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Measuring inflammation in patients with allergic rhinitis using different biomarkers

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Abstract

Allergic rhinitis is an inflammatory disease affecting the mucosal lining of the nose of individuals of all ages. The allergen in the air involves attaching by Immunoglobulin E that causes the release of inflammatory chemicals from mast cells. This study aimed to measure the serum level of biomarkers and to determine the correlation between them in mediating activate cell in patients with AR. In this study, blood samples were taken from 88 AR patients and 88 healthy controls (HC) Symptom score was measured using the SNOT-22 questionnaire and blood sample was analyzed for eosinophil counts (B-Eos) using optical flow cytometry, IgE by electrochemiluminescence immunoassay, as well as eosinophilic cationic protein, anti-alpha smooth muscle actin (anti-SMA), cyclooxygenase- 2 (COX-2R), phosphatase and tensin (PTEN) homolog and Tryptase Alpha/Beta 1(TPSABI) by ELISA. B-Eos, IgE, ECP, anti-SMA, and symptom scores were significantly higher in AR patients than in HC. As a result, we found that there was correlation between B-Eos and ECP ($r = 0.66$, $p = 0.001$). There was also a correlation between IgE and symptom score ($r=0.64$, $p=0.01$). COX-2 also correlated with symptoms score ($r = 0.52$, $p = 0.04$). There was a positive correlation between phosphatase and tensin homolog and TPSAB1 ($r = 0.76$, $p<0.001$) and PTEN and ECP ($r = 0.53$, $p = 0.03$). In conclusion, we realized that in allergic rhinitis, the airway inflammation was characterized by high numbers of eosinophil, IgE, ECP and anti-SMA. B- Eos, IgE and COX-2 were related to symptom scores. The study highlights the importance of systemic inflammation in AR.

1. Introduction

Rhinitis is a disorder characterized by an inflammation of the nasal mucosa. This disorder affects more than 20% of the population [1]. There are various subtypes of rhinitis where allergic rhinitis (AR) [2] is the most common, affecting 1 in 6 persons [3]. AR can be further subdivided into seasonal or perennial AR [4]. Previously, AR was presumed to be a disorder limited to the nose and nasal passages. However, recent evidence shows that AR maybe part of a systemic airway disease involving the whole respiratory tract [5].

AR is characterized by accumulation of inflammatory cells in the nasal mucosal membrane. Biopsies from patients with AR show an important presence of mast cells and eosinophils in AR pathogenicity [6]. Increased levels of eosinophil granulocytes and eosinophil cationic proteins in blood are common [2]. Allergic responses in AR are considered to be due to lymphocyte T-helper type 2 [7] that induce allergic inflammation and the production of allergen-specific immunoglobulin E [8, 9].

The two major cytoskeletal proteins implicated in cell motility are actin and myosin. Actin and myosin are constituents of many cell types and are involved in a myriad of cellular processes including locomotion, secretion, cytoplasmic streaming, phagocytosis, and cytokinesis The monoclonal Anti-a Smooth Muscle Actin (Anti-SMA) may help in the description of stromal cell heterogeneity in various organs, and in differentiating smooth muscle cells from fibroblasts in mixed cultures [10].

COX-2 is a key mediator of *inflammatory* pathways and its elevated expression has been found in several human cancers as well [11]. COX-2 is associated with inflammation, and its expression has been found to lead to an increased inflammation in the airway epithelium, submucosal inflammatory infiltrate, and to induce production of sputum in allergic patients. COX-2 has been implicated in various inflammatory conditions, such as increase vascular permeability in the nose and skin, an increase in nasal sinus pressure, airway narrowing and eosinophil infiltration in the nose and skin [7, 12].

In humans, phosphatase and tensin homolog [13] gene elevation level and mutation can promote the development of numerous cancers. The raised levels in systemic and pulmonary leptin are related to lung injury and lung cancer. PTEN is a protein which encoded by the PTEN gene. This protein has been found in most mammals for which complete genome data exist [14].

Tryptase alpha-1 and tryptase beta-1 [15] are enzymes that in humans are encoded by the same TPSAB1 gene. Beta tryptases appear to be the main isoenzymes expressed in mast cells; whereas in basophils, alpha tryptases predominate. Human mast cells and basophil contain tryptase which is an active cytoplasmic granule trypsin-like serine proteinase that is enzymatically active only as heparin-stabilized tetramers that observed in damaged tissues and they are resistant to all known endogenous proteinase inhibitors [15, 16]. However the physiologic role of tryptase is still uncertain. After locally activation of mast cells, they release tryptase into their microenvironment, and they can act on the numerous extracellular targets in allergic diseases [15 - 17].

The aim of the present study was to measure different potential biomarkers in AR, and healthy controls; and to correlate biomarker levels in patients with AR with symptom scores.

