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Research Article

Serum Osteopontin Levels in School-Age Children with Allergic Conjunctivitis

Senay Aşık Nacaroglu[1]*, Ovgu Buke[2], Serdar Al[3] and Hikmet Tekin Nacaroglu[3]

[1]*Department of Ophthalmology, Bagcilar Training and Research Hospital, Istanbul, Turkey*

[2]*Department of Pediatrics, Bagcilar Training and Research Hospital, Istanbul, Turkey*

[3]*Department of Pediatric Allergy and Immunology, Istanbul Medipol University, Istanbul, Turkey*

***Corresponding Author:** Senay Aşık Nacaroglu, Department of Ophthalmology, Bagcilar Training and Research Hospital, Istanbul, Turkey.

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Abstract

Introduction: The pathogenesis of allergic conjunctival diseases (ACD) is not fully understood. Osteopontin (OPN) is a protein expressed during the inflammatory processes related to Th2 lymphocyte activity. It was demonstrated in previous studies that OPN plays role in asthma and response to venom immunotherapy. As far as we know, there is no other study regarding serum OPN levels in school-age patients with allergic conjunctivitis. In this study, we investigated the serum OPN levels in school-age children with ACD.

Material and Methods: Patients aged between 6 - 16 years who were admitted to Bagcilar Training and Research Hospital Pediatric Ophthalmology outpatient clinic and were diagnosed with ACD were included in the study. The children in the same age range who were admitted to the healthy child outpatient clinic of our hospital and had no symptoms of an allergic disease were included in the control group. The complete blood count parameters and OPN levels of the patients were studied.

Results: The average age of the patients with allergic conjunctivitis was 10.68±3.07 years, 64.2% of them were female, and all patients had mite sensitivity. No statistically significant difference was observed between the osteopontin levels of the patients ($p > 0.05$). No correlation was found between the IgE levels and eosinophil count and OPN levels ($p > 0.05$).

Conclusions: We think there is a need for larger scale studies including patients with chronic and severe symptoms in which clinical symptoms and more parameters are evaluated to clarify the pathophysiological role of OPN in allergic conjunctival diseases.

Keywords: *Allergic Conjunctivitis; Allergic Conjunctival Diseases; Children; Osteopontin*

Abbreviations

ACD: Allergic Conjunctival Diseases; OPN: Osteopontin; VKC: Vernal Keratoconjunctivitis

Introduction

Allergic ocular diseases are the most common cause of chronic conjunctivitis, and they are observed in approximately 15 - 20% of the population in developed countries. Allergic ocular diseases are also called “allergic conjunctivitis” since inflammation is involved in the conjunctiva, and they include a group of inflammatory ocular diseases that generally cause itching, stinging pain, redness, oedema and tearing in the eye [1]. The risk of other allergic diseases such as allergic rhinitis and asthma is increased in children with allergic conjunctivitis, and 60% of children with allergic rhinitis have eye complaints [1-6].

Allergic conjunctival diseases (ACD) are classified as simple allergic conjunctivitis (acute, seasonal and perennial allergic conjunctivitis), vernal keratoconjunctivitis, atopic keratoconjunctivitis, giant papillary conjunctivitis and contact blepharoconjunctivitis. These diseases

have different characteristics in terms of pathogenesis, clinical findings and course [7-10]. The pathogenesis of ACD has not been completely understood yet. Nevertheless, the important role that a number of cytokines play in ocular allergy has been demonstrated by recent progress in immunological studies [11].

OPN is a phosphoglycoprotein expressed by various immune and non-immune cells. It has been demonstrated that particularly B-cells, T-cells, macrophages, eosinophils, neutrophils, natural killer cells, CD11c-positive dendritic cells (DCs) and bronchial epithelium produce OPN [12]. OPN is a protein, that is expressed in the inflammatory processes related to Th2 lymphocyte activity. Additionally, OPN expression enhances Th1 and Th17 immune response [12-15]. It has previously been demonstrated that OPN plays a crucial role in allergic airway inflammation [12,13]. The upregulation of OPN expression occurs in the samples of nasal tissue obtained from asthmatic patients with chronic rhinosinusitis, and in the tear fluids of patients with allergic ocular diseases [11,14,15]. Possible candidate for OPN production, is the small number of CD3+ T cells seen in conjunctival follicles [11]. Furthermore, corneal herpes simplex infection in mice is induced by OPN [17]. Nevertheless, the role of OPN in allergic abnormalities, such as ocular allergy, remains undiscovered.

In this study, we planned to investigate the serum OPN levels in school-age children with simple allergic conjunctivitis. As far as we know, there is no other study regarding serum OPN levels in school-age patients with allergic conjunctivitis.

