Comparison of eosinophilic cationic protein, adiponectin and leptin levels in patients with allergic rhinitis and local allergic rhinitis

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Abstract
Aim: Local allergic rhinitis (LAR) is a type of rhinitis with unclear treatment and unknown incidence and mechanism, which is characterized by perennial rhinitis complaints. A nasal provocation test (NPT) is recommended in the diagnosis of LAR. Eosinophil cationic protein (ECP), leptin, and adiponectin tests are markers reported to be used in the diagnosis and treatment of many allergic diseases. In this study, we aimed to investigate whether there is a difference in serum adiponectin, leptin, and ECP levels between patients with allergic rhinitis (AR) and LAR.

Material and Methods: Adult patients aged 18-65 years, who had been complaining of rhinitis for at least two years, had perennial rhinitis complaints were included in the study.

Results: NPT was positive in 30% of the patients; 30% (9 patients) of the patients who had non-allergic rhinitis and underwent NPT were found to have a positive nasal provocation test. A significant difference was determined between the groups in terms of ECP and adiponectin levels (p: 0.001 and p: 0.007). There was a significant difference between the patients with AR and NPT (-) LAR in terms of ECP levels (p: 0.001). Furthermore, a significant difference was determined between patients with NPT (+) LAR and NPT (-) LAR and patients with NPT (+) LAR and the control group in terms of ECP levels (p: 0.001 and p: 0.001).

Discussion: Although ECP and adiponectin levels differ between patient groups, it is obvious that larger studies are needed to evaluate the correlation of these parameters with the severity of rhinitis in patients with rhinitis.

Keywords
Local Allergic Rhinitis; Leptin; Adiponectin; Nasal Provocation Test; Eosinophil Cationic Protein
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Introduction
Local allergic rhinitis (LAR) is a type of rhinitis with unclear treatment and unknown incidence and mechanism, which is characterized by perennial rhinitis complaints. LAR is included in the non-allergic rhinitis group and is diagnosed with the exclusion of other causes of rhinitis. It is thought to result from nasal local immunoglobulin (Ig) E (entopy) response, despite similar symptoms to AR and negative allergy skin tests and/or negative allergen-specific IgE levels in the blood [1].

In patients with suspected LAR, a nasal provocation test (NPT) is recommended to be beneficial for diagnosis. NPT is a method demonstrating the function and immune response of the nasal mucosa, and provocation tests can be performed using allergens, lysine-aspirin, and nonspecific stimulants (methacholine, capsaicin, cold air, etc.). NPT is recommended in the diagnosis of LAR; however, this method is difficult to apply in routine practice and, thus, studies on markers, which may be beneficial in the diagnosis of LAR, are easy to apply and can be recommended for every patient, continue. Nasal eosinophil cationic protein, nasal specific IgE, basophil activation tests are markers reported to be used in the diagnosis and treatment of many allergic diseases [2]. Adiponectin and leptin are protein-nature hormones released by adipocytes [3]. There are studies suggesting that serum leptin and adiponectin levels are correlated with the severity of allergic rhinitis and may be used in the determination of the severity of AR [4]. Eosinophil cationic protein (ECP) level is an indicator of eosinophil inflammation. In many studies, ECP was reported to be higher in patients with AR compared to the control group [5]. However, there is no sufficient literature data on the use of leptin, adiponectin, and eosinophil cationic protein, which have been studied in allergic rhinitis and were found to be correlated with disease severity, in local allergic rhinitis.

In this study, we aimed to investigate whether there is a difference in serum adiponectin, leptin, and ECP levels not only between patients with AR and LAR, but also between patients with NPT (+) LAR and NPT (-) LAR, and to demonstrate a potential correlation between these parameters and severity of rhinitis.

Material and Methods
Adult patients aged 18-65 years who admitted to Cumhuriyet University Faculty of Medicine Outpatient Clinic of Immunological and Allergic Diseases, had been complaining of rhinitis for at least two years, have had perennial rhinitis complaints, whose rhinitis complaints emerge in domestic and dusty environments, who had not received treatment for rhinitis in the last six weeks were included in the study. The patients with a history of anaphylaxis with any of the allergens to be used, history of oral/oropharyngeal angioedema, and a history of taking medicines that may exacerbate lower respiratory tract complaints (ACE inhibitors, β-blockers, etc.) or influence test results (antihistamines, allergen-specific immunotherapy, etc.) were excluded from the study.