2. Materials and Methods

2.1. Patient sampling and setting of the study

This cross-sectional study was carried out on 88 perennial AR patients and 88 healthy persons of all age groups and both sexes from the Ali Kamal Health Centre, Sulaymaniyah city. These individuals were outpatients from March to July 2016. The study protocol was approved by the Ethics Committee of the Faculty of Medical Science/School of Medicine. The informed permission about the study was obtained from each patient who agreed to contribute to the study in the form of a verbal agreement. The AR was identified based on history, symptoms, and clinical examination by using total serum IgE and complete blood count and blood level of the biomarkers.

Furthermore, an ear, nose, and throat specialist checked the AR diagnosis of the patients according to the British Society for Allergy and Clinical Immunology criteria. Healthy control subjects were normal individuals with no history of any chronic diseases. The control group was made up of people who came with the patients attending with AR. Patients who had used oral anti histamine and intranasal steroid spray within the last 14 days before the blood was drawn were excluded.

2.2. Measurements

An Optical Flow Cytometry Kit was used for detecting eosinophil numbers in the blood (B-Eos). Commercial enzyme‐linked immunosorbent assays [9] were applied to measure serum levels of each anti-SMA (Catalogue number: EH4028), PTEN (Catalogue number: EH1661), COX-2 (Catalogue number: EH2876), and TPSAB1 (Catalogue number: EH1204) (Fine Test, China), while IgE was obtained from Roche, USA and ECP from Diagnostic Development, Uppsala, Sweden. The kit preparation, concentration of each antibody and procedure was done in line with the manufacturer's recommendations.

The SNOT-22 test is used in order to evaluate the severity of nasal symptoms and their influence on the quality of life. The questionnaire is composed by 22 chronic rhinosinusitis related questions, which evaluate the severity of symptoms and their influence on quality of life. All questions are based on a scale from 0.0 to 4.0, where 0.0 defines no problem and 4.0 defines maximal problems [18, 19].

2.3. Statistical analysis

For the basic comparison of biomarkers and symptoms between two groups of patients and control, an independent t-test was used. The relationship between markers in terms of age, IgE, and disease duration in AR were analyzed by bivariate correlation and Pearson coefficient. A one-way ANOVA test was used. Results were expressed as mean, standard error and analyzed using the Student's t-test or the Mann-Whitney-U test for two groups, or one-way analysis of variance (ANOVA). The level of significance was considered as $P \leq 0.05$. Data were analyzed using the Graph Pad Prism 7.0 program.

3. Results

In this study, we have examined 88 AR patients comprising 66 women and 22 men, with a mean age of 33±12 years and 88 healthy controls (HC) (62 women and 26 men), with an age mean of 34±12 years. There were no difference in age comparing patients and HC. Patients had significantly higher level of IgE measurable in the serum than the healthy controls (160.98 IU/mL compared to 34.88 IU/mL, $p = 0.0001$). The clinical assessment showed high symptom score in the patients (1.80 ± 0.4, HC: 0.01, $p = 0.0001$). Of the total patients, 80% had perennial and the remainder seasonal AR (**Table 1**).

Table 1: Characteristics of patients and healthy controls according to age, sex, symptoms, eosinophil, and serum total IgE.

Item	Allergic Rhinitis (N=88)	Healthy Control (N=88)	p-value
Age (year, M±SD)	33.16±11.89	34±11.62	0.363
Sex (Female/Male)	66/22	62/26	
Eosinophil (10^9/L, M±SD)	3.5±2.9	2.29±1.05	0.0004
Serum total IgE (IU/ml, M±SD)	160.98±220.81	34.88±25.52	0.0001
Symptom	1.80±0.40	0.01	0.0001

On the other hand, patients with AR had significantly higher levels of B-Eos, IgE, ECP and anti-SMA than the control group. Additionally, no significant differences between the AR and control groups were found for PTEN, COX-2, and TPSAB1. COX-2 also correlated significantly with the symptoms score. There was a positive correlation between PTEN and TPSAB1 and also PTEN and ECP levels. There was no statistically significant correlation between ASM and each of PTEN, TPSAB1, COX-2, IgE, and ECP levels and as well as symptoms score **(Table 2).**

Table 2: Shows the correlations and p-value between the markers and symptoms in AR patients.

