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RESEARCH

# THE USE OF SERUM EOSINOPHILIC CATIONIC PROTEIN IN THE DIAGNOSIS OF ALLERGIC RHINITIS

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**SUMMARY**

Objective: Early diagnosis and treatment are very important in patients with allergic rhinitis (AR) in order to provide them a higher life quality and to stop the progress of their illness. We have tested whether the high levels of serum eosinophilic cationic protein (ECP) concentrations can distinguish between patients with AR and those with healthy controls. The relationship between other serum inflammation markers was also evaluated. Materials and Methods: In our study, serum ECP concentrations of 49 patients with AR were measured and compared with control group including 26 healthy people. Every subject underwent the skin prick test, serum total eosinophil count, phadiotop, total IgE and specific IgE level measurements. Serum ECP levels were measured by the commercially available immunoassay technique (Pharmacia CAP). Results: Studies have indicated that; serum ECP levels in patients with AR are higher than in healthy human. (Mean ECP level was 12.75µg/l in patient group and 6.62µg/l in control group). The sensitivity of serum ECP was found as % 81.6 and specificity was found as % 57.6. Conclusion: From this observational study it is concluded that serum ECP might be used as a marker which can easily be measured in a clinical setting in the diagnosis of AR when it is used together with skin prick tests. Although it could diagnose AR with a high sensitivity, additional tests will be needed for exact diagnosis.

*Keywords: Allergic rhinitis, eosinophilic cationic protein*

**ALLERJİK RİNİT TANISINDA SERUM EOZİNOFİLİK KATYONİK PROTEİNİN KULLANIMI**

**ÖZET**

Amaç: Allerjik rinitin erken tanı ve tedavisi, bu hastalara iyi bir yaşam kalitesi sunabilmek ve hastalığın ilerlemesini önleyebilmek açısından çok önemlidir. Bu çalışma, bir inflamasyon belirteci olan serum eozinofilik katyonik protein (ECP) ölçümünün allerjik rinit hastalığında tanısal değerinin olup olmadığının ve diğer in vitro ve in vivo testlere üstünlüğünün olup olmadığının ortaya konması amacıyla yapılmıştır. Hastalar ve Yöntemler: Çalışma, 49 allerjik rinitli hasta ve 26 sağlıklı kişi üzerinde vaka-kontrol karşılaştırması şeklinde yapılmıştır. Atopik hastalık tanısı için; deri testleri, serum total eozinofil sayımı, total IgE, spesifik IgE ve phadiotop ölçümleri yapılmıştır. ECP değerleri ölçümleri Pharmacia CAP sistemi kullanılarak yapılmıştır. Bulgular: Allerjik rinitli hastalar normal sağlıklı kişilere oranla yüksek serum ECP değerleri göstermişlerdir. Ortalama ECP değeri hasta grubunda12.75µg/l, control grubunda 6.62µg/l olarak bulunmuştur. Serum ECP ölçümünün duyarlılığı ve özgüllüğü sırayla %81.6 ve %57.6 bulunmuştur. Sonuç: Serum ECP değerleri ölçümü, deri testleri ile birlikte değerlendirildiğinde, allerjik hastalığın tanısını koymada kolay ve ucuz bir yöntem olarak kullanılabilir. Tek başına kullanıldığında yüksek sensitivite ile tanı koydursa da kesin tanı için ek testlere başvurulmalıdır.

*Anahtar Sözcükler: Allerjik rinit, eozinofilik katyonik protein*

## INTRODUCTION

AR is IgE mediated type-I hypersensitivity reaction of the nasal mucosa that involves 10-25% of the general population. Classical symptoms are nasal obstruction, watery rhinorrhea, sneezing and itching at the nose, palate and nasopharyngeal region. Although it does not threaten life, early diagnosis and proper management is essential, because of its serious complications and school and workday losses[1-2].

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Most of the known diagnostic methods for atopy and AR are not highly sensitive and specific. This indicates the need to improve new diagnosis techniques. Serum ECP measurement is a feasible and practical method that is non-traumatic and resulting in a short time with a great sensitivity.

## MATERIAL and METHODS

Patients with history of atopy and complaints of rhinitis who were admitted to ear-nose-throat (ENT) and allergy clinics were evaluated. Forty-nine consecutive patients that were found allergic according to results of in vitro and in vivo tests and those patients that were diagnosed as allergic rhinitis according to their history and otorhinolaryngological examination were enrolled in the study (mean age; was 32.18; 28 women and 21 men). All patients were

informed about the study. The patients whose symptoms have been repeated at least once within 6 months and lasted at least 3 weeks, provocated by allergen exposure. Seasonal variations were accepted as positive history of atopy.

