



The Effect of Allergen Immunotherapy on Serum Periostin Levels in Children with Allergic Rhinitis

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ABSTRACT

Objective: Periostin, an extracellular matrix protein, is related to the eosinophilic airway inflammation. There is no specific marker in allergen immunotherapy to evaluate clinical response. We aimed to investigate the serum periostin levels in the children who receive allergen immunotherapy.

Materials and Methods: Sixteen patients between 8-18 years (12.7±2.8 years) with allergic rhinitis and/or asthma due to grass pollen hypersensitivity and 30 healthy subjects (11.7±2.6 years) were included. Demographic data, eosinophil counts, skin prick tests and the specific IgE levels of the patients are recorded. Symptom scores, visual analog scales, medication scores were determined and the serum periostin levels were measured in the beginning, 4th and 12th months of the allergen immunotherapy.

Results: The symptom scores for rhinitis in the 4th month showed significant improvements in all of the patients (p<0.05). Nine patients with allergic rhinitis accompanied by asthma, showed significant improvements in 12th month symptom score for asthma (p=0.018). A significant correlation was detected between the initial serum periostin levels and the symptom scores for the eye (r=0.668, p=0.005). No significant difference was found in serum periostin levels between the patient and the control groups. There were no significant differences in serum periostin levels in 4th and 12th months compared to the ones in the beginning.

Conclusion: In this study, there were no significant differences in serum periostin levels of children during the allergen immunotherapy. The association of serum periostin levels with symptom scores for the eye needs to be confirmed in more children with allergic rhinoconjunctivitis.

Keywords: Allergen immunotherapy, allergic rhinitis, asthma, serum periostin

ABBREVIATIONS: AA: Allergic asthma, AIT: Allergen immunotherapy, AR: Allergic rhinitis, AD: Atopic dermatitis, Breg: Regulatory B cells, DCs: Dendritic cells, sIgE: Allergen specific IgE, SLIT: Sublingual immunotherapy, SSA: Symptom scores for asthma, SSE: Symptom scores for eye, SSR: Symptom scores for rhinitis, Th1: Type 1 T helper, Th2: Type 2 T helper, Treg: Regulatory T cells, VAS-A: Visual analog scales for asthma, VAS-E: Visual analog scales for eye, VAS-R: Visual analog scales for rhinitis.

INTRODUCTION

Approach to treatment of allergic diseases such as allergic rhinitis and asthma involve control of allergens and triggering factors, patient education, drug therapy and immunotherapy (1). Allergen immunotherapy (AIT) is a treatment method whose effectiveness and reliability

have been proven in cases where disease symptoms persist despite drug therapy and preventive care. It has been used as the most effective treatment since the early 1900s to cure allergic asthma (AA), allergic rhinitis (AR) and atopic dermatitis (AD) associated with respiratory system allergic sensitivity as well as anaphylaxis caused by venom (1-5).

Allergen immunotherapy (AIT) is based on application of gradually increasing amounts of allergens in order to stimulate immunological mechanisms against Type 1-mediated hypersensitivity reactions. Numerous clinical tests and meta analyses have proven the efficacy of AIT (6,7). It inhibits allergic inflammation with early and late phase effects. These effects involve remission of the disease in the long-term, prevention of development of new allergen sensitizations, prevention of the disease from proceeding from allergic rhinitis to asthma, and prevention of anaphylaxis in venom allergies. It is the only method of treatment that alters the course of allergic diseases and it has been demonstrated that immune-responses have changed in favor of T helper 1 (Th 1) and regulatory T cells during the treatment (8). However, there are no routinely used markers to test clinical responses in allergen immunotherapy.

