Salivary Eosinophil Cationic Protein in Allergic Rhinitis

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Original Investigation

Abstract

Objective: Eosinophil cationic protein (ECP) plays a significant role in the pathogenesis of atopic diseases such as allergic rhinitis (AR) and asthma. Using saliva as a diagnostic material is a non-invasive, simple method. Analysis of ECP in saliva was shown as an alternative diagnostic contribution in patients with asthma. In this study we aimed to assess a possible association between the levels of salivary ECP and the diagnosis of AR by comparing serum ECP and salivary ECP levels.

Methods: Thirty-five allergic rhinitis patients (study group) sensitive to Dermatophagoides farinae (D2) in skin prick test (SPT) and 35 nonallergic, SPT negative, healthy volunteers (control group) were included in the study. Salivary ECP, serum ECP and specific IgE D2 levels were measured.

Results: Distribution of age and gender were similar in the study and the control groups (p>0.05). Serum specific IgE D2 levels were significantly higher in the study group compared to the control group (p<0.001). ECP levels in saliva and serum did not show any significant difference in between study and control groups (p=0.738; p=0.796, respectively). No significant difference was found between the levels of ECP in between the serum and the saliva of study and control groups. (p=0.504; p=0.589, respectively). There was no significant correlation between saliva and serum ECP levels of both groups.(r=-0.191/ p=0.114).

Conclusion: Serum and saliva ECP levels seem close to each other and were comparable in both groups, but we did not find any correlation between them Although we hypothesized that saliva ECP may be used as a non-invasive method for the diagnosis of AR, it seems that this parameter is not helpful in diagnosis of AR.

Keywords: Allergic rhinitis, eosinophil cationic protein, saliva, skin prick test, allergy

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Cite this article as: *Kırgezen T, Araz Server E, Savran Turanoğlu F, Yiğit Ö, Uzun H, Durmuş S. Salivary Eosinophil Cationic Protein Levels in Allergic Rhinitis. Turk Arch Otorhinolaryngol 2019; 57(2): 91-4.*

This study was presented at the 38th Congress of Turkish National Otorhinolaryngology Association, October 26-30, 2016, Antalya, Turkey.

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Received Date: 27.01.2019
Accepted Date: 13.05.2019

DOI: 10.5152/tao.2019.4127

Introduction

Allergic rhinitis (AR) is a disease with repetitive symptoms such as nasal obstruction, increased nasal serous secretion and nasal irritation. To determine the correlation between the symptoms and allergic rhinitis, and to get a proper diagnosis, a proper medical history should be taken, and both clinical and laboratory evaluation should be performed (1). While allergic rhinitis was classified as seasonal and perennial (throughout the year) in the past, currently it is classified as intermittent or persistent in line with the ARIA (Allergic Rhinitis and its Impact on Asthma) guidelines. Inhaled allergens are the main causes of allergic rhinitis. Perennial allergic rhinitis is caused by an IgE-mediated inflammatory response to year-round environmental aeroallergens such as dust mites, mold, animal, or certain occupational allergens (2). The major cause of perennial rhinitis is house dust mites. Dermatophagoides pteronyssinus (D1) and Dermatophagoides farinae (D2) are the most commonly seen mites in our country (3). These allergens lead to allergic rhinitis symptoms due to an IgE-mediated reaction. At the beginning of the late reaction phase, mediators and cytokines produced by the inflammatory cells, particularly by eosinophils, play the major role. Major basic protein (MBP) released from eosinophils plays an important role in the production of eosinophil cationic protein (ECP). ECP is the most well-known of these proteins and used as a marker in allergic rhinitis (4).

Eosinophil cationic protein can be measured in plasma, saliva, sputum, nasal bronchoalveolar lavage fluid, digestive tract mucosa, feces, and urine (5, 6). Saliva samples can be taken easily from children and adults alike and used for non-invasive diagnostic testing. ECP has been measured in saliva in asthmatics, and a significant correlation has been observed with the severity of the condition (7). Based on the role of ECP in the pathogenesis of allergic rhinitis, we aimed to examine the contribution of a non-invasive method, 'saliva ECP measurement', in patients positively reacting to D2 allergen, and diagnosed with perennial allergic rhinitis.