For atopy diagnosis, each subject underwent the skin tests, serum total eosinophil count, total and specific IgE measurements and phadiotop measurements. None of the patients was treated with antihistaminic, anti-inflammatory or bronchodilator drugs. In addition, none of them was treated with immunotherapy. Any type of infection including parasitic disease was excluded. Skin tests and specific IgE results accepted as reference tests. Skin prick tests (SPT) (Soluprick ALK, Denmark) were performed on the flexor aspect of forearm. The tested allergens were D.pteronyssinus, D.farinea, grass mix, tree mix, wool, cow's milk, hen's egg, cacao, dog epithelium, cat pelt and mixed feathers. Reactions were recorded after 15 minutes. SPT results were considered positive if the wheal was larger than 2mm or an area at least 25% that of a reference histamine reaction (1 mg/ml histamine chloride).

| | Case (%) ( n=49) | Control (%) ( n=26) |
|---|---|---|
| Positive ECP | 40 (81.8) | 11 (42.3) |
| Negative ECP | 9 (18.2) | 15 (57.7) |

According to $p<0.05$ $X^2=4.03$;
**Table I.** Serum ECP positivities in case and control groups**.**

The patients with positive skin test results were enrolled the study. As control group, 26 healthy non-atopic people were evaluated. None of them had a history or symptoms of atopy. The results of allergic evaluation were negative and they had the same demographical characteristics with the patient group.

ECP was selected as a marker of eosinophil activation because it is more specific for eosinophils than other frequently mentioned eosinophil derivatives. ECP in serum was determined by means of a double-antibody radio-immunoassay technique (Pharmacia CAP, Upsala, Sweden). Blood samples were saved at room temperature for an hour, and then stored at temperature of -29°C until ECP measurements have been done. Reference values for serum ECP levels were taken as 4.4-15 µg/l; because it is the most accepted reference value. Levels under 4.4 µg/l were evaluated as negative or minimal activation of eosinophils. Medium activation of eosinophils was evaluated between 4.4-15 µg/l, higher levels of activation of eosinophils was greater than 15 µg/l. The type of activation of eosinophils defines the medical therapy needed. Cut-off value is 4.4 µg/l [3-6].

The statistical program SPSS/PC+ 5.0 was used for the analysis of data. Chi-square, Fischer's exact chi-square and Mc Nemar's chi-square tests were used in comparative analysis. $p<0.05$ were considered statistically significant.

## RESULTS

Forteen (28.5%) patients had positive family history. Patients were identified as being AR by positive skin test results, together with otorhinolaryngological examination The presence of other allergic diseases together with AR are as follows: 11 of 49 patients had been diagnosed as allergic conjunctivitis, 7 as allergic dermatitis and 1 of them had been diagnosed as both allergic dermatitis and allergic conjunctivitis together. Sensitivity, specificity and correlations were determined by comparing serum ECP values with serum total eosinophil counts, total IgE and phadiotop values.

The results of the laboratory findings of the patients are as follows: Phadiotop values were all positive except 3 patients (93.6%). Total IgE values were above 200ku/l in 38 (77.5%) patients. Total eosinophil counts were above 200/mm in 39 (79.5%) patients. Nine (18.3%) patients showed minimal, 23 (46.8%) patients showed medium and 17 (34.6%) patients showed maximal activation of ECP according to reference values (4.4-15 µg/l). Serum ECP positivities in case and control groups were shown in table-1. Maximum ECP value was 39.6 µg/l and mean ECP value was 12.75µg/l in patient group. Those values were found 14.0 and 6.62µg/l in control group respectively. Forty (81.8%) patients and 11 (42.3%) healthy adults showed positive serum ECP results. Serum ECP values of patient group were found significantly higher as compared with control group ($p<0.05$). As all patients showed positive skin test and spesific IgE results; these test values were accepted as reference values.

| | Serum Phadiotop* | | Eosinophil Count** | | Total IgE*** | |
|---|---|---|---|---|---|---|
| | Positive | Negative | Positive | Negative | Positive | Negative |
| **ECP ( + )** | 37 | 3 | 35 | 5 | 32 | 8 |
| **ECP ( - )** | 9 | 0 | 4 | 5 | 6 | 3 |

* p =0.4 , ** p =0.004, *** p =0.4
**Table II.** The relationship between serum ECP positivity and serum phadiotop, eosinophil count and total IgE positivities**.**

93.8% phadiotop positivity, 77.5% total IgE positivity, 79.5% total eosinophilia positivity were obtained. The relationship between serum ECP positivity and serum phadiotop, eosinophil count and total IgE positivities in case and control groups were shown in table-2. According to these results; there was correlation between serum ECP and eosinophil count values (p=0.004). No correlation was found between serum ECP concentrations and serum phadiotop and between total IgE (p=0.4).