Periostin is a fibrosis-related extracellular matrix protein that was first identified in osteoblasts. It is a new biomarker that is secreted extracellularly and plays a part in the continuation of allergic inflammation and chronic Th2-mediated inflammation (9). Microarray studies have shown that it is expressed at greater rates in the epithelia of asthma patients in comparison to healthy control group patients (10). It has also been determined that periostin is expressed in bronchial epithelia of healthy people and lung fibroblasts and is induced by IL-4 and IL-13 (11). It was indicated in studies conducted on the patients resistant to steroids that periostin levels were markedly increased in patients with eosinophilic airway inflammation, which means that periostin facilitates eosinophilic inflammation. Woodruff et al. (12), investigated periostin as a clinical biomarker for the first time and demonstrated that periostin gene (POSTN) expression increased in patients with asthma and decreased following steroid treatment. Kanemitsu et al. (13) monitored 224 patients with asthma for 4 years and found that high periostin levels (>95ng/ml) were correlated with low FEV1 levels. Mansur et al. (14) showed that periostin was significantly associated with reduced lung function as a biomarker of airway remodeling. Studies have shown that periostin is the best marker compared to serum IgE, tissue eosinophilia, FeNO and blood eosinophil level in eosinophilic airway inflammation (15,16).

Although studies describing serum periostin levels as the best markers indicating allergic airway inflammation are on the increase, there are no studies showing their

use to test clinical response in allergen immunotherapy. Therefore, it has been hypothesized that periostin may play a role in monitoring AIT. The aim of this study was to determine the serum periostin levels in the children who receive allergen immunotherapy. Serum periostin levels were measured periodically in patients who received subcutaneous AIT and their correlation with clinical responses was investigated.

MATERIALS and METHODS

Study Design and Participants

16 patients aged 8-18 years who had allergic rhinitis and/or asthma (7 patients only had AR and 9 patients had AR accompanied by AA) and a control group of 30 healthy individuals were included in the study. The control group was selected among healthy children who did not suffer from an allergic disease, an active infection and immune deficiency.

Diagnosis of AR was made in accordance with the Allergic Rhinitis and its Impact on Asthma (ARIA) Guideline (17) and the diagnosis of asthma was made in accordance with the Global Initiative for Asthma (GINA) Guideline (18). Following observation for at least one year, cases with persistent and moderate-severe AR were evaluated according to European Academy of Allergy and Immunology (EAACI) criteria, written informed consents were obtained and then AIT was begun (19). Presence of heavy and uncontrolled asthma, chronic disease and immune-deficiency in patients were determined as criteria for exclusion from the study. Demographic data (age, gender) and familial history of atopic diseases were obtained from their records and the durations of symptoms and accompanying AD or symptoms of AR were recorded.

The study was approved by the University Ethical Board (2015/62). The patients and their families were informed before the study and their written consents were obtained.

Clinical Evaluation

All the patients were given a form in which to record asthma, rhinitis and eye symptoms during allergen immunotherapy. Daily symptoms (for asthma, cough, wheezing, shortness of breath; for rhinitis nasal congestion, itching, sneezing; for conjunctivitis, itching burning or stinging in the eye) were evaluated using a scoring system. In this scoring system, cases without

symptoms were scored 0, whereas mild symptoms were scored 1, moderate symptoms 2 and severe symptoms 3. Total scores for rhinitis, asthma and eye symptoms were named rhinitis, asthma and eye symptoms scores. The drugs ingested by the patients were recorded in the daily form (β_2 agonist and antihistaminic, 1 point; inhaler/nasal steroids, 2 points). The total asthma and rhinitis treatment scores were calculated by dividing them by the number of days.

The severity of AR was determined using a simultaneous visual analog scale (between 0 and 10). Symptom scores, visual analog scale, and rhinitis and asthma medication scores were recorded at baseline, and at the 4th and 12th months of AIT.

Laboratory Evaluation

The tests that were routinely conducted (percentage of eosinophils, number of eosinophils, total IgE and level of specific IgE measured using the fluoroimmunoassay method) during diagnosis were recorded from the patients' files. Specific IgE level; food products for children (cow milk, egg yolk, cod, wheat flour, peanuts and soy beans), mites, trees, grass and mold were measured quantitatively as specific IgE. Specific IgE positivity is classified between 1 and 6 and those with positivity levels of 2 and higher ($>0,7\text{kuU/ml}$) were accepted as positive.

