RESEARCH ARTICLE

Biomarkers of Allergic Asthma and their Association with Serum Parameters

Suhayla H. Shareef[1*], Kawa Amin[1,2]

[1]*Department of Microbiology, Immunology, College of Medicine, University of Sulaimani, Sulaimani, Iraq.*
[2]*Department of Medical Science, Respiratory Medicine, and Allergology, Uppsala University and University Hospital, Uppsala, Sweden.*

Received: 19th March, 2020; Revised: 28th April, 2020; Accepted: 26th May, 2020; Available Online: 25th June, 2020

ABSTRACT

Background and objective: Asthma is not a single disease but, rather, a heterogeneous inflammatory disorder with various pathogenic mechanisms. A key element of severe asthma (SA) has been stated to be eosinophilic inflammation (EI). However, there is no serum biomarker that can reliably predict EI in SA. In this regard, the present study was carried out to evaluate the biomarkers of allergic asthma (AA), and their association with serum parameters.

Methods: The present cohort study consisted of 66 AA and 61 healthy controls (HC) from January 2017 to November 2018. Data on variables, like all types of leukocyte cells, total and specific serum IgE, ECP, tryptase, IL-18, CD-20, and FEV1 were obtained. For comparison between the unpaired variables, the Mann-Whitney U test, χ^2 test, and Kruskal Wallis H test was used.

Results: Symptoms (cough, wheezes, dyspnea) and family history were found as significant indicators for AA ($p < 0.05$). The AA and HC were significantly different in terms of blood and serum parameters (i.e., WBC, lymphocyte, monocyte, neutrophil, eosinophil, basophil, total IgE, external counterpulsation (ECP), tryptase, IL-18, and CD-20) ($p < 0.05$); therefore, these factors might be reliable indicators of AA. Also, most of the biomarkers were significantly correlated with the serum parameters, which reveal the reliability of these variables as indicators of AA.

Conclusion: All types of leukocyte cells were found as reliable biomarkers to diagnose AA patients from HC. Also, FEV1%, Total WBC, and serum total IgE, ECP, Tryptase, IL-18, and CD-20 were found as reliable biomarkers for the severity of AA.

Keywords: Allergy, Asthma, Biomarkers, Leukocyte Blood Cells, Lung Function, Serum Parameters.

International Journal of Pharmaceutical Quality Assurance (2020); DOI: 10.25258/ijpqa.11.2.16

How to cite this article: Shareef SH, Amin K. Biomarkers of allergic asthma and their association with serum parameters. International Journal of Pharmaceutical Quality Assurance. 2020;11(2):281-288.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Over 300 million people all over the world are suffering from asthma, which is referred to as a chronic inflammatory disease with multifactorial etiologies. Asthma has long been regarded as common airway EI, caused by allergic sensitization, leading to acute bronchoconstriction and airway hyper-responsiveness (AHR).[1] AA is initiated as a result of contact with aeroallergens, like house dust mite and pollen, which have proteolytic features and a small number of bacterial components like lipopolysaccharides.[2]

Asthma is a heterogeneous disease that is detected through its intermittent signs, including chest tightness, cough, and wheeze, which are resulting from reversible airflow obstruction.[3] Several phenotypes of asthma have been defined based on disease severity, the presentation of symptoms, the age of their onset, and the existence of other conditions, like eosinophilia and allergy with various long-term complications, and response to corticosteroids (CS) therapy.[4] Although asthma phenotypes have been defined, the international global initiative for asthma (GINA) guidelines emphasize the severity of asthma as a basis for its management.[4]

Type 2 cytokines like IL-4, IL-5, and IL-13 play a significant role in eosinophilic asthma (EA), which can be controlled by CS as a key anti-inflammatory agent to control inflammation associated with this genotype of asthma.[5,6] Fractional excretion of nitric oxide (FeNO) concentration, serum periostin level, sputum eosinophil count, and total eosinophil cell (TEC) are the traditional biomarkers that are used to monitor EI in asthma.[7] In cases with SA, TEC is a biomarker that can predict favorable responses to anti-IL-5 antibody treatment.[8] It is vital to identify an appropriate biomarker with the capacity to effectively reflect EI in asthma. It has been reported that periostin is related to type 2 airway inflammation in asthma, and eosinophil derived neurotoxin

Author for Correspondence: suhayla.shareef@yahoo.com

(EDN) represents eosinophil activation in asthma in children; however, its value as a serum biomarker in adult asthma has not been assessed yet.[9]

Defining appropriate biomarkers is significant in order to be able to predict the disease course and the response to therapy.[4,10] A biomarker is defined as a measurable indicator through which normal or pathological biological processes or pharmacologic responses to therapeutic intervention are assessed.[4] For a biomarker to be valid, it needs to have some key features, including cost-effectiveness, being quantifiable with certain performance, ease of collection in the real-world setting, reliability, and reproducibility, changeability with disease progression, normalization with successful treatment, being informative about disease prognosis and clinical outcomes, and high predictive ability to distinguish between disease and health.[11,12]

Although research studies have tried to identify applicable biomarkers in clinical practice for managing of asthma, only a few predictive biomarkers have been described for asthma, including periostin, FeNO, eosinophils in blood and/or sputum, IgE, and there are still controversies over their utility in diagnosis, prognosis, and therapy.[10,13] In this regard, the present study was carried out to identify AA biomarkers and their association with serum parameters in patients with AA.