Methods

Thirty-five patients who were admitted to our clinic in the period from May 2015 to June 2016 with allergic rhinitis symptoms and diagnosed with allergic rhinitis based on nasal examination and a positive skin prick test (SPT) for D2 allergen were included in the study as the study group. The control group consisted of 35 volunteers without allergic symptoms, such as rhinitis or dermatitis, and with a negative SPT and no comorbidities such as eosinophilic rhinosinusitis or parasitic diseases that could affect ECP levels. The ethics committee approval was obtained with the decision number 653, dated May 15, 2015 (İstanbul Training and Research Hospital Review Board). All patients and volunteers included in the study provided their informed consent as per the Helsinki Declaration.

We obtained medical histories, performed physical examination and SPT. Exclusion criteria were being out of 18-65 age range, pregnancy, drug use that would affect SPT results, having upper respiratory tract infection within the last 30 days, having a structural abnormality in the upper respiratory tract or the body area used in SPT, diagnosis of asthma, smoking, and the candidate's rejection to take part in the study.

Both groups underwent detailed physical examination and their allergic symptoms and drug use histories were recorded in detail. The SPT was performed in all subjects. SPT included eight allergens: D1, D2, tree mix, weed mix, grass mix, Alternaria alternata, dog and cat dander. Following SPT, saliva was obtained for ECP measurement, and blood samples were obtained for serum specific IgE D2 measurement.

Skin prick test (SPT)

Skin prick test was applied to both groups in accordance with the guidelines of the European Academy of Allergy and Clinical Immunology. Patients were told to discontinue the use of short acting antihistamines and tricyclic antidepressants, at least two weeks prior to the SPT. The patients were also advised to discontinue their inhaled steroids, short acting systemic steroids and topical steroids for a period of at least two weeks. Patients who were subjected to immunotherapy were excluded because of the therapy's possible effect of blunting the test response.

Normal saline was used as negative control and 10 mg/mL of histamine was used as positive control. Fifteen minutes after the application, tests of patients whose positive control was greater than the negative control, and the patients who had a skin response greater than 5 mm were evaluated. While the response for D2 allergen was evaluated, a reaction larger than 3 mm compared to negative control was accepted as SPT positive. In the control group, negative result was obtained for all allergens.

Serum IgE D2, ECP and saliva ECP

Following the SPT test, 5 mL of venous blood was obtained from both the participants of study and the control groups, centrifuged (10 min/1300 rpm), and supernatants were stored at -80°C until analysis. For saliva sampling, patients were told to not eat or drink anything for 1 hour prior to the test (8). They were also asked not to brush their teeth 15 minutes prior to the test and advised to rinse their mouth with water 3 times. Saliva samples (2 mL) were collected in dry tubes and centrifuged for two minutes at 10000 x g speed. Samples were separated into smaller volumes and kept at -80°C until analysis. ECP levels were determined with a commercial labelled immune sorbent analysis kit (Cat. Nr: YHB1093Hu, YH Bioresearch Laboratory, Shanghai, China). The intra-assay and inter-assay variation coefficients of the kit were lower than 10% and 12%, respectively, and the limit of detection was found as 0.25 ng/ml. Analysis was done in accordance with the kit protocol. Optical density values were measured in 10 minutes by using microplate reader (ELX800, Bio-Tek Instruments, Inc., Vermont, USA). The serum ECP limit value of our laboratory was 24 ng/ml and serum IgE D2 limit value was taken as <0.35 kU/L.