		PTEN	TEPSAB1	COX-2R	ECP	IgE	Total Eosinophil	Symptoms
ASMA	Correlation Ratio	0.063	0.061	-0.04	-0.11	0.025	-0.041	0.0075
	P-value	0.56	0.57	0.71	0.31	0.82	0.71	0.94
PTEN	Correlation Ratio		**0.76**	-0.14	**0.53**	0.058	-0.091	-0.14
	P-value		**0.0005**	0.18	**0.03**	0.59	0.4	0.2
TEPSAB1	Correlation Ratio			0.082	-0.088	-0.0066	-0.13	0.045
	P-value			0.45	0.42	0.95	0.24	0.67
COX-2R	Correlation Ratio				0.15	0.11	-0.034	**0.52**
	P-value				0.17	0.32	0.75	**0.04**
ECP	Correlation Ratio					0.14	**0.66**	0.13
	P-value					0.19	**0.001**	0.24
IgE	Correlation Ratio						**0.55**	**0.64**
	P-value						**0.02**	**0.01**
Total Eosinophil	Correlation Ratio							**0.51**
	P-value							**0.05**

ASMA: Anti-Smooth Muscle Antibody; PTEN: Phosphatase and tensin homolog; TPSAB1: Tryptase alpha/beta-1; COX-2R Cyclooxygenase-2 Receptor; ECP: Eosinophil cationic Protein; NS: no significant

Moreover, there was statically significant correlation between B-Eos and ECP, as well as there was a significant correlation between symptom score and each of IgE and B-Eos levels **(Figure 1).**

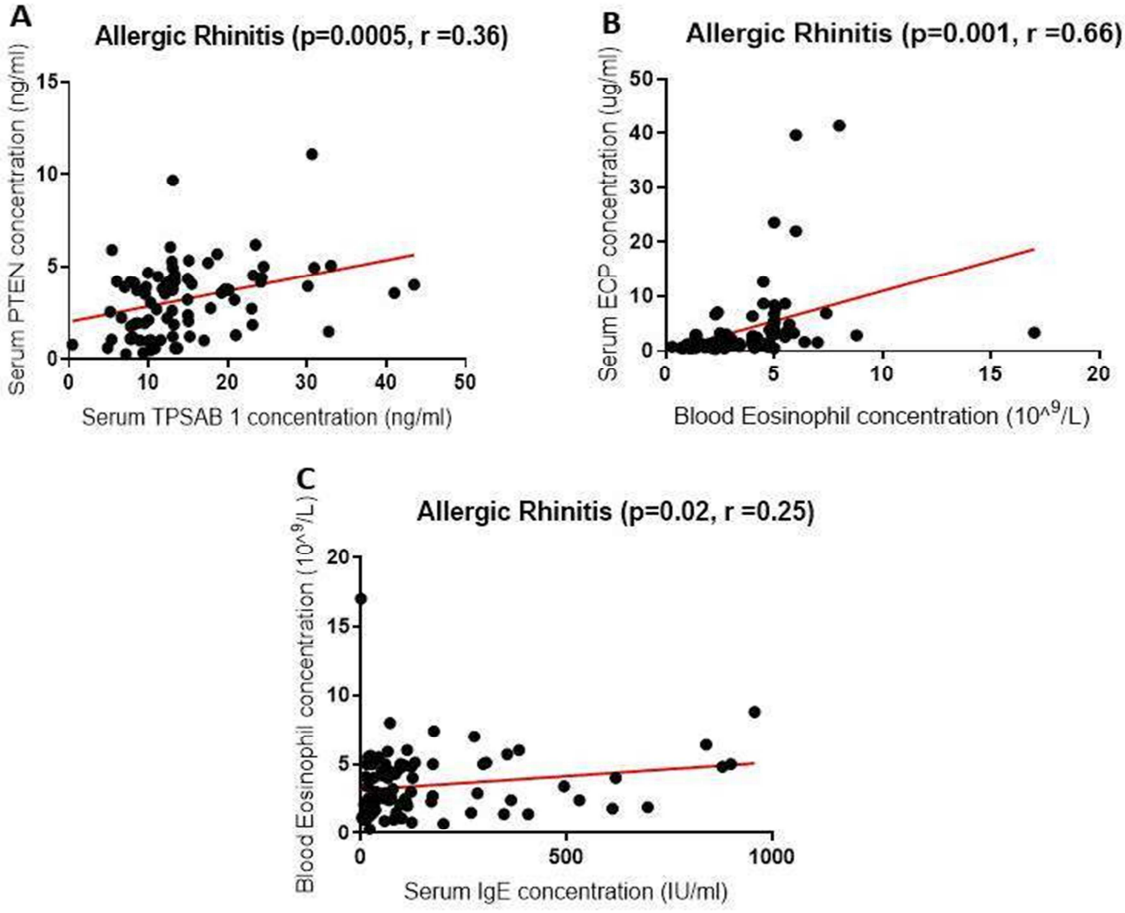

Figure 1. The histogram shows the correlation between different markers positive cells in patients with allergic rhinitis. Spearman nonparametric correlation test was used.

4. Discussion

The main findings of this study was that the patients with AR have increased levels of B-Eos, IgE, ECP, and anti-SMA compared to healthy controls. In the AR group B- Eos, IgE, and COX-2 was related to symptom scores.