MATERIALS AND METHODS

Study Design and Setting

The present cohort study was conducted at the Allergy and Asthma Center in Sulaimani, the Kurdistan region-Iraq from January 2017 to November 2018.

Study Population

The present study consisted of 66 non-smoking patients with AA. Patients were diagnosed and its severity was determined by a specialist physician based on the GINA guideline criteria.[14] Other inclusion criteria were positive for specific IgE, elevation in total serum IgE levels, a pulmonary function test (PFT), and a minimum of two symptoms consistent with asthma (assessed for cough, wheeze, and dyspnea). Individuals with other lung-related or medical illnesses, exacerbated asthma, respiratory infection, systemic diseases, chronic pulmonary disorders other than asthma, gastroesophageal reflux disease, cardiac disease, and those treated with systemic CS within 4 weeks before this study were excluded. The study also consisted of 61 HC who were age- and sex-matched with the patients and were chosen based on the following criteria: (1) no history of respiratory or other diseases that might interfere with the results, and (2) baseline forced expiratory volume in 1 s (FEV1) > 80% predicted and FEV1/forced vital capacity (FVC) ratio > 0.7.

Data Collection

Six ml of peripheral blood was taken from each subject. 3 mL were separated and placed into EDTA tubes for total blood cell count. The remaining 3 mL was placed in a gel tube to obtain the serum. The tubes were centrifuged at 10,000 rpm for 10 minutes, and the serum was put into 1.5 mL Eppendorf tube and stored at –80°C until cytokine level estimation was carried out.

Blood samples were collected for the measurement of total and specific serum IgE using a human IgE ELISA kit (quantitative solid-phase sandwich enzyme immunoassay technique) provided by (cloud-Clone Corp, USA) (Catalog number: SEA545Hu) and Inhalation Middle East (IgE) (Euroimmun, UK) (DP 3117-1601E), according to the manufacturer's protocol. Specific IgE was measured against *Dermatophagoides pteronyssinus, Dermatophagoides farinae, Penicillium notatum, Cladosporium herbarum, Aspergillus fumigatus, Candida albicans, Alternaria alternate,* sweet vernal grass, timothy grass, cultivated rye, alder, birch, oak, olive tree, common ragweed, mugwort, dandelion, cockroach, cat, and hamster. The detection of specific IgE of ≥ 0.35 kU/L was used as a definition of sensitization to a specific allergen. IgE sensitization was defined as sensitization to at least one of the investigated allergens.

Levels of serum concentration eosinophil cationic protein (ECP) ng/mL, tryptase pg/mL, IL-18 pg/mL, and membrane spanning 4 domains subfamily a, member 1 (MS4A1)/(CD-20) ng/mL were determined by ELISA, and the Cloud Clone Corp kit for tryptase and IL-18 (Cloud-Clone Corp, USA) (Catalog No: SEB070Hu) and (Catalog No.: SEA064Hu), while for ECP (CUSABIO, USA) (Catalog No.: CSB-E11729h) and CD-20 (MyBioSource, USA) (Catalog No.: MBS455171) were used according to the manufacturer's protocol.

Computerized spirometer (Jaeger-Toennier, Germany) was used as the PFT to measure FEV1 predicted percent of the patients at their enrollment in the study, and when indicated according to studies design. Also, a standard spirometry method was used to measure the lung function parameters (i.e., FEV1, FVC, and FEV1/FVC).

Statistical Analysis

The Mann-Whitney U test, t-test, contingency χ^2 test, and Kruskal Wallis H test were used to compare the unpaired continuous and dichotomous variables. Spearman's rank coefficient determines and calculates the cut-off points. Rank correlation analysis defined correlations among the continuous variables. All statistical analyses were performed through Prism 7.0 software (Graphpad, La Jolla, California, USA). $p < 0.05$ was considered significant.

Ethical considerations

Informed consent was obtained from all of the subjects prior to participation. Also, the study protocol was approved by the Ethics Scientific Committee of the College of Medicine, Sulaimani University, Iraq.

RESULTS

The results of the study indicated that the mean age of AA patients and HC were respectively 39.09 and 39.95 years, and they were not significantly different in this regard ($p = 0.54$). Females dominated both groups with 63.64 and 65.57% in the AA and HC, respectively, and they were not significantly

different in this regard (p = 0.81). The two groups were significantly different in terms of their symptoms (p < 0.0001) and family history (p = 0.0058). The duration of asthma was found to be 9.0 ± 1.113 years, with a range from 1 to 34 years (Table 1).