The patients were divided into three groups based upon allergy skin test results: allergic rhinitis (AR), non-allergic rhinitis (NAR), and control group. The patients included in the non-allergic rhinitis group were further grouped based upon nasal provocation test results: NPT (+) LAR and NPT (-) LAR.

Each case was subject to an allergy skin test with standardized inhalant allergens (ALK, Madrid, Spain). Skin prick test was performed using general inhalant allergens, house dust mite (Dermatophagoides (D) farinae, D. pteronyssinus), cat (Felis domesticus), dog (Canis familiaris), cockroach (Blattella germanica), fungi (Alternaria, Cladosporium, Aspergillus), and pollen mixtures (tree, weed, grass). It was carried out in all patients in which systemic atopy could not be demonstrated with skin prick tests and specific IgE.

The patients were asked to evaluate their rhinitis symptoms quantitatively and to display their responses to the question “how much does your rhinitis complaints bother you?” on a 0-10-cm scale. As reported in the literature, <5 cm values on the scale were evaluated as mild rhinitis and ≥6 cm values as moderate-severe rhinitis [6].

The nasal provocation test (NPT) was performed for qualitative measurement of nasal reactivity against an allergen. Thusly, a mixture of house dust mite was selected as a suspected allergen in patients who have complained of perennial rhinitis in domestic and dusty environments. NaCl (0.9%) was used as a control step. In addition, a nasal allergen provocation was performed with a mixture of house mite dust (D. pteronyssinus, ALK, Madrid, Spain). Dusty allergens were diluted to 1 BU/mL, 2 BU/mL, 4 BU/mL, and 10 BU/mL. The house dust mite solution was applied into two nostrils with a metered-dose pump spray by spraying upwards and towards the concha.

Firstly, a non-allergenic and neutral pH solution was applied in order to determine the response to nonspecific stimulants. When no symptoms developed, a nasal provocation test with an allergen was performed. Following each allergen application, symptom scoring and visual analog scale were filled. The nasal provocation test was completed in a total of 75 minutes at 15-minute intervals using 0.9% NaCl and allergens (1, 2, 4, 10 BU/mL).

Serum ECP levels were measured using Unicap ECP FEIA kits (Pharmacia & Upjohn Diagnostics AB Uppsala, Sweden) with a CAP System on an automated device.

Leptin (Leptin sandwich DRG, DRG Instruments, Marburg, Germany) was measured quantitatively using the sandwich enzyme immunoassay technique and expressed as nanogram/milliliter.

Serum adiponectin (Assay Max Human Adiponectin ELISA Kit, Missouri USA) levels were measured using "The enzyme-linked immunosorbent assay (ELISA)" method and expressed as microgram/milliliter.

The study was carried out with financial support granted by the Scientific Research Unit following the decision of the ethics committee (Decision No: 2010/99) dated 07.07.2010 (CUBAP Number: T-459). Written informed consent was obtained from each patient.

Statistical evaluation was performed using the SPSS (Statistical Package for the Social Sciences)-16 program. Normally distributed parameters were presented as mean ± standard deviation, and data that were not normally distributed were expressed as median (minimum-maximum). Nominal (unclassified) variables between the AR, NAR, and control groups were analyzed using the x2 independence test. The normality
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of numerical data was firstly evaluated with the Shapiro-Wilk test. Variance analysis was performed using one-way ANOVA for normally distributed data and Kruskal-Wallis test for non-normally distributed data. For inter-group comparison, post hoc analysis was performed with the Tukey-ANOVA test. Changes in symptom scores of the groups were compared with the Kruuskal-Wallis test, as they exhibited abnormal distribution. For nonparametric data, the Kruskal-Wallis independent samples test was used for multiple comparisons, and the Mann-Whitney U independent samples test was used for paired comparisons. For parametric data, the Levene test was used for multiple comparisons, and the Student T-test was used for paired comparisons. A p<0.05 was considered statistically significant.

