healthy controls.

Unal et al. investigated the serum leptin levels in allergic rhinitis and healthy controls, and they reported that the serum leptin levels were significantly higher in patients with allergic rhinitis.

To the best of our knowledge, this is the first report investigating the relation of serum and tear leptin level in PAC.

In this study, we hypothesized that the levels of leptin in serum and tear were increased in patients with PAC. However, serum and tear leptin concentrations were not found to be significantly different in patients and controls. These results suggest that serum and tear leptin levels do not change in patients with PAC. Although our results do not confirm any significant alteration in serum and tear leptin concentrations in patients with PAC, it is possible that leptin may play a role in the pathophysiology of PAC as it is pro-inflammatory and eosinophil survival factor. The insignificant results concerning with the levels of serum leptin in our study may be due to local inflammation. However, in this condition, an elevated leptin levels is expected in the tear. The level of tear leptin might not always reflect the clinical diagnosis as the eosinophil count does in tear. It should be noted that the studies reported only systemic circulating leptin levels. So, as with other regulators of the inflamma-
tory response, leptin function may be modulated by the ratio between free and bound leptin, by the expression of different forms of the receptors, the ratio between signaling and non-signaling receptors, and the presence of specific inhibitors. These factors have to be taken into account to evaluate the possible role of leptin in ocular allergic disease.

Further studies are needed to have more information molecular regulation and effects of leptin, and to investigate the levels of free and bound leptin and to determine leptin’s role in the pathogenesis of allergic conjunctivitis.

LIMITATIONS OF STUDY
There are some limitations in this study. There were only 30 (15 patient and 15 controls) subjects who participated in the complete study. Our hospital is an university hospital and a tertiary health institution in our country. Thus, the admissions to our out-patient clinic of the patients with perennial alergic conjunctivitis are limited. Additionally, because only the patients which are symptomatic and without medications enrolled into this study, the number of the partipicants is limited.

Competing interests: The authors declare that they have no competing interests.

REFERENCES