Comparing the three AA groups with symptoms scores (SS) of 1, 2, and 3, revealed that they were significantly different in terms of FEV1% predicted (p < 0.0001), Total WBC (TWBCs) cells/mcL (p = 0.001), Total lymphocyte (TLC) cells/mcL (p = 0.0004), Total monocyte (TMC) cells/mcL (p = 0.031), Total eosinophile (TEC) cells/mcL (p = 0.017), Total basophile (TBC) cells/mcL (p = 0.006), serum total IgE ng/mL (p = 0.0002), ECP ng/mL (p = 0.041), Tryptase pg/mL (p = 0.026), IL-18 pg/mL (p = 0.0003), and CD-20 ng/mL (p < 0.0001). However, there was no significant difference between them regarding their total neutrophile (TNC) cells/mcL (p = 0.072) (See Table 2).

Further, comparison of the group with AA with the HC, revealed they were significantly different in terms of TWBCs, TMC, TNC, TEC, TBC, IgE, ECP, Tryptase, IL-18, and CD-20 (p < 0.001). As shown in Table 3 and Figure 1, the mean values of all these biomarkers and parameters were significantly higher in the AA group than the HC. While the mean value of FEV1 decreased in AA than HC. Also, the two groups were not significantly different regarding TLC (p > 0.05) (Table 3 and Figure 1).

Table 1: Demographic characteristics of the study subjects

Groups / *Variables*	*AA patients* (*n = 66*)	*Healthy control (n = 61)*	*Statistical test* t/X^2	*p value*
Age (yr; mean ± SE)	39.09 ± 1.19	39.95 ± 0.7	t = 0.608	0.543 ns
Gender (N, %)				
Male	24 (36.36)	21(34.43)	X^2 = 0.052	0.819
Female	42 (63.64)	40(65.57)		ns
Symptoms (mean ± SE) (cough, wheezes, dyspnea)	2.591 ± 0.08	0.131 ± 0.043	t = 26.21	< 0.0001****
1 symptom	6 (9.09)			
2 symptom	15 (22.72)			
3 symptom	45 (68.18)			
Family History (mean ± SE)	0.681 ± 0.12	0.245 ± 0.095	t = 2.806	0.0058**
Non Relative	39 (59.09)	54 (88.52)		
1st Relative	15 (22.72)	2 (3.28)		
2nd Relative	6 (9.09)	2 (3.28)		
3rd Relative	6 (9.09)	3 (4.92)		
Duration of asthma (yr; mean ± SE)	Range (1–34) 9 ± 1.113	NA	–	-–

Table 2: Correlation between lung function, all types of blood leukocyte cells and serum parameter in the three SS groups in AA patients

Groups / *Variables*	*Group A* (*Symptom score 1*) *N = 6*	*Group B* (*Symptom score 2*) *N = 15*	*Group C* (*Symptom score 3*) *N = 45*	*p value*
		Kruskal-Wallis test		
FEV1% predicted		23.64		< 0.0001****
Total WBC (cells/mcL)		13.63		0.0011**
Total lymphocyte (cells/mcL)		15.74		0.0004***
Total monocyte (cells/mcL)		6.946		0.031*
Total neutrophile (cells/mcL)		5.239		0.0729
Total eosinophile (cells/mcL)		8.072		0.0177*
Total basophile (cells/mcL)		10.03		0.0066**
Serum total IgE (ng/mL)		16.92		0.0002***
Serum ECP (ng/mL)		6.343		0.0419*
Serum Tryptase (pg/mL)		7.228		0.0269*
Serum IL-18 (pg/mL)		16.46		0.0003***
Serum CD-20 ng/mL		20.43		< 0.0001****

Table 3: Comparison of all types of blood leukocyte cells across study groups

Groups	*Allergic asthma*	*Healthy control*			
Variables	*(N = 66) Mean ± SE*	*(N = 61) Mean ± SE*	*95% CI*	*t-test*	*p-value*
Total leukocyte (cells/mcL)	8,126 ± 214.6	6,608 ± 190.3	946.4 – 2089	5.257	<0.0001 ****
Total monocyte (cells/mcL)	5,01.4 ± 16.77	3,77.4 ± 16.42	77.5 – 170.6	5.273	<0.0001 ****
Total lymphocyte (cells/mcL)	2,285 ± 92.5	2,054 ± 70.19	–1.607– 463.6	1.965	0.051 ns
Total neutrophile (cells/mcL)	4,739 ± 144.7	4,075 ± 142.7	260.5 – 1067	3.259	0.001 **
Total eosinophile (cells/mcL)	5,44.9 ± 40.79	89.9 ± 5.859	370.3 – 539.8	10.63	<0.0001 ****
Total basophile cells/mcL	76.68 ± 4.181	37.15 ± 3.564	28.57 -50.48	7.139	<0.0001 ****

