ORIGINAL ARTICLE

Correlation of serum periostin level with the clinical severity and other biomarkers in allergic rhinitis patients

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ABSTRACT

Background: Allergic rhinitis (AR) is a common and chronic IgE-mediated respiratory airway disease. AR is characterized by heterogeneous group of symptoms like rhinorrhea, sneezing, itching and obstruction. The prevalence of AR has increased over the years. Several biomarkers have been identified as indicators of the pathogenesis and prognosis of AR. However the correlation between these biomarkers hasn't been yet investigated. Herein, we investigated the correlation between periostin that is considered as a marker for Th2 mediated inflammation and other biomarkers like eosinophils, eotaxin and soluble interleukin 2 receptor (sIL-2R). **Methods**: This cross sectional study included the investigation of 23 AR patients before the beginning of allergen immunotherapy. First, symptom scores were recorded for all patients then; skin prick test was done to verify the diagnosis of allergy. Blood samples were collected for eosinophilic count and their sera were analyzed for periostin, eotaxin, and sIL-2R levels. ELISA was used to quantify all these biomarkers. **Results**: This study revealed that periostin is a promising diagnostic biomarker in AR patients and correlated with the severity of AR. Blood eosinophils count is a good biomarker as well, and correlated with the severity of symptoms. There is a negative correlation between periostin and sIL-2R levels whereas no correlation was found between serum periostin level and eotaxin or eosinophils. **Conclusion**: Periostin is a novel mediator in AR can be used as a biomarker for AR severity.

Keywords: Allergic Rhinitis; Human periostin protein; Eosinophils; IL-2 Receptor; Eotaxin-1

INTRODUCTION

Allergic rhinitis (AR) is the most common IgE-mediated respiratory airway disease. It is characterized by variety of symptoms; sneezing, watery rhinorrhoea, itching and nasal blockage [1]. The prevalence of AR has eventually increased over the years; about 20-30% of the population in Africa is suffering from this disease [2]. In Egypt, the prevalence of physician-diagnosed allergic rhinitis was 15.3%, while the prevalence of asthma was 9.4% [3]. AR is a risk factor for asthma and about 15% to 38% of the patients with nasal allergy concomitantly suffer from asthma [4]. During the last years, the sustained research efforts have revealed some biomarkers that could be useful in clinical practice for the diagnosis and prognosis of allergic diseases however, only a few biomarkers can serve for the diagnosis of AR (e.g. IgE, eosinophils, IL-5, IL-6, IL-13 and macro-phage inflammatory protein (MIP) 1β, periostin, eotaxin, sIL-2R). The utility of these biomarkers in diagnosis, prognosis and responsiveness to therapy is still controversial [5]. Periostin is an extracellular matrix protein which is secreted by airway

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epithelial cells or fibroblasts in response to IL-13 [6]. Periostin has been considered as a biomarker for Th2 inflammation [7]. Also, the production of periostin is upregulated in allergic rhinitis, chronic rhinosinusitis with nasal polyps, and aspirin-induced asthma [8]. Several studies have utilized serum periostin level to predict the response to asthma biologics like omalizumab therapy [9, 10]. According to Global Initiative for Asthma (GINA), blood eosinophil count is neither a specific nor a sensitive diagnostic marker for asthma or AR, but it can serve as a prognostic biomarker to predict several therapeutic responses and be used with other markers to confirm the diagnosis [11]. Baseline blood eosinophils count can be used as a biomarker to predict the clinical efficacy of anti-IL5 antibody (mepolizumab), and anti-IL4 receptor antibody (dupilumab) [12]. Previous reports suggested that blood eosinophilia ($\geq$ 300 cells/μL) is associated with better response to anti-IgE antibody (omalizumab) [13]. Recent studies of the correlation between serum periostin concentration and sputum or blood eosinophilia have reported contradictory results [14]. Eotaxin is a selective chemokine that has been implicated in the pathogenesis of allergic diseases [15, 16]. Multiple studies have confirmed the concordance between an elevated concentration of eotaxin and allergen exposure [16]. From these studies, eotaxin is a promising marker of allergic inflammations. In addition, several reports have shown that the release of a soluble form of interleukin 2 receptor (sIL-2R) can result from the reactions of inflammatory and immunoregulatory cells so it can be used as a marker for some allergic and inflammatory diseases [17]. All these studies described different biomarkers in the pathogenesis of allergic diseases especially AR and asthma but the concordance between these biomarkers hasn't been explained yet. In this study we sought to find the relationship between periostin serum level and other biomarkers like sIL-2R, blood eosinophils and eotaxin in AR patients before the beginning of immunotherapy, also we investigated the concordance between periostin serum level and the severity of AR.