Materials and Methods

Patient Population

Twenty-eight Patients aged between 6 - 16 years who were admitted to Bagcilar Training and Research Hospital Pediatric Ophthalmology outpatient clinic and were diagnosed with allergic conjunctivitis were included in the study. The diagnosis of allergic conjunctivitis was made by clinical history, symptoms and physical examination findings [10]. The patients were referred to the pediatric allergy outpatient clinic to investigate their accompanying systemic allergic diseases and to perform atopy tests. The complete blood count parameters and osteopontin levels of the patients were studied before starting the medical treatment. The children in the same age range who were admitted to the healthy child outpatient clinic of our hospital and had no symptoms of an allergic disease were included in the control group. The specified blood parameters were compared with the control group.

The exclusion criteria were as follows: the presence of asthma, receiving systemic steroids within the last month, the presence of an acute/chronic infection, and having any other systemic disease, including hepatic, renal, cardiovascular diseases, diabetes mellitus, cancer, sepsis and systemic inflammatory disorders. Informed consent was taken from all patients, and approval from the Research Ethics Committee of Bagcilar Training and Research Hospital was obtained. The study was conducted in accordance with the Declaration of Helsinki. İnformed consent was obtained from all study participants and/or their parents.

Skin prick test

Skin prick tests were performed on the anterior surface of the forearm when patients were not under the influence of antihistamines. A Stallerpoint device was used for conducting skin prick tests in order to test for common aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), a mixture of grain pollens (oats, barley, wheat, corn), a mixture of grass pollens (*Lolium perenne*, *Phleum pratense*, *Dactylis glomerata*, *Poa pratensis*, *Anthoxanthum odoratum*, *Holcus lanatus*, *Festuca elatior*, *Agrostis vulgaris*, *Cynodon dactylon*, *Avena sativa*, *Avena fatua*, *Lotus Corniculatus*), a mixture of tree pollens (*Acer pseudoplatanus*, *Robinia pseudoacacia*, *Aesculus hippocastanum*, *Tilia platyphyllos*, *Platanus vulgaris*), weed-mix pollens (*Medicago sativa*, *Brassica nigra*, *Trifolium pratense*, *Urtica dioica*, *Rumex acetosa*), *Alternaria alternaria*, cockroaches (*Blattella germanica*), and cat and dog dander (Stallergenes SA, 92160 Antony, France). Histamine (10 mg/ml) and physiological saline were utilized as positive and negative references, respectively. The assessment of skin reactions was performed after 20 minutes. Wheal diameter ≥ 3 mm was considered as a positive reaction. Atopy was classified as minimum one positive reaction in the skin test.

Counting Blood Samples

For the determination of osteopontin level, peripheral venous blood samples were obtained following overnight fasting for a period of 10h, and they were centrifuged at 3000 rpm for a period of 10 minutes within the first hour of collection, and the plasma samples isolated were kept at the temperature of -80°C until the assay was performed. The concentration of serum osteopontin was studied with micro Elisa method using micro Elisa kits obtained from Affymetrix eBioscience Company. An intra-assay coefficient of variation of the ELISA system was determined to be 3 - 9%, while its inter-assay coefficient of variation was determined to be 4 - 10.2%.

Statistical analysis

The 2007 program Number Cruncher Statistical System (NCSS) (Utah, USA) was utilized for statistical analyses. In the evaluation of the data, in addition to descriptive statistical methods (mean, standard deviation), the independent t-test was used in the paired comparison of variables exhibiting normal distribution, the Mann-Whitney U test was used in the paired comparison of variables not exhibiting normal distribution, and the chi-square test was used in the comparison of qualitative data. The Pearson correlation test was used to determine the relationships of variables with each other. Evaluation of the results was performed at the significance level of $p < 0.05$.

Results

The average age of the patients with allergic conjunctivitis was 10.68 ± 3.07 years, 64.2% of them were female, and all patients had mite sensitivity. The symptoms of allergic rhinitis were present in 57.1% (n = 16) of the patients. No statistically significant difference was observed between the average age, gender distribution, birth weight, birth week, mode of delivery, smoking during pregnancy, smoking at home, the presence of atopic dermatitis, having pets at home and heating distribution of the healthy patients and the patients with allergic conjunctivitis ($p > 0.05$). The presence of atopy in the family and the presence of damp in the house were found to be statistically significant in the patients with allergic conjunctivitis ($p < 0.001$) (Table 1). Blood IgE levels and eosinophil count were significantly higher in patients with allergic conjunctivitis than healthy group ($p = 0.003$, $p<0.001$ respectively). No statistically significant difference was observed between the osteopontin levels of the patients ($p > 0.05$) (Table 2). No correlation was found between the IgE levels and eosinophil count and OPN levels ($p = 0.746$, $p = 0.20$) (Figure 1 and 2).