Results
A total of 90 cases, 30 patients with allergic rhinitis (patients with sensitivity to D. pteronyssinus and/or D. farinae detected in the skin test), 30 patients with non-allergic rhinitis (patients without sensitivity to D. pteronyssinus and/or D. farinae detected in skin prick and intradermal tests). Thirty cases (33.3%) were male and 60 (67.7%) were females (Figure 1) (Table 1).

There was no difference between patients in the AR and NAR groups in terms of duration of rhinitis and the severity of rhinitis symptoms (p: 0.688). When rhinitis was classified in both groups according to the ARIA (Allergic Rhinitis and its Impact on Asthma) classification, frequencies of all four groups of rhinitis severity were observed to be similar (p: 0.460 and p: 0.220, respectively).

NPT was positive in 30% of the patients; 30% (9 patients) of the patients who had non-allergic rhinitis and underwent nasal provocation test with D. pteronyssinus antigen were found to

Table 3. Post hoc analysis of the groups for adiponectin, ECP and leptin levels

![Figure 1. Demographic characteristics of the study population](image1)

![Figure 2. Correlation of visual analogue scale with adiponectin, leptin and ECP in patients with local allergic rhinitis](image2)
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have a positive nasal provocation test. The mean ECP, leptin, and adiponectin levels in patients in all 4 groups included in the study are summarized in Table 2. A significant difference was determined between the groups in terms of ECP and adiponectin levels (p: 0.001 and p: 0.007, respectively). There was no significant difference in terms of leptin levels (p: 0.142).

As a result of post hoc analyses, a significant difference was determined between the patients with allergic rhinitis and the control group in terms of ECP levels (p: 0.001). There was a significant difference between the patients with allergic rhinitis and NPT (-) LAR in terms of ECP levels (p: 0.001). Furthermore, a significant difference was determined between patients with NPT (+) LAR and NPT (-) LAR, and patients with NPT (+) LAR and the control group in terms of ECP levels (p: 0.001 and p: 0.001, respectively). There was no significant difference between the patients with allergic rhinitis and NPT (+) LAR in terms of ECP levels (p: 0.540). It was shown that there was no significant difference between patients with NPT (-) LAR and the control group in terms of ECP levels (p: 0.937) (Table 2).

As a result of post hoc analyses, a significant difference was determined between patients with allergic rhinitis and the control group in terms of adiponectin levels (p: 0.005). It was demonstrated that there was no significant difference between the patients with allergic rhinitis and NPT (+) LAR (p: 0.740), patients with allergic rhinitis and NPT (-) LAR (p: 0.185), patients with NPT (+) LAR and NPT (-) LAR (p: 0.963), and patients with NPT (-) LAR and the control group (p: 0.618) in terms of adiponectin levels (Table 3).

When the severity of rhinitis and serum ECP levels were evaluated in the LAR group, no significant association between the severity of rhinitis and serum ECP levels was determined in the NPT (+) and NPT (-) LAR groups (r: 0.283, p: 0.460 and r: 0.135, p: 0.230, respectively).

When the severity of rhinitis and serum leptin levels were evaluated in the LAR groups, no significant association between the severity of rhinitis and serum leptin levels was determined in the NPT (+) and NPT (-) LAR groups (r: 0.025, p: 0.949 and r: 0.322, p: 0.155, respectively).

When the severity of rhinitis and serum adiponectin levels were evaluated in the AR and LAR groups, no significant association was determined (r: -0.095, p: 0.808 and r: -0.274, p: 0.230, respectively) (Figure 2).

Discussion

In this study, we investigated whether there is a difference between AR, LAR, and the control groups in terms of ECP, leptin, and adiponectin levels, and the correlation of these parameters with the severity of rhinitis. ECP was found to be increased in both patients with allergic rhinitis and NPT (+) local allergic rhinitis compared to the control group. Furthermore, ECP was shown to be increased in patients with NPT (+) LAR compared to the patients with NPT (-) LAR. Adiponectin levels were determined to be significantly higher in patients with AR compared to the control group. No correlation was determined between all 3 parameters and the severity of rhinitis.

