Serum and Tear Leptin Levels in Patients with Allergic Conjunctivitis

Allerjik Konjonktivitli Hastalarda Serum ve Gözyaşı Leptin Düzeyleri

Burak TURGUT, MD,[a]
Jülide KURT, MD,[a]
Nevin İLHAN, MD,[b]
Süleyman Serdar KOCA, MD,[c]
Tamer DEMİR, MD,[a]
Ülkü ÇELİKER, MD[a]

Department of [a]Ophthalmology, [b]Biochemistry, [c]Rheumatology, Fırat University Faculty of Medicine, Elazığ

Geliş Tarihi/*Received:* 16.09.2008
Kabul Tarihi/*Accepted:* 06.01.2009

Yazışma Adresi/*Correspondence:*
Burak TURGUT, MD
Fırat University Faculty of Medicine, Department of Ophthalmology, Elazığ, TÜRKİYE/TURKEY
drburakturgut@yahoo.com

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/m^2) 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/m^2) 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 n g/ml vs. 10.49 ± 7.26 n g/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 enflamatuar hastalıkta artış gösteren pro-enflamatuar bir sitokindir. Biz, bu çalışmada perennial allerjik konjonktivitli hastalarda serum ve gözyaşı leptin düzeylerini araştırmayı ve sağlıklı bireylerdeki düzeyleriyle karşılaştırmayı amaçladık. **Gereç ve Yöntemler:** Perennial allerjik konjonktivitli 15 hasta (8 kadın ve 7 erkek) ile 15 sağlıklı birey (8 kadın ve 7 erkek) çalışma kapsamına alındı. Ortalama yaş hasta grubunda 21.95 ± 6.3 yaş, kontrol grubunda 24.8 ± 7.9 yaş idi. Vücut kitle endeksi hasta grubunda 24.17 ± 5.64 kg/m^2, kontrol grubunda ise 22.47 ± 3.56 kg/m^2 idi. Serum ve gözyaşı leptin düzeyleri enzyme-linked immunosorbent assay (ELISA) yöntemiyle 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:** Perennial allerjik konjonktivitli hastalarda serum ve gözyaşı leptin düzeylerinde farklılık olmadığı görüldü. Serbest ve bağlı leptin seviyelerinin araştırıldığı çalışmalarla allerjik göz hastalıklarında leptinin rolü anlaşılabileceği kanaatindeyiz.

Anahtar Kelimeler: Leptin; allerjik konjonktivit; inflamasyon

Turkiye Klinikleri J Med Sci 2010;30(1):233-8

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.[1,2] 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 antihistaminics, 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 intraassay 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).

TABLE 1: Demographic features, serum and tear leptin levels in perennial allergic conjunctivitis and controls groups.

	Control (mean ± S.D.)	Patient (mean ± S.D.)	p
Age(year)	24.80 ± 7.90	21.95 ± 6.30	p= 0.281
BMI (kg/m²)	22.47 ± 3.56	24.17 ± 5.64	p= 0.336
Serum Leptin (ng/mL)	10.49 ± 7.26	9.79 ± 7.73	p= 0.833
Tears Leptin (pg/mL)	8.90 ± 1.81	8.68 ± 1.70	p= 0.805

BMI; body mass index. P values were determined by Mann-Whitney U test.

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).