		Healthy Group n:52		Allergic Conjunctivitis Group n:28		p
Age		11.81 ± 3.07		10.68 ± 3.07		0.121
Gender	Female	34	65.38%	18	64.29%	0.922
	Male	18	34.62%	10	35.71%	
Birth weight		3332.9 ± 693.37		3394.6 ± 584.07		0.704
Number of siblings		2.52 ± 1.13		2.08 ± 0.72		0.088
Mode of delivery	Normal	15	30.00%	12	48.00%	0.126
	Cesarean Section	35	70.00%	13	52.00%	
Birth week	≤ 37 weeks	3	6.00%	3	12.00%	0.367
	Term	47	94.00%	22	88.00%	
Smoking during Pregnancy	Present	7	13.73%	8	32.00%	0.061
	Absent	44	86.27%	17	68.00%	
Smoking at Home	Present	33	64.71%	18	72.00%	0.525
	Absent	18	35.29%	7	28.00%	
Atopy in the family	Present	2	3.92%	11	44.00%	< 0.001
	Absent	49	96.08%	14	56.00%	
Atopic Dermatitis	Present	3	5.88%	5	20.00%	0.061
	Absent	48	94.12%	20	80.00%	
Pets at home	Present	6	11.76%	5	20.00%	0.338
	Absent	45	88.24%	20	80.00%	
Damp in the house	Present	4	7.84%	7	28.00%	0.019
	Absent	47	92.16%	18	72.00%	
Heating	Natural gas	46	92.00%	22	91.67%	0.961
	Stove	4	8.00%	2	8.33%	

Table 1: *Distribution and comparison of the patients' demographic characteristics. Independent t-test, Chi-square test, $p < 0.05$ is significant.*

	Healthy Group n:52	Allergic Conjunctivitis Group n:28	p
Hemoglobin (g/dl)	13.52 ± 1.06	13.48 ± 0.91	0.881ǂ
White Blood Cell ($\times 10^3/\mu L$)	8.73 ± 2.45	9.06 ± 2.78	0.592ǂ
Eosinophil Count ($\times 10^3/\mu L$)	148 ± 140	377 ± 272	< 0.001ǂ
IgE (IU/L)	60 ± 39	212 ± 236	0.003ǂ
Osteopontin (ng/mL)	45.69 ± 21.79	49.81 ± 27.12	0.698*

Table 2: *Comparison of the patients' laboratory parameters.*

*ǂ Independent t-test Chi-square test * Mann-Whitney U test p < 0.05 is significant.*