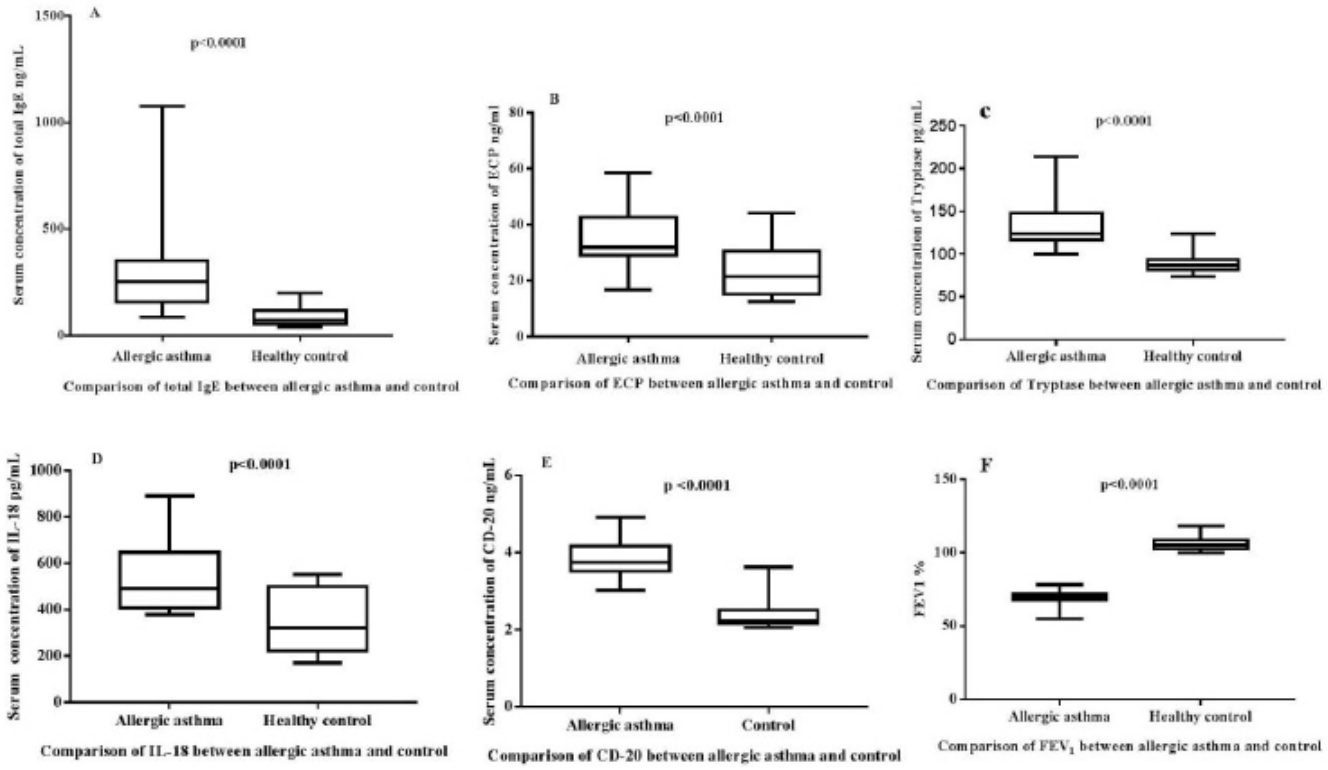

Figure 1: Box-whiskers plot of sero-biomarkers concentration and PFT across study groups. A) Total serum IgE; B) ECP; C) Tryptase; D) IL-18; E) CD-20 and F) FEV1 respectively.

Analyzing the association between the biomarkers in the AA patients indicated that TWBC had a significant correlation with all other biomarkers (i.e., TMC, TLC, TNC, TEC, and TBC) ($p < 0.001$). TMC had a significant correlation with TLC, and TBC ($p < 0.0001$). TLC was significantly correlated with TEC ($p < 0.05$) and TBC ($p < 0.0001$). TNC was found to be significantly correlated with TEC ($p < 0.05$). Finally, the TEC had a significant correlation with TBC ($p < 0.05$) (Table 4).

Analyzing the correlation between the biomarkers and other parameters indicated that TWBC had a highly significant correlation with total IgE, ECP, tryptase, IL-18, and CD-20 ($p < 0.001$). Also, TMC had a significant correlation with ECP ($p < 0.001$), IL-18, and CD-20 ($p < 0.05$). TLC was found to be significantly correlated with total IgE, ECP, tryptase, IL-18, and CD-20 ($p < 0.05$). Moreover, TNC had a significant correlation with total IgE, ECP, tryptase, IL-18, and CD-20 ($p < 0.05$). In addition, TEC was significantly correlated with total IgE, ECP, tryptase, and IL-18 ($p < 0.05$). Finally, TBC had a significant correlation with total IgE, ECP, Tryptase, IL-18, and CD-20 ($p < 0.05$) (Table 5).