METHODS

Our study included 23 patients with AR. Participants were enrolled from the Allergy and Immunology Unit, Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, Egypt. Inclusion criteria included adult patients (21-45 years) with mild to moderate AR. Exclusion criteria included the presence of severe persistent asthma, patients with associated Broncho-pulmonary disorders and other respiratory or systemic diseases. Steroid-dependent patients or who taking immunotherapy before the start of the study were not included in this study.

The diagnosis of allergy was verified by a history of exposure to allergens, family history for allergic diseases and careful clinical examination. Clinical history of chronic AR over two years was present in all cases. AR patients recorded the nasal symptoms of rhinorrhea, sneezing, itching and obstruction. The following scale was used for each symptom scoring: 0 = no symptom, 1 = mild (symptom was of short duration and not annoying), 2 = moderate (symptom was frequently annoying but did not interfere with normal daily activity or sleep), or 3 = severe (symptom interfered with normal daily activity or sleep). The total nasal symptom score (TNSS) was the sum of the scores for the individual symptoms. TNSS values (0–12) were categorized as mild (0–4), moderate (5–8), and severe (9–12) [18, 19].

After that, skin prick test was done by utilizing different Coca's extracted allergens. The tested allergens were extracted from house dust mites (HDM) (Dermatophagoidesfarinae and Dermatophagoidespteronyssinus), tobacco leaf, ryegrass, cottonwood mix, Aspergillus species mix, date palm pollen (Phoenix dactylifera, Pho), and ash mix. Aqueous solutions were prepared from each allergen extract using the weight/volume unit as follow: the allergens were eluted for a time, and then the solid materials were filtered out, leaving aqueous solutions that were subjected to further

filtration to be used as stock solutions. The diluted doses were prepared using saline (0.9%) in 10 mL vials which were prepared under complete aseptic technique at the Allergy & Immunology Unit, Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University [20, 21]. Skin prick test was performed on the inner aspect of the forearm. After disinfection of the skin by 70% ethyl alcohol, the forearm was coded with a marker pen according to the allergens being tested along with the positive control (Histamine hydrochloride 10mg/ml) and negative control (saline). Then, a drop of each allergen solution, negative and positive controls was placed beside each mark and a small prick was made through the drop using a sterile prick lancet. The results for allergens were red after 15-20 minutes [22]. Positive reaction to an allergen was observed when the skin becomes itchy, red and swollen with a wheal in the center. A wheal of 3 mm or greater indicates the presence of specific IgE to the allergen tested.

Eosinophil counting was done as follows: A drop of well mixed blood was placed on the base of a slide with a pipette tube. A spreader slide was placed in front of the blood and moved backwards to touch the drop of blood. Then, a smear was made that covered two-thirds of the slide length with an oval feathered end. The smear was properly air dried and floored with Leishman stain for about 5-10 minutes, then double diluted with buffered water and allowed for another 5–10 minutes for the cells to pick the stain. After this, the slides were properly rinsed under running water and air dried. The slide was finally viewed one millimeter away from the tail (the monolayer part) with the microscope [23].

Quantitative measurement of periostin by ELISA (Abcam, Cambridge, UK): A 100 µL standard or sample was added to the wells and incubated at 37 °C for 90 min, and then the plate content was discarded and 100 µL biotinylated antibodies was added to all wells and incubated at 37 °C for 60 min. Each well was then washed three times with 300 µL 0.01 M PBS. Thereafter, a 100 µL ABC working solution was added and incubated at 37 °C for 30 min. Sequentially, wash five times with 300 µL 0.01 M PBS, add 90 µL of prepared TMB color developing agent; and incubate at 37 °C in dark for 15-20 min. The reaction was stopped by the addition of 100 µL TMB stop solution. The OD was read at 450 nm using an automated reader.