Skin Prick Test

The sensitizations of the patients to 10 most frequent allergens were determined using the routine skin prick test. These allergens were determined to be *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Alternaria*, *Cladosporium*, *Betulaceae*, a mixture of 5 trees (alder, hazal, poplar, elm, willow), a mixture of 4 cereals (oat, wheat, barley, rye), a mixture of 6 herbs (velvet grass, orchard grass, rye grass, timothy, blue grass, meadow fescue), *Salicaceae* and latex (*Allergopharma*, Reinbek,

Germany). Histamine (10 mg/mL) and physiological saline solution were used as the positive and negative controls. The results were measured 15 minutes later. 3 mm and bigger induration diameters were accepted as positive.

Determination of Serum Periostin Levels

The sera of all the patients participating in the study were centrifuged and kept at -40°C . Periostin levels were tested using the ELISA method (Human Periostin ELISA, BioVendor-Laboratori medicina, a.s, Karasek, Czech Republic).

Statistical Analysis

Statistical analyses were conducted using SPSS (Statistical Package for Social Sciences) program (version 22, SPSS Inc., USA). $P < 0.05$ was accepted as statistically significant. Minimum, maximum, mean \pm standard deviation and median were given as descriptive statistics. Assumption of normality was checked using the Kolmogorov-Smirnov test. Student's t-test was used to test whether there was a difference between means of 2 groups. The Friedman test was conducted to test the difference of the groups according to the time. Multiple comparisons were made concerning the Friedman test for groups where there was a difference. Spearman correlation coefficient was used. The results were presented in the form of tables and graphs.

RESULTS

Patients and Control Groups

The mean age of the 16 patients was 12.7 ± 2.8 years (range 8-18). The control group was composed of 30 healthy individuals (15 female, 15 male) aged 8-18 with a mean age of 11.7 ± 2.6 years. There was no significant difference between the mean ages of the groups ($p = 0.238$). The demographic data of the patients and the control group are given in Table I.

Table I. Demographic characteristic in patients with allergic rhinitis.

| | Patient | | Control | | p |
|-------------------------------------|---------|----------------|---------|----------------|-------|
| | n | Mean \pm SD | n | Mean \pm SD | |
| Sex (female/male) | 10/6 | | 15/15 | | |
| Age of AIT treatment (year) | | 12.7 \pm 2.8 | | 11.7 \pm 2.6 | 0.238 |
| Allergic rhinitis + asthma / asthma | 9/7 | | - | | - |
| Family history of allergy | 12 | | - | | - |

Mean \pm SD: Mean \pm standard deviation

Symptom Scores for Asthma, Allergic Rhinitis and Eye

According to the result of the Friedman test, a significant difference was found in the symptom scores for asthma of the patients with AA and in the symptom scores for rhinitis of the patients with AR ($p=0.03$, $p=0.002$). Asthma symptom scores for baseline and the 12th month were statistically significantly different ($p=0.018$). As for the symptom scores for rhinitis, on the other hand, the differences between the baseline and the 4th month and between the baseline and the 12th month were found to be statistically significant ($p=0.03$, $p=0.031$). No significant difference was found for all the patients between symptom scores for eye at the baseline, the 4th month and the 12th month ($p=0.171$) (Figure 1A-C), (Table II, III).

Medication Scores for Asthma and Allergic Rhinitis

The evaluation of medication scores for rhinitis and asthma showed that 10 patients received regular AR treatment at the beginning of the AIT, whereas it was observed that rhinitis treatment continued only in 4

patients in the 4th month of the immunotherapy. In the 12th month, on the other hand, no patient was receiving this treatment.

The evaluation of medication scores for rhinitis and asthma also showed that while 4 patients were receiving regular asthma treatment at the beginning of the AIT, asthma treatment continued in 3 patients in the 4th month of the immunotherapy. The symptom score for asthma was 1.9 in these patients but decreased to 0.86 in the 4th month. None of the patients were receiving asthma treatment in the 12th month.

Visual Analog Scale (VAS)

According to the results of the Friedman test, no significant difference was observed in the VAS of the patients with asthma ($p=0.327$). A statistically significant decrease was found in the VAS of the patients with AR in the 4th month ($p=0.002$). When the patients were evaluated in terms of VAS for the eye, there was a statistically significant decrease in the 4th month compared with the baseline ($p=0.017$). (Figure 2A-C), (Table II, III).