Table 4: Correlation between the studied biomarkers in AA patients

Parameters	*Total monocyte cells/mcL*	*Total lymphocyte cells/mcL*	*Total neutrophile cells/mcL*	*Total eosinophile cells/mcL*	*Total basophile cells/ mcL*
Total WBC (cells/mcL)					
Spearman r	0.421	0.528	0.818	0.494	0.535
95% CI	0.192 – 0.606	0.322 – 0.687	0.714 – 0.886	0.280 – 0.661	0.330 – 0.692
Sig. (2-tailed)	0.0004	<0.0001	<0.0001	<0.0001	<0.0001
p value	***	****	****	****	****
Total monocyte (cells/mcL)					
Spearman r		0.713	0.079	0.245	0.643
95% CI		0.564 – 0.817	-0.173 – 0.321	-0.003- 0.466	0.469-0.769
Sig. (2-tailed)		<0.0001	0.528	0.0467	<0.0001
p value		****	ns	*	****
Total lymphocyte (cells/mcL)					
Spearman r			0.026	0.256	0.748
95% CI			–0.223 – 0.273	0.008 – 0.475	0.613 – 0.840
Sig. (2-tailed)			0.832	0.037	<0.0001
p value			ns	*	****
Total neutrophile (cells/mcL)					
Spearman r				0.264	0.182
95% CI				0.016 – 0.481	–0.069 – 0.412
Sig. (2-tailed)				0.0320	0.142
p value				*	ns
Total eosinophile (cells/mcL)					
Spearman r					0.277
95% CI					0.030 – 0.492
Sig. (2-tailed)					0.024
p value					*

Table 5: Correlation between the studied biomarkers and serum parameters in AA patients

Parameters	*Serum total IgE (ng/mL)*	*Serum ECP (ng/mL)*	*Serum Tryptase (pg/mL)*	*Serum IL-18 (pg/mL)*	*Serum MS4A1 (ng/mL)*
Total WBC cells/mcL					
Spearman r	0.411	0.483	0.662	0.506	0.445
95% CI	0.181 – 0.599	0.267 – 0.654	0.494 – 0.782	0.294 – 0.670	0.221 – 0.625
Sig. (2-tailed)	0.0006	<0.0001	<0.0001	<0.0001	0.0002
p value	***	****	****	****	***
Total monocyte cells/mcL					
Spearman r	0.239	0.437	0.214	0.280	0.251
95% CI	– 0.010 – 0.460	0.211 – 0.618	– 0.036 – 0.44	0.033 – 0.494	0.002 – 0.471
Sig. (2-tailed)	0.052	0.0002	0.083	0.022	0.041
p value	ns	***	ns	*	*
Total lymphocyte cells/mcL					
Spearman r	0.361	0.514	0.391	0.312	0.370
95% CI	0.124 – 0.560	0.304 – 0.676	0.157 – 0.583	0.068 – 0.520	0.133 – 0.566
Sig. (2-tailed)	0.002	<0.0001	0.0012	0.010	0.002
p value	**	****	**	*	**
Total neutrophile cells/mcL					
Spearman r	0.361	0.365	0.592	0.469	0.355
95% CI	0.124 – 0.560	0.127 – 0.562	0.402 – 0.733	0.249 – 0.642	0.116 – 0.555
Sig. (2-tailed)	0.002	0.002	<0.0001	<0.0001	0.003
p value	**	**	****	****	**
Total eosinophile cells/mcL					
Spearman r	0.274	0.269	0.305	0.342	0.229
95% CI	0.027 – 0.489	0.021 – 0.485	0.061 – 0.515	0.102 – 0.545	– 0.020 – 0.452
Sig. (2-tailed)	0.025	0.028	0.012	0.004	0.064
p value	*	*	*	**	ns
Total basophile cells/mcL					
Spearman r	0.381	0.424	0.365	0.531	0.307
95% CI	0.146 – 0.575	0.196 – 0.608	0.128 – 0.563	0.325 – 0.689	0.063 – 0.516
Sig. (2-tailed)	0.001	0.0004	0.002	<0.0001	0.0120
p value	**	***	**	****	*

DISCUSSION

The characteristics of asthma have been understood well through recent large-scale studies, which have confirmed that asthma is significantly correlated with some unique clinical/demographic characteristics, including respiratory comorbidities, atopy (increased aeroallergens sensitization) rate, low baseline lung function, frequent exacerbations, female sex, smoking, and older age.[15] The results of the present study are in line with those that demonstrated that asthma characteristics would be affected by an increase in age.[16] On the other hand, female sex and asthma were not significantly correlated. These results are in line with the results of the present study, which demonstrated that the factor of sex could not have any significant effects on the development of AA. The association between these characteristics and asthma was also confirmed by Youngsoo *et al*.[17] The AA patients in the present study were found to have symptoms like cough, wheezes, and dyspnea. Similarly, Merin *et al*. reported that patients with AA have certain symptoms, such as, chest tightness, cough, and shortness of breath, wheezing, and variable airflow obstruction.[18]