In the present study B-Eos was higher among AR patients compared to control subjects. Similar result was found by Pal *et al.* used B-Eos as a diagnostic standard for AR [20]. Also, Kampe *et al*. and Shreepad *et al.* reported that the B-Eos was significantly higher in patients with AR compared to HC subjects [21, 22]. IgE levels were also significantly higher in AR patients as compared to HC. Similar results have been reported by other researchers [23, 24]. The elevation of serum IgE is a diagnostic marker of immediate-type allergic reactions.

In the present study, we observed that the ECP level was significantly higher in the AR group compared with the healthy group. Several other studies have been recorded the same results [21]. Cheng and co-workers, however, had assumed that the level of ECP correlated with the severity and diagnosis of AR. However, they did not observe a significant increase in patients with chronic rhinosinusitis and nasal polyposis [25]. In the inflammation site, primed eosinophil immediately discharges four preformed, highly cytotoxic, cationic granule proteins: eosinophil cationic protein, eosinophil peroxidase [2], eosinophil derived neurotoxin [26] previously eosinophil protein X (EPX) and major basic protein (MBP) in addition to the chemokines, cytokines and growth factors [21]. Thus, the serum ECP level increases rapidly according to eosinophil discharge in the inflammation site.

We also found that anti-SMA levels were significantly higher in AR patients as compared to control subjects. No previous studies have made a comparison between anti-SMA levels in AR patients and healthy individuals. The anti-smooth muscle antibodies (anti-SMA) are supposed to be focused against either actin, tubulin or the intermediate filaments of the cell [27]. In one investigation, it was observed that the AR leads to mucosal inflammation, and this specificity is accompanied by an increase in the number of inflammatory cells in the nasal mucous membrane. This has been confirmed in biopsy investigations in patients with AR. This causes an accumulation of activated mast cells and eosinophils in the nasal mucosa, and it is found to be important in the pathogenesis of AR [7]. Mast cells have an important role to play in inflammatory and immediate allergic reactions by their ability to release potent inflammatory mediators such as histamine, chemotactic factors, proteases, cytokines and metabolites of arachidonic acid. These act on the vasculature, smooth muscle, connective tissue, mucous glands, and inflammatory cells [7]. In, our study, we observed that anti-SMA was significantly higher in AR patients compared to control individuals, and this could be due to the action of mast cell and its mediators.

A positive correlation between B-Eos and IgE levels in the AR patients was found. This result indicates that the eosinophils are in contact with IgE by its surface receptors and causes a release of pro-inflammatory mediators from the eosinophils [28]. Similar results was found in another study [29]. We also found a significant positive correlation between serum ECP and B-Eos in the AR patients. Such positive correlation was also found by Li *et al*. who suggested that ECP could be an important mediator in the pathogenesis of AR as indicated by this positive correlation of serum ECP with eosinophilia in AR patients [29].

In the present study, we found a significant negative correlation between serum ECP and PTEN levels in the AR patients. No previous studies have proved a correlation between ECP and PTEN levels in such patients. Two previous reports have, however, focused separately on ECP and PTEN correlations with allergic inflammations [21, 30]. The concentrations of TPSAB 1 and PTEN levels are significantly correlated in the group of AR patients. No previous studies have indicated a correlation between TPSAB 1 and PTEN levels in AR patients. PTEN is implicated in allergic inflammation as it correlated inversely with inflammation [31].

There was a positive correlation between B-Eos and symptom score. This is in contrast to the finding of Pal *et al*. who did not find a significant correlation between nasal eosinophilia and symptoms [20], but in another report, there was a statistically significant association between B-Eos and the symptoms, rhinorrhea

and sneezing [32]. In this study, we also found a significant correlation between IgE levels and symptom scores. The serum IgE levels in AR raised during the emergence of acute symptoms, in related sinonasal polyposis and fungal involvement [29]. As a result of the release, allergic mediators such as histamine can cause nasal both abstraction and sneezing-rhinorrhea or one of them upon cross-linking of the allergen-specific IgE bound to the surface of mast cells in AR patients [33].

Additionally, we found a significant positive correlation between COX-2 and symptom in the AR patients. This was also found in another study that involved COX-2 in the case of allergic nasal inflammation in rats. Our results are in accordance with a study showing a significant inhibition of nasal symptom among pollinosis patients when a selective COX-2 inhibitor, meloxicam was administered in combination with an antihistamine [12].

5. Conclusion

In allergic rhinitis, the airway inflammation was characterized by high numbers of eosinophils, IgE, ECP and anti-SMA. B- Eos, IgE, and COX-2 was related to symptom scores. The study highlights the importance of systemic inflammation in AR.

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