Table II. Asthma symptom scores and visual analog scales of asthmatic patients at baseline, the 4th month and the 12th month of AIT treatment.

| | Asthma (n=9) | | | | | | | | | P |
|-------|--------------|-----------|--------|-----------------------|-----------|--------|------------------------|-----------|--------|--------------------|
| | Baseline | | | 4 th month | | | 12 th month | | | |
| | Min-Max. | Mean±SD | Median | Min-Max. | Mean±SD | Median | Min-Max. | Mean±SD | Median | |
| SSA | 0-3.5 | 1.03±1.15 | 0.89 | 0-1.95 | 0.73±0.75 | 0.54 | 0-0.85 | 0.17±0.33 | 0 | 0.030 ^a |
| VAS-A | 0-5 | 1.78±2.17 | 0 | 0-1 | 0.33±0.5 | 0 | 0-7 | 0.89±2.32 | 0 | 0.327 |

| ASS | 0-4 | 0-12 | 4-12 |
|-----|----------|--------------------|----------|
| | $p>0.05$ | 0.018 ^a | $p>0.05$ |

^a $p<0.05$ calculated with the Friedman test.

^a $p<0.05$, multiple comparisons of the Friedman test. **SSA:** Symptom scores for asthma, **VAS-A:** Visual analog scales for asthma, **Min-Max:** Minimum-maximum, **Mean±SD:** Mean±standard deviation.

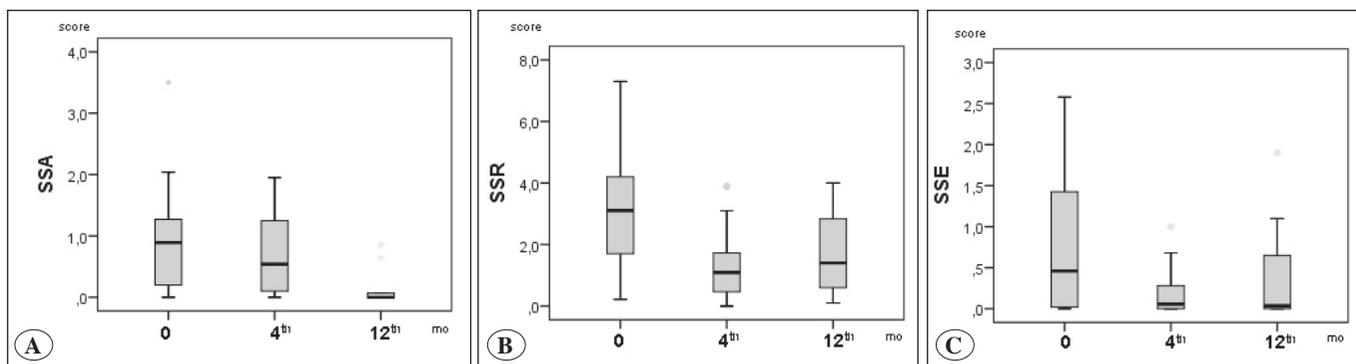


Figure 1. Symptom scores for asthma (A), rhinitis (B) and eye (C) at baseline, and 4th and 12th month after AIT treatment.

Serum Periostin Levels

According to the result of Student's t test, the difference between the mean ages of the patients (49.59±15.57) and the control group (56.38±14.78) was not statistically significant (p=0.152).

The patients were divided into two groups, one group consisting of those with AR accompanied by asthma (7 patients) and the other with AR only (9 patients) and they were analyzed again for serum periostin levels. There was no statistically significant difference between serum periostin levels of patients with asthma (n=9) and patients with AR (n=7) at baseline, the 4th and the 12th months (p=0.625, p=0.368) (Figure 3,4), (Table IV).

There was no significant relationship between the symptom scores for rhinitis and asthma and serum

periostin levels according to the Spearman correlation coefficient. The correlation between the baseline symptom scores for the eye and baseline serum periostin values was found to be statistically significant (r=0.668, p=0.005).