SIL-2R and eotaxin were also measured by ELISA kits (Abcam, Cambridge, UK) according to the manufacturer's guidance.

Ethical Considerations

This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans. Written informed consents were obtained from the study participants. Approval by IRB research committee of Zagazig Faculty of Medicine was also included.

Statistical analysis

We analyzed the data with Graph Pad Prism 8 software (San Diego, California, USA) and the data are presented as means and range. Student's t-test was applied when two values were compared. Pearson correlation coefficient was used to assess the concordance between the studied parameters. Probability values (p) of <0.05 were considered significant. The sample size was calculated by the open epi program with a confidence level of 95 % and power of 80%.

RESULTS

This study included 23 AR patients (10 females and 13 males) with mean age 37.7 ± 6.91. Demographic and laboratory characteristics of the patients at the beginning of the study are shown in Table 1.

Results of skin prick test

Skin prick test shows that all the study participants have mixed sensitizations. As can be expected, the most prevalent antigen detected by skin prick test in those AR patients was date palm pollen (95%) followed by house dust mite (65%) and ash mix sensitization (56%). Whereas, cottonwood was the least sensitization observed in the study participants

with percentage (39%). Furthermore, we didn't find any correlation between serum periostin level and the results of skin prick test.

Correlation between AR severity and periostin serum level

In this study we observed that, there is a positive correlation between the severity of symptom scores and the level of periostin in the serum of those patients (r= 0.875, p=0.0001). As can be seen in figure 1, the patients with mild AR has lower serum periostin level than the patients with moderate AR, indicating that serum periostin level can be used as a marker for the severity of AR.

Correlation between periostin serum level and sIL-2R

Figure 2 shows the correlation between periostin serum level and sIL-2R concentration in the patients with AR. As can be observed there is a negative correlation between the two

markers (r= -0.6007, R squared=0.3609) and this correlation was significant (p=0.05).

Correlation between serum periostin level and eotaxin concentration

Unexpectedly, there was no significant correlation between periostin serum level and eotaxin concentration in the study participants (r= -0.045, p= 0.89).

Correlation between serum periostin level and eosinophils count

Although most of the study participants had eosinophilia (eosinophils>300), there is no correlation between serum periostin level and eosinophils count. There is a positive correlation between eosinophils count and the severity of AR (r=0.943, p <0.0001). Eosinophils count in cases of moderate AR was higher than in cases of mild AR as can be seen in figure 3.

Table 1. Demographic and laboratory characteristics of the study participants

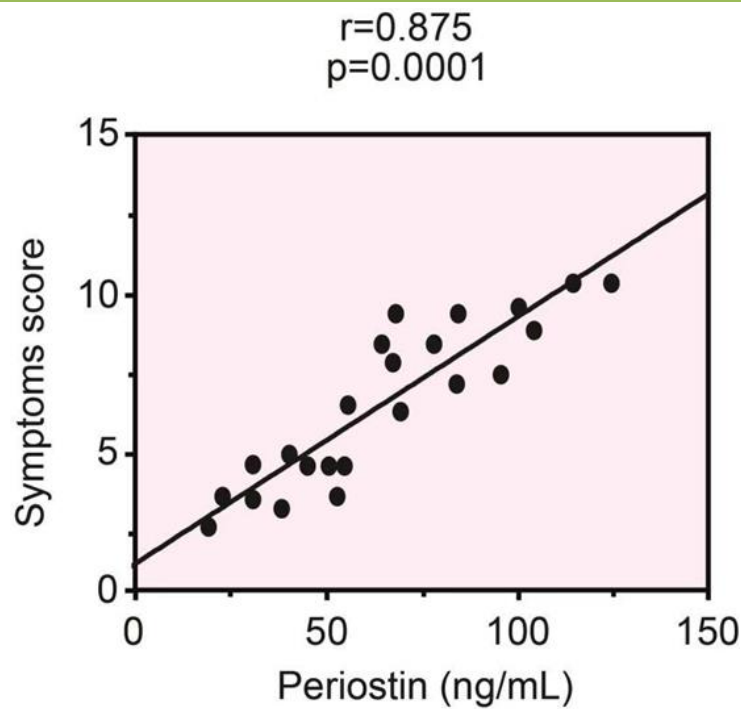| Characteristics	Mean (range)
N (female/male)	23 (10/13)
age (years)	37.7 (21-45)
symptom scores	9.2 (2-12)
eosinophilic count (cells/mcL)	590.9 (250-750)
periostin conc (ng/ml)	59.63 (19-125)
sIL-2R conc (pg/ml)	2044 (1155-2077)
eotaxin conc (pg/ml)	151.6 (83-349)

*Date expressed as mean (range).