Statistical Analysis

For statistical analysis, Statistical Package for the Social Sciences (SPSS) version 15.0 for Windows program (SPSS Inc.; Chicago, IL, USA) was used. For numeric variables, descriptive statistics were given as mean, standard deviation, minimum, maximum, and median. Since the comparison of numeric variables in two independent groups did not have a normal distribution, the Mann-Whitney U test was used. Paired t test was used when numeric variables had different normal distribution conditions in dependent groups and the Wilcoxon test was used when they did not have normal distribution conditions. The ratio of categorical variables between the groups was tested via chi square test. Statistical alpha significance level was taken as $p<0.05$. Spearman test was used for correlation analysis.

Results

The study group included 17 males and 18 females with a mean age of 26.8 years. The control group included 25 males and 10 females with a mean age of 32.5 years. Age and gender distribution of the study and the control groups were similar (p=0.052 and p=0.051, respectively).

No significant differences were found between the study and the control groups in means of saliva ECP and serum ECP levels (p=0.738 and p=0.796, respectively). There was no significant difference between serum and saliva ECP levels in both of the groups (p=0.504; p=0.589). There was no significant difference between these groups' saliva ECP and serum ECP levels (Table 1). Mean serum lgE D2 of the study group was significantly higher compared to the control group (p<0.001) (Table 2).

In the study group; there was no significant correlation between saliva and serum ECP levels (r=-0.191/ p=0.114 and; between Saliva ECP level and Serum Ig E level (r=-0.085/ p=0.484and

Table 1. Salivary and serum eosinophilic cationic protein levels between the two groups

	Study Group		Control Group		
	Mean±SD	Min-Max/Median	Mean±SD	Min-Max/Median	p
Salivary ECP	30.3±22.5	2.2-80.5/23.3	26.6±15.3	2.0-52.6/20.6	0.738
Serum ECP	34.5±26.3	8.8-115/28.3	30.3±19.1	9.24-94.2/26	0.796
p	0.504		0.589		

ECP: eosinophil cationic protein; SD: standart deviation

Table 2. Serum specific lgE D2 and eosinophilic cationic protein values of the groups

		Study Group		Control Group		
		n	%	n	%	p
Serum Ig E D2	<0.35	1	2.9	27	77.1	<0.001
	≥0.35	34	97.1	8	22.9	
Serum ECP	<24	14	40.0	16	45.7	0.629
	≥24	21	60.0	19	54.3	

ECP: eosinophil cationic protein; Ig E D2: Ig E dermatophagoides farinae

Table 3. Correlation analysis between saliva and serum eosinophilic cationic protein levels

		Serum ECP	Serum Ig E
Salivary ECP	r	-0.191	-0.085
	p	0.114	0.484
Serum ECP	r		0.148
	p		0.223

Speramna Correlation

ECP: eosinophil cationic protein; Ig E: dermatophagoides farinae (D2) Ig E

also; between Serum ECP level and Serum Ig E level (r=0.148/ p=0.223) (Table 3).

Discussion

Although specific diagnosis of allergic rhinitis cannot be made by measuring allergic markers, they may provide objective support to the diagnosis (9). Allergic rhinitis patients have been reported to have higher serum total lgE, ECP and eosinophil levels compared to nonallergic people (10). In our study, serum and saliva ECP levels were measured both in the study group (patients who were found to be allergic to D2 after physical examination, SPT and serum specific lg E D2) and in the control group (nonallergic volunteers).

Eosinophils, which are the source of ECP, are found in nasal mucosa in allergic rhinitis (11). ECP level is not specific and increases in atopic diseases, such as allergic rhinitis and recurrent wheezing, and in chronic infections like chronic rhinosinusitis (12, 13). Therefore, we excluded patients with other atopic diseases or infections from our study.

Eosinophil cationic protein exists in various body fluids: serum, plasma, nasal lavage fluid, sputum, bronchoalveolar lavage fluid and urine (5, 6). Saliva ECP concentration has been given as 250-450 μg/L (14). Naturally, it is easier to get the sample from saliva. Not many studies were conducted on the ECP levels of saliva. Correlation between serum and saliva ECP values are not yet confirmed (15).