DISCUSSION

Allergen immunotherapy is recommended as an effective method in the treatment of allergic diseases such as AR and AA which can not be controlled through allergen avoidance and medical treatment (8). Serum periostin level was tested as a biomarker in this study to evaluate clinical response to AIT showing a correlation between serum periostin level and symptom scores for eye.

Five of the patients with allergic rhinitis/asthma to whom immunotherapy was applied in this study had accompanying conjunctivitis and one of them had vernal

Table III. Symptom scores and visual analog scales for the patients with allergic rhinitis and eyes at baseline, the 4th month and the 12th month of AIT treatment.

| | Allergic rhinitis (n=16) | | | | | | | | | p |
|-------|--------------------------|-----------|--------|-----------------------|-----------|--------|------------------------|-----------|--------|--------------------|
| | Baseline | | | 4 th month | | | 12 th month | | | |
| | Min-Max. | Mean±SD | Median | Min-Max. | Mean±SD | Median | Min-Max. | Mean±SD | Median | |
| SSR | 0.2-7.3 | 3.20±1.91 | 3.11 | 0-3.89 | 1.29±1.13 | 1.09 | 0.1-4 | 1.66±1.23 | 1.4 | 0.002 ^a |
| SSE | 0-3 | 0.81±0.86 | 0.46 | 0-1 | 0.19±0.30 | 0.06 | 0-2 | 0.38±0.56 | 0.04 | 0.171 |
| VAS-R | 0-10 | 5±2.99 | 5 | 0-5 | 1.38±1.59 | 1 | 0-8 | 2.81±2.23 | 3 | 0.001 ^a |
| VAS-E | 0-8 | 2.19±2.54 | 1.5 | 0-5 | 0±1.32 | 0 | 0-5 | 1±1.75 | 1 | 0.006 ^a |

^ap<0.05 calculated with the Friedman test.

| | 0-4 | 0-12 | 4-12 |
|-------|--------------------|--------------------|--------|
| SSR | 0.030 ^a | 0.031 ^a | p>0.05 |
| VAS-R | 0.002 ^a | p>0.05 | p>0.05 |
| VAS-E | 0.017 ^a | p>0.05 | p>0.05 |

^ap<0.05, multiple comparisons of the Friedman test. VAS-E: Visual analog scales for eye, VAS-R: Visual analog scales for rhinitis, Min-Max: Minimum-maximum, Mean±SD: Mean±standard deviation, SSR: Symptom scores for rhinitis, SSE: Symptom scores for eye.

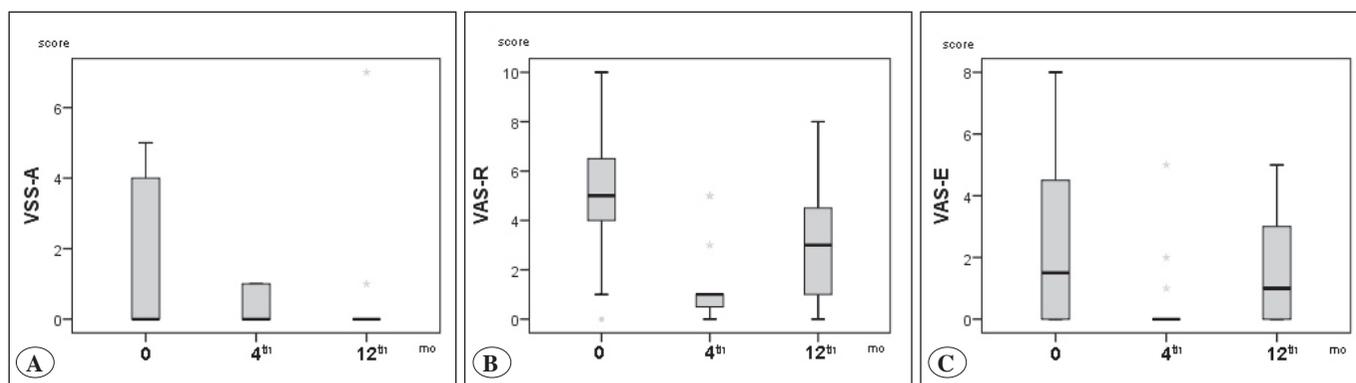


Figure 2. Visual analog scales for asthma (A), rhinitis (B) and eye (C) at baseline, and the 4th and the 12th month of AIT treatment.

conjunctivitis. Vernal conjunctivitis is a chronic and severe allergic inflammatory disease in the eye, threatening sight (20). It has been reported that allergic rhinitis and vernal conjunctivitis substantially affect the quality of life in patients (14). A significant improvement was observed in the visual analog scores for rhinitis and the eye in these patients as a result of AIT.