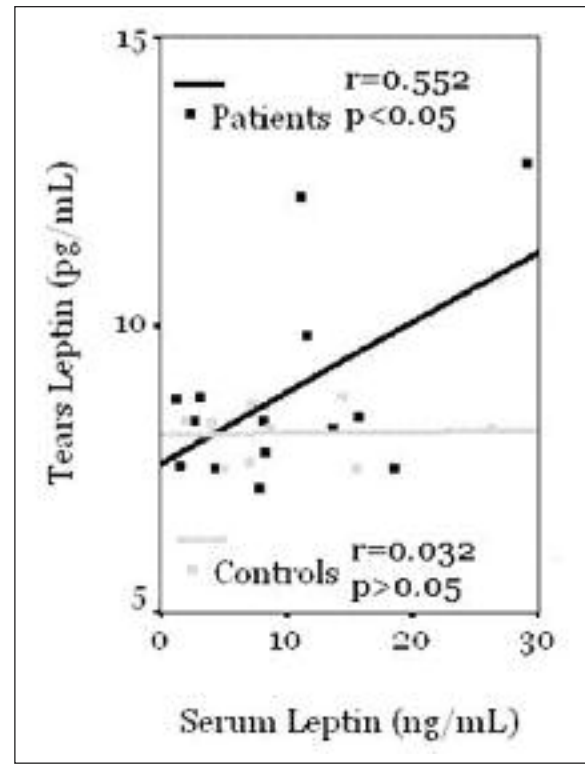

FIGURE 1: Tear and serum leptin levels were positively correlated in the study group with perennial allergic conjunctivitis but this correlation was not seen in the healthy control subjects.

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.[15,16] 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.[15-18] 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.[10,15,19] 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.[19]

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.[20]

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.[21,22]

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.[23,24]

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.[25,26] In chronic obstructive pulmonary disease, circulating leptin levels have been reported to be either physiologically regulated or related to the inflammatory status.[27,28]

Guler et al investigated the serum leptin levels in asthmatic children and healthy controls, and found significantly higher levels in children with atopic asthma.[10]

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.[29]

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.[30]

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 hypothetized 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 allergic conjunctivitis are limited. Additionally, because only the patients which are symptomatic and without medications enrolled into this study, the number of the partipiciants is limited.