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r=0.875
p=0.0001

Figure 1. Correlation between periostin concentration and the severity of AR
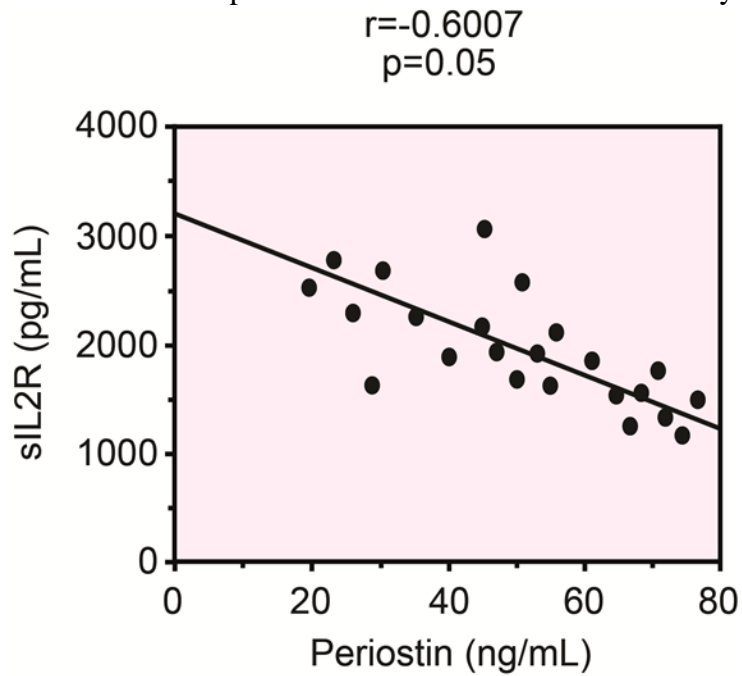
r=-0.6007
p=0.05

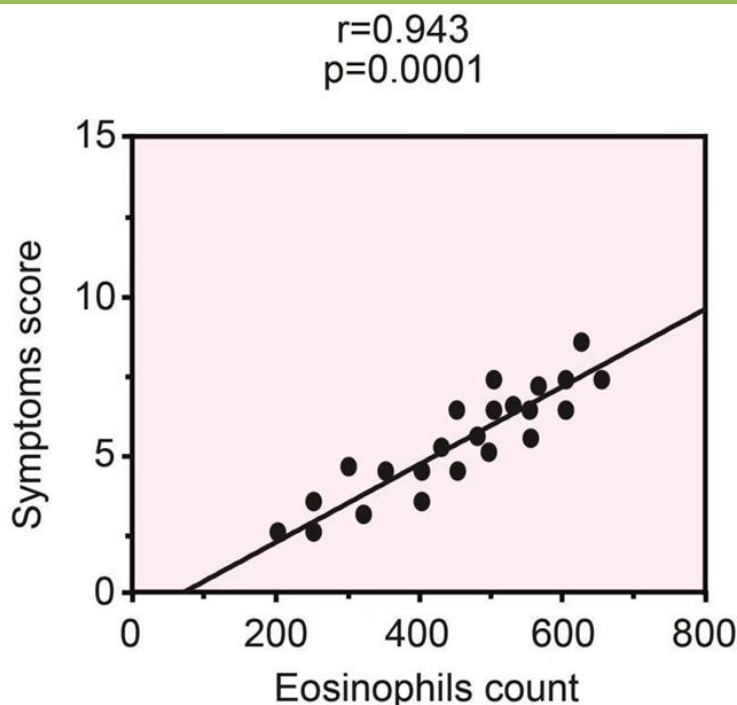**Figure 2.** Correlation between serum periostin level and sIL-2R