In our study, early allergic responses that developed within the first one hour after the nasal provocation test were evaluated. After exposure to an allergen or provocation with allergens, an early response develops due to mast cell, and a late response develops due to IL-13, and neurogenic pathway leads to only early response. In the literature, 30-62.5% of positivity after provocation with tree or grass pollen, 54% of positivity after provocation with house dust mite were reported in patients with local allergic rhinitis, whereas positivity after provocation with any allergen was reported to be 62% [7]. In our study, the NPT response, determined by the total symptom score and VAS was found to be positive in 30% in the local allergic rhinitis group.

It has been suggested that ECP may lead to the development of local allergy via some mechanisms, including the induction of mast cell granulation, the involvement of lymphocytes, and the stimulation of tissue "remodeling". Bellussi et al [8] reported that serum ECP levels were higher in patients with NPT-positive non-allergic rhinitis. It was also suggested that ECP and IgE measurement in nasal lavage fluid of patients with non-allergic rhinitis and rhinosinusitis is a useful marker for determination of entopy [9]. Tomassini et al. [10] reported that serum ECP levels significantly increased in patients with AR during the pollen season. However, Rondon et al. could not find a significant difference between patients with persistent rhinitis with positive NPT with Dermatophagoides pteronyssinus antigen and patients with NPT- negative persistent rhinitis in terms of serum ECP levels [11]. ECP levels in nasal lavage fluid were significantly higher in both groups compared to the control group, whereas serum ECP levels were higher in patients with persistent allergic rhinitis, although similar to the NAR group [12]. As a result of our study, similar to Rondon et al., we did not determine a significant difference between patients with NPT (+) and patients NPT (-) local allergic rhinitis in terms of ECP levels.

Sin et al. [13] reported that serum ECP levels were associated with the severity of rhinitis. Especially when serum IgE, ECP and eosinophilia are evaluated together, it is emphasized that this association is more significant. In patients with suspected AR with high serum ECP levels, although it is emphasized that other clinical and laboratory examinations should be performed, low serum ECP levels alone do not exclude entopy [14]. However, in our study, due to the relatively low number of patients with NPT (+) local allergic rhinitis and, probably, the asymptomatic course of patients in our study, we could not determine such a correlation in our study.

Leptin has been shown to play a role in both innate and adaptive immunities and allergic sensitization. Ciprandi et al. [15] found that outside pollen season serum leptin levels in patients with seasonal AR were similar to healthy individuals. When serum leptin levels of the same patients were evaluated during pollen season, they determined higher leptin levels compared to both the values outside pollen season and healthy controls. On the contrary, in a study by Erel et al. [16], in which they compared serum leptin levels of 43 patients with AR and healthy controls, no difference could be demonstrated between both groups. In our study, no significant difference was determined between all 4 groups in terms of serum leptin levels. The fact that some patients were evaluated during the asymptomatic period was thought to cause a lack of difference.
In other studies, evaluating the association between severity of airway disease and leptin, it was suggested that there was a positive association between severity of asthma and serum leptin levels in both adults and children, and leptin was suggested to play a role as an inflammatory mediator [17]. However, there are also studies revealing no correlation between the severity of symptoms and serum leptin levels in patients with seasonal allergic rhinitis [18].

In a study by Hsueh et al. [19], conducted with 97 pediatric patients with AR, a positive correlation was shown between serum leptin levels and severity of allergic rhinitis, and it was suggested that leptin may have a predictive value in predicting the severity of allergic rhinitis. In contrast to these aforementioned studies, such a correlation could not be shown in our study, probably as our study included an adult age group. Adiponectin plays a regulatory role not only in glucose and energy metabolism, but also in the immune system and allergic inflammation. It is well known that adiponectin is associated with sensitization and its levels are influenced by allergic diseases [20]. Ciprandi et al. [21] reported higher serum adiponectin levels in patients with seasonal allergic rhinitis compared to those outside pollen seasons. In another study conducted with 103 patients with seasonal allergic rhinitis, in the same group, serum adiponectin levels were significantly higher compared to the control group [22]. In another study, although higher serum adiponectin levels were found in 41 patients with pollen-sensitive allergic rhinitis compared to the control group, this difference was reported to be insignificant [23]. Although the difference was determined between the patients with AR and the control group in terms of adiponectin levels, this situation was not detected in patients with local allergic rhinitis. These results suggest the idea that serum adiponectin has pro-inflammatory effects, and its levels increase in allergic diseases, although examination and measurements of cytokines that are the regulator of expressions of genes related particularly to body weight and human serum adiponectin levels are needed for corroboration of this assumption.