Schmekel et al. (7) found that salivary ECP levels were higher in asthmatics than in healthy adults and decreased with increased doses of inhaled corticosteroids. In asthma patients, salivary ECP amount was found to be useful for the evaluation of the severity of disease and response to treatment (7). In our study, we aimed to investigate the possible role of salivary ECP as an alternative to serum ECP analysis in the evaluation of allergic rhinitis patients.

As expected, mean D2-specific serum lgE of allergic rhinitis patients were found significantly higher compared to healthy adults. Altough the amount of ECP in saliva and serum were measured higher in study group; we found no significant difference in these levels between the control and the study groups, and this may be associated with the effect of many factors such as circadian rhythm, age and seasonal factors on ECP levels (16). We found no significant correlation between serum and saliva ECP levels and serum IgE level in study group. Our control group consisted of 35 volunteers without allergic symptoms such as rhinitis, dermatitis, with a negative SPT, and without comorbidities (that could affect ECP levels such as eosinophilic rhinosinusitis and diseases due to parasites). It is known that this type of comorbidities may affect ECP levels. We obtained medical histories, performed examinations and SPT, but we did not request any imaging modality or any other diagnostic biochemical or microbiological laboratory tests.

Standardization is needed for the routine measurement of salivary ECP. Different results may be obtained by reducing variables in different patient and control groups and depending on the severity of the symptoms.

Eosinophil cationic protein, which was demonstrated to be a signal of bronchial asthma activity in the study of Schmekel et al. (7), was found elevated also in the oral mucosa because of the generalization of the inflammation. In our study, we ex-

cluded bronchial asthma patients and those with other allergic conditions. We found that the allergic rhinitis reaction that is relatively localized one did not cause a significant increase of ECP in saliva.It may be suggested that limited inflammation in nasal mucosa does not cause sufficient eosinophilic activation in the oral mucosa, and that ECP in the circulation does not show significant passage to oral mucosa via the blood.

Many researchers analyzed serum ECP levels as a marker of atopy in various diseases, however, investigations on the correlation between serum ECP level and AR are rare in the literature. Many authors noted an association between serum ECP and AR, whereas some others failed to show such relation (17).

Li et al. (18) found that serum ECP was higher in AR patients and correlated with the blood eosinophil count in AR patients. They also suggested that ECP might be a major protein of eosinophil in upper respiratory tract inflammation and could be a noteworthy mediator in AR pathogenesis. Serum ECP level was found positively correlated with eosinophilia in AR patients in their study (18).

In our study, ECP levels did not demonstrate significant differences in either of the groups in terms of saliva or serum. However, these levels were found to be higher in allergic rhinitis patients. In the treatment of allergic rhinitis, there are many studies demonstrating the importance of serum ECP level for the evaluation of the effect of the dose, the severity of the disease and in clinical follow-up (16). We aimed to determine the presence, hence the significance of salivary ECP in the evaluation of allergic rhinitis, to explore whether or not it can be used as a parameter (for example, in monitoring, diagnosing, following response to treatment) in allergic rhinitis. As per our findings, ECP should be supported by other test methods.

Conclusion

Serum ECP is an auxiliary laboratory constituent used for the evaluation of allergic rhinitis. Serum and saliva ECP levels were not found significantly different between the groups. Further, we found no correlation between the levels of serum and saliva ECP in total. Although we hypothesized that saliva ECP may be used as a non-invasive method for allergic rhinitis, it did not present superior properties that would contribute to the diagnosis.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of İstanbul Training and Research Hospital Institutional Review Board (Decision number: 653, Date: 15 May 2015).

Informed Consent: Written informed consent was obtained from trainers and trainees who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - T.K.; Design - T.K., E.A.S.; Supervision - Ö.Y.; Data Collection and/or Processing - F.S.T., S.D., H.U.; Analysis and/or Interpretation - T.K., E.A.S., H.U.; Literature Search - F.S.T., S.D.; Writing - T.K.; Critical Reviews - Ö.Y.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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