Potential biomarkers that could demonstrate clinical efficacy of allergen immunotherapy were defined by EAACI (21) and they were divided into seven groups: 1. IgE (total IgE, ratio of specific IgE and sIgE/Total IgE) 2. Specific IgE/Specific IgG4 ratio, 3. Serum inhibitor activity for IgE (IgE-FAB and IgE-BF), 4. Basophil activation, 5. Cytokines and chemokines, 6. Cellular markers (Regulatory T cells, regulatory B cells and dendritic cells), 7. In vivo markers (provocation tests). It was stated that each defined biomarker had its own advantages and disadvantages and that there was no single generally agreed upon method to determine the efficacy of immunotherapy.

The allergen-specific IgE (sIgE) level is the most relevant biomarker for allergen immunotherapy (22). During immunotherapy allergen-specific IgG subclasses, in particular IgG4, have been demonstrated to be increased compared to baseline values in correlation with clinical response to treatment in some studies. However, the IgE-FAB assay is of benefit but technically more complex and is currently performed in specialized laboratories (23). The basophil activation test can be studied by using flow cytometry evaluating the expression of two surface markers on basophils: CD63 and CD203c (22,23). However, basophil responses after AIT are variable and only a limited number of studies of basophil activation are available. The measurement of cytokines and chemokines in nasal fluid through multiplex cytokine analysis with improved technology has shown an increase in response to allergen provocation. So far, no cytokines or chemokines have been shown to predict the clinical outcome in individual patients before the onset of AIT (21).

Table IV. Serum periostin levels at baseline, and the 4th and the 12th month of AIT treatment in the patients with allergic rhinitis and/or asthma.

| | Baseline | | | 4 th month | | | 12 th month | | | P |
|--------------------------|-----------|-----------|--------|-----------------------|------------|--------|------------------------|-------------|--------|--------------------|
| | Min-Max. | Mean±SD | Median | Min-Max. | Mean±SD | Median | Min-Max. | Mean±SD | Median | |
| Asthma | 28-63.5 | 46.8±12.5 | 51 | 30-68 | 43.8±11.27 | 39.5 | 26-73 | 45.67±13.85 | 43.5 | 0.625 ^a |
| Allergic rhinitis | 29.5-78.5 | 53.2±19.2 | 47.5 | 33-56 | 41.86±9.38 | 39.5 | 32-58 | 43.07±9.14 | 41 | 0.368 ^a |

^a: p<0.05 calculated with the Friedman test, **Min-Max**: minimum-maximum, **Mean±SD**: Mean±standard deviation.

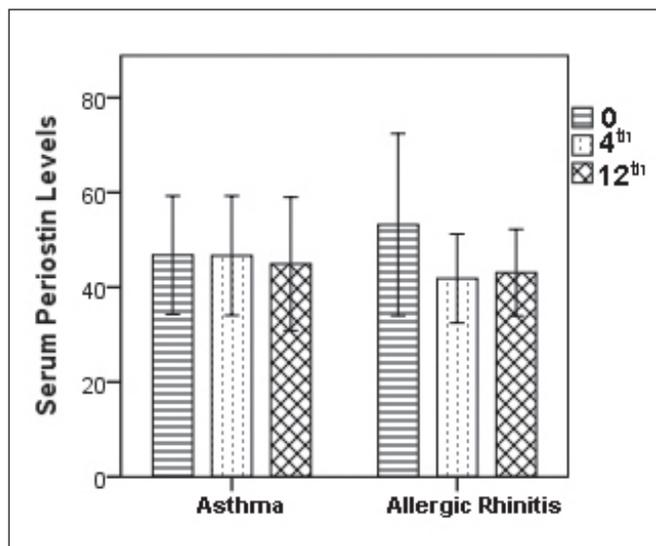


Figure 3. Serum periostin levels at baseline, and the 4th and the 12th month of AIT treatment in the patients with allergic rhinitis and/or asthma.