Hsueh et al. [24] showed a negative correlation between serum adiponectin levels and the severity of allergic rhinitis. Ciprandi et al., [21] however, did not determine any correlation between the severity of rhinitis and serum adiponectin levels in patients with seasonal allergic rhinitis. Similarly, in our study, no correlation was observed between the severity of allergic rhinitis and adiponectin levels.

In our study, responses that developed within the first one hour after the nasal provocation test were considered to be the early responses. The first limitation of our study is the inability to perform NPT with other indoor allergens that play a role in perennial rhinitis in patients. Nevertheless, the incidence of LAR due to isolated sensitivity to other allergens is lower compared to local sensitivity to Dermatophagoides pteronyssinus (sensitivity to Dermatophagoides pteronyssinus 60%, Alternaria alternata 17.3%, cat dander 2.7%) [25]. In addition, among other limitations, there is a small population and the inclusion of only one center.

**Conclusion**

In conclusion, our study is important due to the very limited number of studies investigating serum ECP, leptin, and adiponectin levels in patients with AR, NPT (+), and NPT (-) LAR. Although ECP and adiponectin levels differ between patient groups, it is obvious that larger studies are needed in order to evaluate the correlation of these parameters with the severity of rhinitis in patients with rhinitis.
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2007 Nov-Dec;35(6):232-8. doi: 10.1157/13112988. PubMed PMID: 18047813.

17. Wen Y, Zhou L, Li Y, et al. Role of leptin in allergic rhinitis during sublingual immunotherapy. European archives of ot-rhino-laryngology : official journal of the European Federation of Otology-Rhinology-Laryngology Societies (EUFOS) : affiliated with the German Society for Otology-Rhinology-Laryngology - Head and Neck Surgery. 2018 Nov;275(11):2733-2738. doi: 10.1007/s00405-018-5123-0. PubMed PMID: 30218387; eng.

18. Ciprandi G, De Amici M, Tosca MA, et al. Serum leptin levels depend on allergen exposure in patients with seasonal allergic rhinitis. Immunol Invest. 2009;38(8):681-9. doi: 10.3109/08820130903107965. PubMed PMID: 19860581.

19. Jarti T, Saarikoski L, Jarti T, et al. Obesity, adipokines and asthma. Allergy. 2009 May;64(5):770-7. doi: 10.1111/j.1398-9995.2008.01872.x. PubMed PMID: 19210351.

20. Chwalba A, Machura E, Ziora K, et al. The role of adipokines in the pathogenesis and course of selected respiratory diseases. Endokrynologia Polska. 2019;70(6):504-510. doi: 10.5603/EPa2019.0051. PubMed PMID: 31891413; eng.

21. Ciprandi G, De Amici M, Tosca M, et al. Serum adiponectin levels in patients with seasonal allergic rhinitis. Int Immunopharmacol. 2010 May;10(5):635-8. doi: 10.1016/j.intimp.2010.02.008. PubMed PMID: 20188664.

22. Ciprandi G, Filaci G, Negriini S, et al. Serum leptin levels in patients with pollen-induced allergic rhinitis. Int Arch Allergy Immunol. 2009;148(3):211-8. doi: 10.1159/000161581. PubMed PMID: 18849612.

23. Ciprandi G, Murdaca G, Marseglia G, et al. Serum adiponectin levels in patients with pollen-induced allergic rhinitis. Int Immunopharmacol. 2008 Jun;8(6):945-9. doi: 10.1016/j.intimp.2008.02.004. PubMed PMID: 18442902.

24. Hsu KC, Lin YJ, Lin HC, et al. Serum leptin and adiponectin levels correlate with severity of allergic rhinitis. Pediatr Allergy Immunol. 2010 Feb;21(1 Pt 2):e155-9. doi: 10.1111/j.1399-3038.2009.00878.x. PubMed PMID: 19725899.

25. Rondon C, Campo P, Galindo L, et al. Prevalence and clinical relevance of local allergic rhinitis. Allergy. 2012 Oct;67(10):1282-8. doi: 10.1111/all.12002. PubMed PMID: 22913574.

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