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

REFERENCES

1. Tomaç N. [Allergic conjunctivitis]. Turkiye Klinikleri J Med Sci 2004;24(4):396-410.
2. Groneberg DA, Bielory L, Fischer A, Bonini S, Wahn U. Animal models of allergic and inflammatory conjunctivitis. Allergy 2003;58(11): 1101-13.
3. Friedlaender MH, Sweet J. Conjunctival provocation tests and naturally occurring allergic conjunctivitis in clinical trials. Int Ophthalmol Clin 1988;28(4):338-9.
4. Fujishima H, Saito I, Takeuchi T, Shinozaki N, Tsubota K. Measurement of interleukin-4 and histamine in superficial cells of conjunctiva in patients with allergic conjunctivitis. Curr Eye Res 1996;15(2):209-13.
5. Fujishima H, Sahashi N, Shimazaki J, Tsubota K. Allergic conjunctivitis caused by sugi (Cryptomeria japonica D. Don) pollen out of season. Asian Pac J Allergy Immunol 1995;13 (2):113-7.
6. Friedlaender MH. Conjunctivitis of allergic origin: clinical presentation and differential diagnosis. Surv Ophthalmol 1993;(38 Suppl):105-14.
7. Fujishima H, Okamoto Y, Saito I, Tsubota K. Respiratory syncytial virus and allergic conjunctivitis. J Allergy Clin Immunol 1995;95(3): 663-7.
8. Otero M, Lago R, Lago F, Casanueva FF, Dieguez C, Gómez-Reino JJ, et al. Leptin, from fat to inflammation: old questions and new insights. FEBS Lett 2005;579(2):295-301.
9. Conus S, Bruno A, Simon HU. Leptin is an eosinophil survival factor. J Allergy Clin Immunol 2005;116(6):1228-34.
10. Guler N, Kirerleri E, Ones U, Tamay Z, Salmayenli N, Darendeliler F. Leptin: does it have any role in childhood asthma? J Allergy Clin Immunol 2004;114(2):254-9.
11. Kimata H. Elevated serum leptin in AEDS. Allergy 2002;57(2):179.
12. Shore SA, Schwartzman IN, Mellema MS, Flynt L, Imrich A, Johnston RA. Effect of leptin on allergic airway responses in mice. J Allergy Clin Immunol 2005;115(1):103-9.
13. Wong CK, Lam CW. Clinical applications of cytokine assays. Adv Clin Chem 2003;37:1-46.
14. Lukacs NW, Tekkanat KK. Role of chemokines in asthmatic airway inflammation. Immunol Rev 2000;177:21-30.
15. Bastard JP, Jardel C, Delattre J, Hainque B, Bruckert E, Oberlin F. Evidence for a link between adipose tissue interleukin-6 content and serum C-reactive protein concentrations in obese subjects. Circulation 1999;99(16):2221-2.
16. Raso GM, Pacilio M, Esposito E, Coppola A, Di Carlo R, Meli R. Leptin potentiates IFN-gamma-induced expression of nitric oxide synthase and cyclo-oxygenase-2 in murine macrophage J774A.1. Br J Pharmacol 2002;137(6):799-804.
17. Marguet C, Dean TP, Warner JO. Soluble intercellular adhesion molecule-1 (sICAM-1) and interferon-gamma in bronchoalveolar lavage fluid from children with airway diseases. Am J Respir Crit Care Med 2000;162(3 Pt 1):1016-22.
18. Wong CK, Cheung PF, Lam CW. Leptin-mediated cytokine release and migration of eosinophils: implications for immunopathophysiology of allergic inflammation. Eur J Immunol 2007;37(8):2337-48.
19. Gabay C, Dreyer M, Pellegrinelli N, Chicheportiche R, Meier CA. Leptin directly induces the secretion of interleukin 1 receptor antagonist in human monocytes. J Clin Endocrinol Metab 2001;86(2):783-91.
20. Church MK, Okayama Y, Bradding P. The role of the mast cell in acute and chronic allergic inflammation. Ann N Y Acad Sci 1994;725:13-21.
21. Grunfeld C, Zhao C, Fuller J, Pollack A, Moser A, Friedman J, et al. Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters. J Clin Invest 1996;97(9):2152-7.
22. Sarraf P, Frederich RC, Turner EM, Ma G, Jaskowiak NT, Rivet DJ 3rd, et al. Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. J Exp Med 1997;185(1):171-5.
23. Arnalich F, López J, Codoceo R, Jim nez M, Madero R, Montiel C. Relationship of plasma leptin to plasma cytokines and human survivalin sepsis and septic shock. J Infect Dis 1999;180(3):908-11.
24. Carlson GL, Saeed M, Little RA, Irving MH. Serum leptin concentrations and their relation to metabolic abnormalities in human sepsis. Am J Physiol 1999;276(4 Pt 1):E658-62.
25. Anders HJ, Rihl M, Heufelder A, Loch O, Schattenkirchner M. Leptin serum levels are not correlated with disease activity in patients with rheumatoid arthritis. Metabolism 1999;48 (6):745-8.
26. Hoppin AG, Kaplan LM, Zurakowski D, Leichtner AM, Bousvaros A. Serum leptin in children and young adults with inflammatory bowel disease. J Pediatr Gastroenterol Nutr 1998;26(5):500-5.

27. Takabatake N, Nakamura H, Abe S, Hino T, Saito H, Yuki H, et al. Circulating leptin in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1999;159(4 Pt 1):1215-9.

28. Schols AM, Creutzberg EC, Buurman WA, Campfield LA, Saris WH, Wouters EF. Plasma leptin is related to proinflammatory status and dietary intake in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1999;160(4):1220-6.

29. Mai XM, Böttcher MF, Leijon I. Leptin and asthma in overweight children at 12 years of age. Pediatr Allergy Immunol 2004;15(6):523-30.

30. Unal M, Eskandari G, Muşlu N, Pata YS, Akbaş Y. Serum leptin levels in patients with allergic rhinitis. Otolaryngol Head Neck Surg 2006;134(2):331-3.