The airway lumen, the airway submucosa, and the peripheral blood have been reported to include eosinophils whose presence is not always consistent. As initially leukocytes dwelling in tissues, blood counts cannot necessarily indicate the whole involvement of eosinophils in the affected tissues.[19,20] However, in the present study, they were significantly different in terms of WBC cells/mcL and TMC cells/mcL. As concluded by Wan *et al*., the number of monocytes in the blood (monocytosis) rises in response to chronic infections in certain cancers, blood disorders, and autoimmune disorders.[21] Langerhans cell histiocytosis, sarcoidosis, and infections can lead to a rise in the number of macrophages in parts of the body like the skin, lungs, and other organs, but not in the blood. Moreover, a bone marrow disorder, chemotherapy, or bloodstream infection can lead to a decrease in the overall WBC count, which in turn causes monocytopenia.[22]

Based on the results of the present study, the number of FEV could significantly affect the SS of patients with AA. These results are in line with Harold *et al*., who demonstrated that a small decrease in FEV affects asthma symptoms. However, the level of serum eosinophil protein and TEC and are indicators of the short-term rise in asthma symptoms like decreased FEV1, wheezing, cough, dyspnea, AHR, and the need for CS treatment in patients with mild to moderate asthma.[23] Independent of asthma status, neutrophils are the most abundant kind of cell found in induced sputum samples.[24] In the present study, there was not a remarkable difference between various serums regarding their TNC cells/mcL. In addition, the number of their airway neutrophils has been reported with a remarkable increase in patients with SA.[25] It should further be noted that serum IL-18 results in neutrophils that produce enzymes and other factors that play a role in the activity of eosinophil.[26] Research has indicated that neutrophilic and EA are not mutually exclusive conditions; however, neutrophilic inflammation is accompanied by lower levels of FEV1.[25] These findings are in line with the significant correlations shown in the present study.

As shown in the present study, serum total IgE ng/mL is one of the main biomarkers of AA. This finding was also reported by Matsui *et al*..[27] The release of proinflammatory mediators like leukotrienes, prostaglandins, histamine, and tryptase is activated by the subsequent binding of allergens to allergen-specific IgE, as a result of which allergic symptoms emerge.[28,29] In agreement with the findings of the present study, levels of serum IgE have a close correlation with the presence and severity of asthma in children, adolescents, and adults.[30] Serum IgE levels are associated with AHR, even in patients who do not have a history of asthma symptoms or atopy.[31] Increased values of serum IgE are associated with wheeze probability and decreased lung function.[31] Responsiveness to anti-IgE therapy is predicted through serum total IgE, which is not, however, useful for monitoring response. In addition, bronchial mucosal IgE and mast cells may be a helpful biomarker.[32]

In accordance with the present study, external counterpulsation (ECP) is one of the main proteins of eosinophil cytotoxic, which could significantly affect the symptoms of AA patients. This result is in line with those reported by Persson[33] who introduced major basic protein (MBP), eosinophil peroxidase (EPX), ECP, and EDN as the eosinophil cytotoxic proteins.[33] According to recent studies, unlike total numbers, eosinophil degranulation products can be a better indicator of the activation status of eosinophil. Research has indicated that wheezy patients and healthy controls can be differentiated by ECP concentration,[34] which is in line with the findings of the present study. Similarly, a recent study with repeated nasal lavage in 147 healthy school children revealed a relatively high intra-individual variability of nasal ECP concentrations.[35]

According to the results of a meta-analysis and of systematic review blood biomarkers, peripheral TEC was found to have a specificity of 77% and sensitivity of 71% for detecting sputum eosinophils >3 %, and the extreme levels of blood eosinophil might be more specific and sensitive for detecting sputum eosinophils.[36,37] For instance, airway eosinophilia is highly unlikely to be seen in patients who have TEC < 90 cells/mL; however, significant sputum eosinophilia is expected to be observed in almost all patients who have >400 cells/mL of blood.[38] As a reference value, TEC > 300 cells/mL and sputum eosinophil levels (>2–3%) might be used to define eosinophilic disease.[39]

Based on the results obtained in the present study, there is a significant association between the TEC of the AA patients and the TWBCs. This finding is in agreement with those reported by Hastie *et al*., who indicated different correlations between peripheral eosinophilia and airway inflammation.[40] Therefore, as shown in research, airway eosinophil activity cannot be fully shown by blood eosinophils,[40] which questions its reliability

as a good biomarker. Based on the result of the present study, the TEC may significantly affect the SS of patients with AA. Moreover, the TEC might not be a sensitive biomarker for diagnosing the most severe T2-high asthmatics, because there is an inverse relationship between elevated disease severity and sputum/blood eosinophilia ratio, particularly in patients with steroid-dependent asthma.[18,40]

CONCLUSION

Reliable diagnosis of patients with AA from healthy individuals can be performed through total blood leukocyte cells (TWBC, TMC, TNC, TEC, and TBC). Moreover, the severity of AA can reliably be determined through biomarkers, such as FEV1%, TWBC, and serum total IgE, ECP, Tryptase, IL-18, and CD-20 (MS4A1).