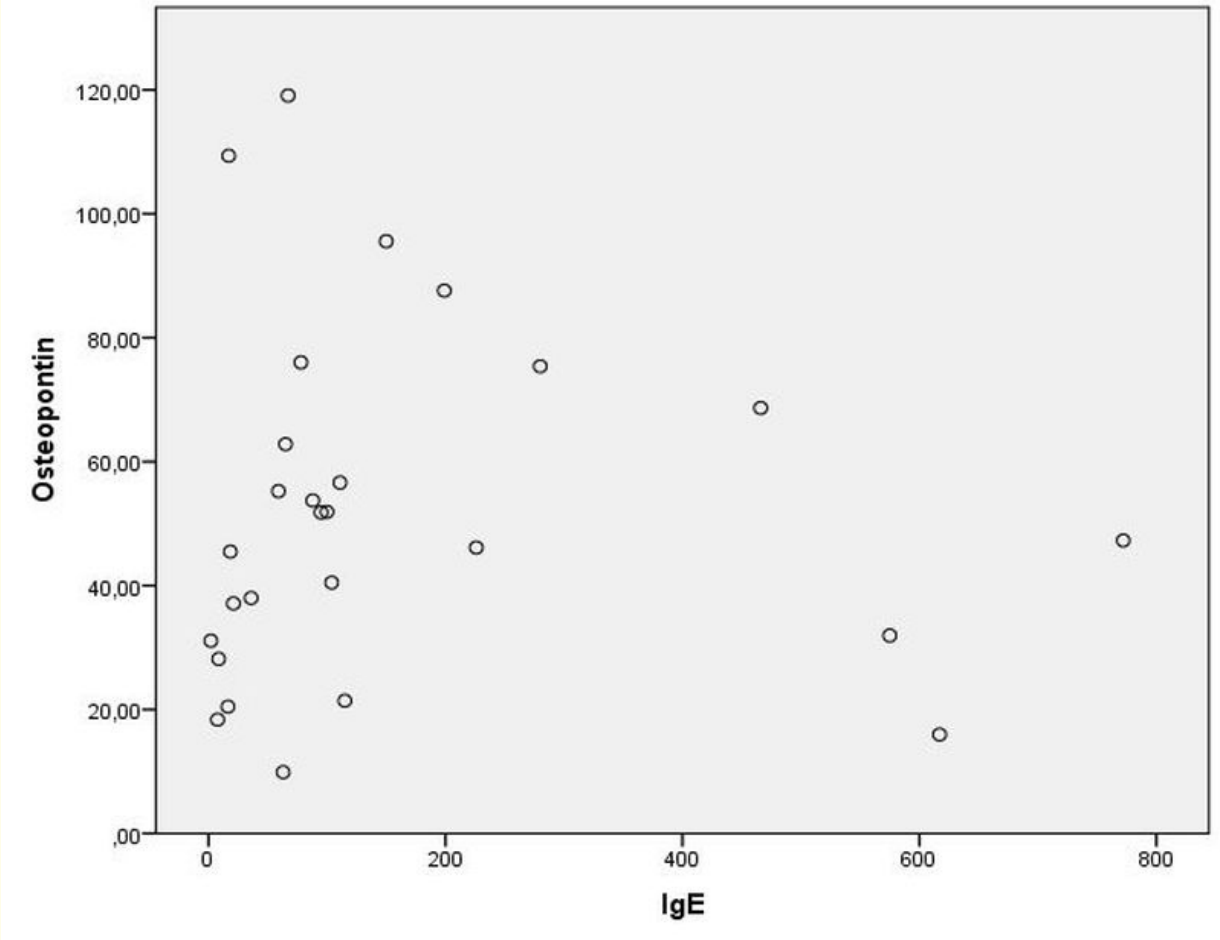

Figure 1: *Correlation graph of Serum Osteopontin and IgE levels. Coefficient of correlation r=0.065, p = 0.746.*