The sensitivity for ECP was found as 81.6% and the specificity for ECP was found as 57.6% . Negative predictivity and positive predictivity values were 62.5% and 78.4% respectively.

**DISCUSSION**

In our study, 14 (28.2%) patients had positive family history of atopy. Wang et al. reported children with bilateral family history had been prone to develop AR up to a rate of 70%, whereas children with unilateral family history may have AR with a rate of 50% [6]. 32.6% of our patients had isolated AR; in 67.4% of patients AR was associated with other types of allergic diseases. In reported studies, bronchial asthma is associated with AR between 20-30% [7,8]. In our study; blood eosinophilia was evident in 79.5% of patients. This result is significantly higher as compared with control group and is similar with literature[8,9].

Patients with AR had significantly higher phadiotop values compared with control group. Erikkson et al showed that phadiotop measurements could diagnose AR with a sensitivity up to 99.6% and the specificity up to 95%. They impressed on the importance of family history, skin tests and phadiotop measurements for diagnosis of atopic diseases[10]. In our study, high total IgE values were evident in 77.5% of patients. This shows patients with AR have significantly higher total IgE values than control group. Benson et al showed that increases in total IgE correlates weakly but positively with AR[11].

We showed serum ECP levels in patients with AR were significantly higher than control group. Recent studies show that serum ECP levels may be a predictor of atopy and it is a useful marker in diagnosing atopic diseases: Koller et al reported that eosinophil activation measured by serum ECP was present in infants with wheezing and this might indicate a predictive value of serum ECP measurements to identify those patients in whom infantile asthma was developing[12]. Takeda et al showed the effect of bronchial provocation test with house-dust mite on serum ECP, and found significantly high results[13]. In another study, 21 patients with allergic asthma had been studied and significantly high serum ECP, EDN (eosinophil derived neurotoxin), eosinophil counts and urinary EDN values had been found[14].

There are a lot of clinical studies about the value of ECP levels in management of AR, dose effectiveness during the treatment, severity of the disease and the clinical follow-up. These studies confirmed that measure of ECP in atopic patient is a simple, non-invasive and a cheap method for diagnosis and determining the severity of the disease and for monitoring of the efficiency of the treatment[15-19]. On the other hand, some investigators suggested that determination of ECP in atopic patients was not useful in diagnosing and monitoring the disease or it was a poor indicator of them[20-21]. Our findings also supported that ECP should be used with other diagnostic tests for the diagnosis of the disease. It may be used for determining the severity of the disease or monitoring the disease, but it needs further studies.

Kandil et al found significantly high serum ECP values in 20 asthmatic children and they suggested that ECP is a highly specific marker for asthma. They also impressed on ECP may be a marker of eosinophilic activity and degranulation that correlates with the severity of bronchial asthma[22]. Koller and colleagues suggested that serum ECP level in children with wheezing could identify development of the infantile asthma with the specificity 95% and the sensitivity 92.3% [12].

According to our results, serum ECP measurements may be a useful marker for predicting the AR. Other laboratory and clinical evaluations should be done to patients with positive serum ECP results in whom AR is suspected. It does not exclude the atopy in patients with negative serum ECP assessment. ECP measurements may give more relevant informations about the severity of the persistant disease, which will need further studies. ECP measurement provides physician no further testing before referring the patient to allergist, so it could be a sensitive and time-saving measure in atopy diagnosis in otolaryngology department for further evaluation and treatment of the atopy.

**CONLUSION**

Our findings demonstrate that patient history, skin tests and serum ECP measurements together can diagnose the allergic disease in a short time and by the most economical way. We suggest that ECP measurement is an appropriate method for screening atopy in allergic rhinitis patients, which would establish a good communication between

otolaryngologist and allergist. Although serum ECP mesurements could diagnose AR with a high sensitivity, additional tests will be needed for exact diagnosis.

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