REFERENCES

1. Yoo K-H, Ahn H-R, Park J-K, Kim J-W, Nam G-H, Hong S-K, *et al*. Burden of respiratory disease in Korea: an observational study on allergic rhinitis, asthma, COPD, and rhinosinusitis. Allergy, asthma & immunology research. 2016;8(6):527-534.
2. Su Z, Lin J, Lu F, Zhang X, Zhang L, Gandhi N, *et al*. Potential autocrine regulation of interleukin-33/ST2 signaling of dendritic cells in allergic inflammation. Mucosal immunology. 2013;6(5): 921.
3. Ray A, Raundhal M, Oriss TB, Ray P, Wenzel SE. Current concepts of severe asthma. The Journal of clinical investigation. 2016;126(7):2394-403.
4. Books S. User menu. Religion. 2015;15:05.
5. Castro M, Zangrilli J, Wechsler ME, Bateman ED, Brusselle GG, Bardin P, *et al*. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. The Lancet Respiratory Medicine. 2015;3(5):355-366.
6. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, *et al*. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. The Lancet. 2012;380(9842):651-659.
7. Kim M-A, Izuhara K, Ohta S, Ono J, Yoon MK, Ban GY, *et al*. Association of serum periostin with aspirin-exacerbated respiratory disease. Annals of Allergy, Asthma & Immunology. 2014;113(3):314-320.
8. Goldman M, Hirsch I, Zangrilli JG, Newbold P, Xu X. The association between blood eosinophil count and benralizumab efficacy for patients with severe, uncontrolled asthma: subanalyses of the Phase III SIROCCO and CALIMA studies. Current medical research and opinion. 2017;33(9):1605-1613.
9. Kim C-K. Eosinophil-derived neurotoxin: a novel biomarker for diagnosis and monitoring of asthma. Korean Journal of paediatrics. 2013;56(1):8.
10. Eguiluz-Gracia I, Tay TR, Hew M, Escribese MM, Barber D, O'hehir RE, *et al*. Recent developments and highlights in biomarkers in allergic diseases and asthma. Allergy. 2018;73(12):2290-2305.
11. Fitzpatrick AM. Biomarkers of asthma and allergic airway diseases. Annals of Allergy, Asthma & Immunology. 2015;115(5):335-340.
12. Kelly RS, Dahlin A, McGeachie MJ, Qiu W, Sordillo J, Wan ES, *et al*. Asthma metabolomics and the potential for integrative omics in research and the clinic. Chest. 2017;151(2):262-277.
13. Villaseñor A, Rosace D, Obeso D, Pérez-Gordo M, Chivato T, Barbas C, *et al*. Allergic asthma: an overview of metabolomic strategies leading to the identification of biomarkers in the field. Clinical & Experimental Allergy. 2017;47(4):442-456.
14. Bateman ED, Hurd S, Barnes P, Bousquet J, Drazen J, FitzGerald M, *et al*. Global strategy for asthma management and prevention: GINA executive summary. European Respiratory Journal. 2008;31(1):143-178.
15. Dolan CM, Fraher KE, Bleecker ER, Borish L, Chipps B, Hayden ML, *et al*. Design and baseline characteristics of the epidemiology and natural history of asthma: Outcomes and Treatment Regimens (TENOR) study: a large cohort of patients with severe or difficult-to-treat asthma. Annals of Allergy, Asthma & Immunology. 2004;92(1):32-39.
16. Moore WC, Bleecker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, *et al*. Characterization of the severe asthma phenotype by the national heart, lung, and blood institute's severe asthma research program. Journal of Allergy and Clinical Immunology. 2007;119(2):405-413.
17. Lee Y, Lee J-H, Yang E-M, Kwon E, Jung C-G, Kim S-C, *et al*. Serum Levels of Eosinophil-Derived Neurotoxin: A Biomarker for Asthma Severity in Adult Asthmatics. Allergy, asthma & immunology research. 2019;11(3):394-405.
18. Kuruvilla ME, Lee FE-H, Lee GB. Understanding asthma phenotypes, endotypes, and mechanisms of disease. Clinical reviews in allergy & immunology. 2019;56(2):219-233.
19. Desai D, Newby C, Symon FA, Haldar P, Shah S, Gupta S, *et al*. Elevated sputum interleukin-5 and submucosal eosinophilia in obese individuals with severe asthma. American Journal of respiratory and critical care medicine. 2013;188(6):657-663.
20. Szefler SJ, Wenzel S, Brown R, Erzurum SC, Fahy JV, Hamilton RG, *et al*. Asthma outcomes: biomarkers. Journal of Allergy and Clinical Immunology. 2012;129(3): S9-S23.
21. Eo WK, Da Wun Jeong HJC, Won KY, Choi SI, Kim SH, Chun SW, *et al*. Absolute monocyte and lymphocyte count prognostic score for patients with gastric cancer. World Journal of Gastroenterology: WJG. 2015;21(9):2668.
22. Batty N, Ghonimi E, Feng L, Fayad L, Younes A, Rodriguez MA, *et al*. The absolute monocyte and lymphocyte prognostic index for patients with diffuse large B-cell lymphoma who receive R-CHOP. Clinical Lymphoma Myeloma and Leukemia. 2013;13(1):15-18.
23. Kim H, Ellis AK, Fischer D, Noseworthy M, Olivenstein R, Chapman KR, *et al*. Asthma biomarkers in the age of biologics. Allergy, Asthma & Clinical Immunology. 2017;13(1):48.
24. Fahy JV. Eosinophilic and neutrophilic inflammation in asthma: insights from clinical studies. Proceedings of the American Thoracic Society. 2009;6(3):256-259.
25. Shaw DE, Berry MA, Hargadon B, McKenna S, Shelley MJ, Green RH, *et al*. Association between neutrophilic airway inflammation and airflow limitation in adults with asthma. Chest. 2007;132(6):1871-1875.
26. Trevor J, Deshane J. Refractory asthma: mechanisms, targets, and therapy. Allergy. 2014;69(7):817-827.
27. Matsui E, Sampson H, Bahnson H, Gruchalla R, Pongracic JA, Teach S, *et al*. Allergen-specific IgE as a biomarker of exposure plus sensitization in inner-city adolescents with asthma. Allergy. 2010;65(11):1414-1422.
28. Holgate S, Smith N, Massanari M, Jimenez P. Effects of omalizumab on markers of inflammation in patients with allergic asthma. Allergy. 2009;64(12):1728-1736.

29. Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. Journal of Allergy and Clinical Immunology. 2003;111(2):S486-S94.
30. Haselkorn T, Szefler SJ, Simons F, Zeiger RS, Mink DR, Chipps BE, *et al*. Allergy, total serum immunoglobulin E, and airflow in children and adolescents in TENOR. Pediatric Allergy and Immunology. 2010;21(8):1157-1165.
31. Gerald JK, Gerald LB, Vasquez MM, Morgan WJ, Boehmer SJ, Lemanske Jr RF, *et al*. Markers of differential response to inhaled corticosteroid treatment among children with mild persistent asthma. The Journal of Allergy and Clinical Immunology: In Practice. 2015;3(4):540-6.e3.
32. Pillai P, Chan Y-C, Wu S-Y, Ohm-Laursen L, Thomas C, Durham SR, *et al*. Omalizumab reduces bronchial mucosal IgE and improves lung function in non-atopic asthma. European Respiratory Journal. 2016;48(6):1593-1601.
33. Persson C. Lysis of primed eosinophils in severe asthma. Journal of Allergy and Clinical Immunology. 2013;132(6): 1459-1460.
34. Stoleski S, Minov J, Karadzinska-Bislimovska J, Mijakoski D, Atanasovska A. Eosinophil Cationic Protein Concentrations among Crop and Dairy Farmers with Asthma. Open Access Maced J Med Sci. 2018 Mar 15;6(3):456-462.
35. De Lima PO, Dos Santos FV, Oliveira DT, Carvalho de Figueiredo R, Pereira MC. Effect of eosinophil cationic protein on human oral squamous carcinoma cell viability. Molecular and clinical oncology. 2015;3(2):353-356.
36. Wagener AH, de Nijs SB, Lutter R, Sousa AR, Weersink EJ, Bel EH, *et al*. External validation of blood eosinophils, FENO and serum periostin as surrogates for sputum eosinophils in asthma. Thorax. 2015;70(2):115-120.
37. Arron JR, Choy DF, Scheerens H, Matthews JG. Noninvasive biomarkers that predict treatment benefit from biologic therapies in asthma. Annals of the American Thoracic Society. 2013;10(Supplement): S206-S213.
38. Westerhof GA, Korevaar DA, Amelink M, de Nijs SB, de Groot JC, Wang J, *et al*. Biomarkers to identify sputum eosinophilia in different adult asthma phenotypes. European respiratory Journal. 2015;46(3):688-696.
39. Coumou H, Bel EH. Improving the diagnosis of eosinophilic asthma. Expert review of respiratory medicine. 2016;10(10):1093-1103.
40. Hastie AT, Moore WC, Li H, Rector BM, Ortega VE, Pascual RM, *et al*. Biomarker surrogates do not accurately predict sputum eosinophil and neutrophil percentages in asthmatic subjects. Journal of allergy and clinical immunology. 2013;132(1):72-80. e12.