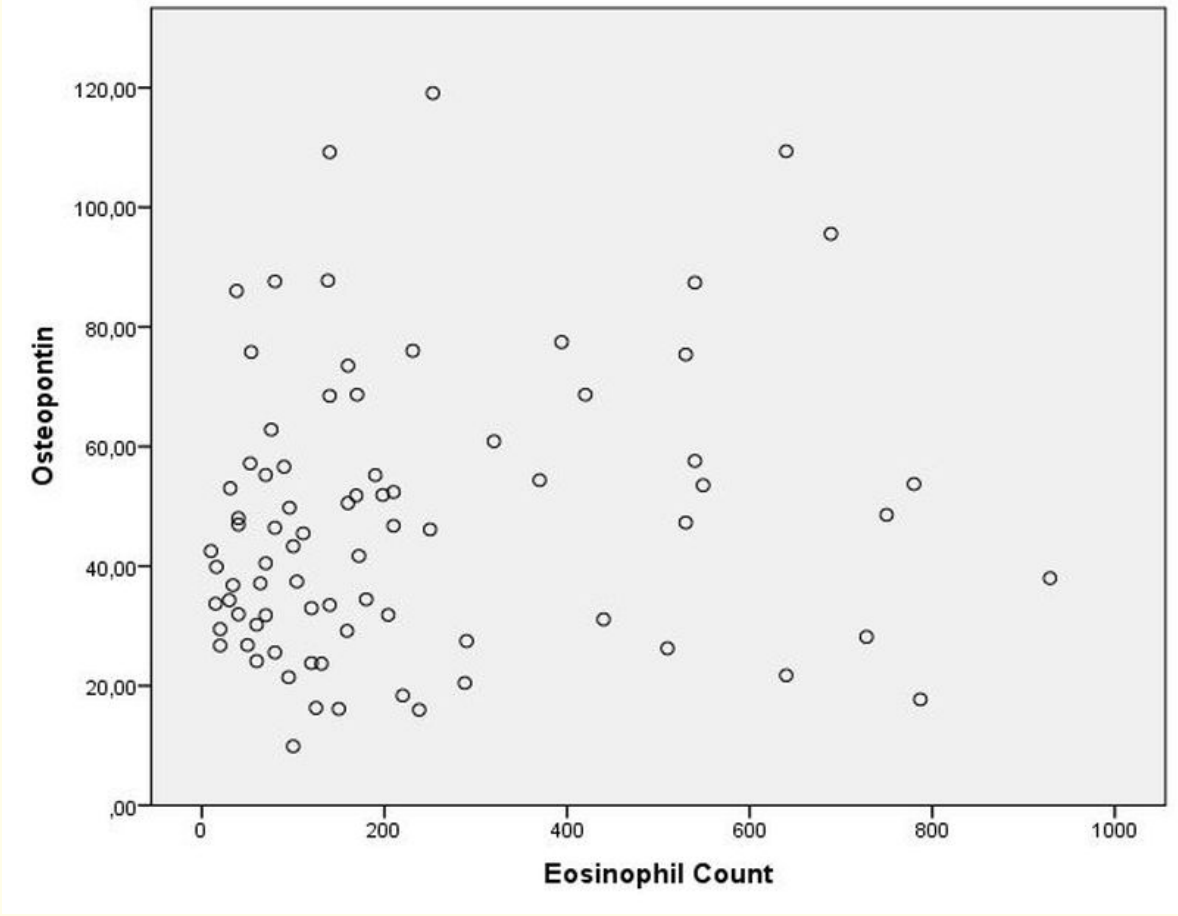

Figure 2: *Correlation graph of Serum Osteopontin level and Eosinophil Count. Coefficient of correlation r = 0.146, p = 0.200.*

Discussion

In this study evaluating the OPN levels in school-age children with simple allergic conjunctivitis, the serum levels measured in the patients were found to be similar compared to the healthy group.

The role of OPN in patients with allergic conjunctivitis in childhood is not exactly known yet. It was demonstrated in previous studies that OPN increases in diseases such as asthma in which Th2 inflammation plays a role [13-15,18-20]. Akelma., *et al.* [18] determined higher OPN levels in asthmatic children above the age of five years compared to the control group. A subgroup analysis indicated similar OPN levels between the control group and children below the age of five years. The researchers indicated the fact that expected temporary wheezing attacks were the reason for this and Th2-mediated inflammation in patients below the age of five years as a reason for the increased OPN levels. Samitas., *et al.* [13] found that the amount of OPN in serum and BAL fluid in stable adult patients was higher compared to the control group. In the same study, the researchers stated that OPN expression was correlated with the reticular basal

membrane thickness and the OPN level was associated with remodeling. High OPN levels of serum, saliva, and BAL liquid samples were determined in other studies conducted on adults with asthma [14,19]. In patients with allergic rhinitis which is another disease group in which Th2 inflammation plays a role, in the study conducted by Liu., *et al*. [14], an increase in OPN levels was determined in patients with allergic rhinitis, which was positively related with the total nasal symptom scoring, total eosinophil count, serum Eosinophil Cationic Protein (ECP), and IL-5.

Studies carried out on patients with allergic conjunctivitis are limited. In the literature, in a study in which Uchio., *et al.* [10] measured the tear OPN levels in patients with ACD, they showed that patients produced a significant amount of OPN in their tears. However, they could not clearly explain the source of produced tear OPN. They also showed that the clinical severity of ACD was significantly correlated with tear OPN levels. However, they stated that there was no significant relationship between the serum total IgE levels and tear OPN levels. Similarly, in our results, no correlation was observed between the serum OPN levels and blood eosinophil count and IgE levels.

In the previous studies, researchers reported that mast cells and OPN played an important role in severe inflammatory reaction and abnormal tissue repair afterwards, in other words, in tissue remodeling [21]. Uchio., *et al.* [11] stated that proliferative lesions such as giant papilla or corneal ulcer observed in severe cases with ACD could be regarded as tissue remodeling characterized by fibroblast and extracellular matrix formation. In the light of this information, they suggested the hypothesis that the increased tear OPN level in severe ACD cases could reflect the tissue remodeling. In our study, the serum OPN levels in children with allergic conjunctivitis were found to be similar with healthy children. This was interpreted as the fact that the patients who were included in the study were patients with simple allergic conjunctivitis and do not have chronic and severe ACD in which tissue remodeling was observed.

We consider including patients with only simple allergic conjunctivitis, and the absence of evaluation of other proinflammatory cytokines, the effect of which on conjunctival inflammation in children has been shown, and tear osteopontin levels as the limitations of the present study.

Conclusions

This is the first study investigating serum osteopontin levels in children with allergic conjunctivitis. We think there is a need for larger scale studies including patients with chronic and severe symptoms in which clinical symptoms and more parameters are evaluated to clarify the pathophysiological role of OPN in allergic conjunctival diseases. We believe that the increase in our knowledge on this issue will provide us with new biological indicators and treatment options in the near future.

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Conflict of Interest

The authors declare no conflict of interest.

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