Figure 3. Correlation between blood eosinophils count and AR severity

DISCUSSION

Despite the sustained research efforts, only few biomarkers useful in the diagnosis and management of allergic diseases such as asthma and AR have been validated. Periostin was discovered as a novel mediator in asthma and other allergic diseases such as AR and atopic dermatitis [24]. Serum periostin was used to stratify asthma patients into Th2 low and Th2 high which is utilized in detection of the suitable treatment and prediction of the response of asthma biologics [25]. It is useful also for stratifying AR patients based on endotypes and has the potential to predict the efficacy of biologics for AR [26, 27]. In this study we found that periostin is correlated to AR severity as higher level of serum periostin was present in the patients with moderate AR and high symptom scores. Our results is in concordance with Kenemitsu and his colleagues who found that higher serum levels of periostin were related to greater decline in lung function in asthma patients [28]. Also, Jonstam et al. [26] has found that high serum periostin level is associated with the severity and certain phenotypes of AR like the presence of nasal polyps. Nagasaki et al. [29] has found

that the identification of patients with high serum periostin levels allowed predicting patients with increased risk of asthma exacerbation. These studies unrevealed the role of serum periostin as a marker for the severity of allergic diseases especially asthma and AR and to predict the risk of exacerbation of asthma in the future years. However, our results were not consistent with Scichilone et al. [30] whose results showed that there is no significant correlation between baseline serum levels of periostin and lung function parameters. Scichilone et al. [30] has illustrated the cause of these results by his small sample size (n=15). In addition, kim et al. [31] showed that serum periostin levels were not associated with allergic rhinitis in children. The possible explanation for this difference is serum periostin levels in adults without allergic disease were significantly lower than in children without allergic disease so increased basal level of serum periostin may mask any increase in serum periostin associated with allergic disease [32]. Surprisingly, Gordon et al. [33] has explored the biological role of periostin in asthma using periostin deficient mice and proposed a protective role for

periostin, probably mediated by TGF-beta induced differentiation of T regulatory cells. Furthermore, the current study showed that serum periostin levels were not correlated with the allergic pattern and this result is consistent with the previous reports [30]. Blood eosinophils have good correlation with the severity of AR. These results are consistent with the previous reports which revealed that blood eosinophils are associated with disease severity and asthma phenotypes [34]. Also, Pal et al. [35] has found that eosinophil count in nasal smears is a highly specific criterion for the diagnosis of AR. Klaewsongkram et al. [36] also found a positive correlation between the nasal eosinophil count and the symptom of nasal stuffiness (p = 0.037). Also, we didn't find any correlation between periostin and blood eosinophils levels. Controversy, serum periostin level was proposed as a systemic biomarker of eosinophilic asthma, by showing a significant correlation with sputum eosinophils [37]. The possible explanations of the difference between our results and these previous reports may be the small sample size of our study also; we measured only blood eosinophils not nasal scraping eosinophils which are more reliable in AR than blood eosinophils. It was previously reported that, there is a significant elevation in the expression of the T-lymphocyte activation markers like sIL-2R in patients with AR [17]. This finding provides further support that cell-mediated immunity is an essential component in the pathogenesis of AR. As well as, eotaxin has been found to play an important role in the pathogenesis of AR [15]. The novelty in this study arises from the correlation between periostin and other biomarkers of AR like sIL-2R and eotaxin. A negative correlation between sIL-2R and serum periostin levels was detected. Further studies are also needed to confirm this correlation and to detect the cause. Additionally, we didn't find any relationship between serum periostin level and eotaxin concentration. This finding is consistent with Asano et al. [38] who didn't find any correlation between periostin and eotaxin in asthma patients associated with rhinitis. The main limitations in this research were the small sample size and measuring blood eosinophils instead of nasal scraping eosinophils. Further studies are also needed to investigate the relationship between periostin and other biomarkers that are important in AR like IgE, IL5, and IL13.

CONCLUSION

Several biomarkers are found to be important in the pathogenesis of AR but the correlation between these biomarkers hasn't been investigated. Periostin a novel mediator in allergic diseases can be used as a biomarker for AR severity. As well as, blood eosinophils are good indicators for AR severity. There is a negative correlation between periostin and sIL-2R however no correlation was found between serum periostin level and eotaxin or eosinophils count.

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