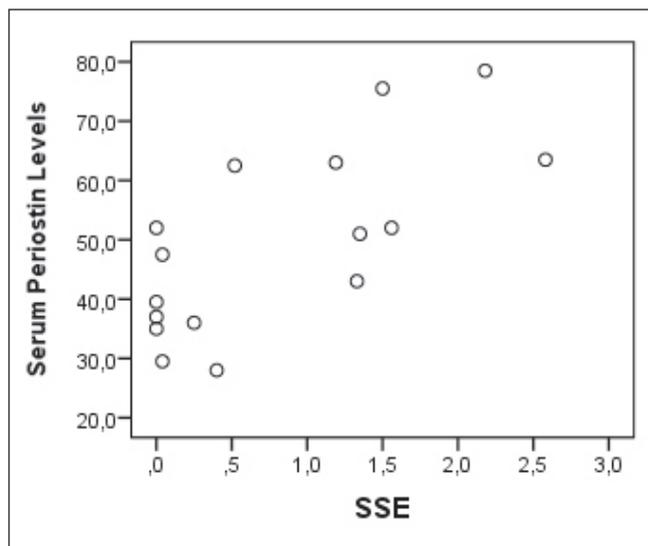


Figure 4. Correlationship between serum periostin and symptom scores for eye.

Various cellular biomarkers defined by EAACI were used in some studies on patients with allergic rhinitis treated with allergen immunotherapy to determine the efficacy of immunotherapy. Allergen immunotherapy has been associated with the induction of several mechanisms that inhibit both early and late phase allergic responses, such as a decrease in mast cell and basophil activity, induction of allergen-specific regulatory T cells (Tregs) and regulatory B cells (Bregs), dendritic cells (DCs) and an increase in allergen-specific IgG4 (21,24). Tregs appear to play a key role in the immunological processes of AIT, mainly skewing the Th2 to Th1 immune response. It appears that a change in allergen-specific B cells in the direction of Breg cells is one of the major alterations in the course of AIT. There are not enough data to link the presence or function of Tregs with clinical efficacy. The frequency of allergen-specific T and B cells is very low: it is technically challenging and currently impossible to use this in clinical practice. Dendritic cell-associated candidate markers of efficacy have been identified in a single short-term SLIT study in practice. Provocation tests have been used as surrogate markers of clinical response to AIT (21). They are recommended for understanding the mechanisms and permit biomarker discovery both at the local level and in peripheral blood. Allergen provocation is not the same as natural exposure: standardization and validation vary for the different challenge protocols (21). In conclusion, although several studies have shown a correlation between some biomarkers and AIT, more data are needed for the use in clinical practice.

Since immunotherapy is a commonly used method of treatment in patients with allergen sensitivity, there is a need for a generally accepted and approved biomarker to determine the efficacy of this treatment method. Therefore, studies in this regard are in progress. Although there are studies in the relevant literature indicating the role of serum periostin in airway inflammation in allergic diseases and asthma, there are no studies in which periostin was used as a biomarker to test the clinical response to allergen immunotherapy. This study investigated whether serum periostin could be used as a biomarker to test the clinical efficacy of AIT in children receiving AIT. The data showed that there was no significant change in serum periostin levels. Serum periostin has been demonstrated to be a biomarker for severe asthma and our study included patients with allergic rhinitis and/or mild asthma. Therefore, these findings may be related to the asthma severity of our patients.

CONCLUSION

There were no significant changes in the serum periostin levels of children with allergic rhinitis and/or asthma receiving allergen immunotherapy. The fact that the serum periostin level was found to be correlated with symptom scores for the eye leads one to think that patients with allergic rhinitis need to be tested for eosinophilic inflammation in the airway.

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