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Original Research Article

Assessing Serum Level of Different Biomarkers in Children with Asthma to Evaluate Their Role in Response to Treatment

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Conflict of interest: Nil

Abstract:

Aim: The aim of the present study was to examine the changes of serum levels of biomarkers which may occur as a result of treatment in children with asthma.

Material & Methods: The present study was conducted for 1 year and 50 children who were referred to Upgraded Department of Pediatrics to evaluate the changes of biomarkers (Immunoglobulin E, Eosinophil and Eosinophil Cationic Protein), 5ml peripheral blood samples were drawn before and after the treatment period of six-month. They were measured by ELISA method. The data were analyzed by SPSS software ver.16.0 using descriptive statistics and Paired Sample t test.

Results: According to the results, 70% of the children were male and 30% were female. In order to investigate the role of biomarkers in the evaluation of the treatment and management of asthma, the results of the analysis showed that serum levels of Periostin, ECP, IgE and number and percentage of eosinophil decreased after six months of treatment. Based on the results of pairedt-test, there was a significant difference between the mean biomarkers studied in beginning of the study and the six months later. Comparison of mean blood cells before and after treatment showed a significant difference in white blood cells and erythrocyte sedimentation rate. In addition to the main findings, regarding the relationship between the demographic variables and the biomarkers levels, result of independent t-test showed that there was no significant relationship between gender and serum biomarkers in pre-treatment and post-treatment measurements (P>0.05). Also, based on Pearson test results, there was no significant relationship between age and serum biomarkers in pre-treatment and post-treatment measurements (P>0.05).

Conclusion: The biomarkers serum levels in the children were reduced after the end of the treatment period. Thus, in this study, the role of selective biomarkers in asthma management was confirmed.

Keywords: Asthma, Biomarkers, Children.

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Introduction

Asthma affects approximately 340 million people worldwide and is the leading noncommunicable lung disease in children. [1] In addition, asthma is the most common chronic disease in the children with its incidence estimated as 5- 10 percent. [2,3] In this disorder, the airways chronic inflammation results in limitations of airflow, which cause various symptoms. [2,4] Genetic and environmental factors influence these disorders which their mechanisms have not been identified completely. [5] Different phenotypes characterize distinct clinical characteristics and specific pathophysiological mechanisms. [6,7] Airway inflammation and hyperresponsiveness are the mainstay of asthma and govern the clinical scenario.

In this regard, type 2 inflammation is the predominant phenotype in asthmatic children and adolescents. [8] Type 2 inflammation occurs when at least one factor is decisive, including blood eosinophils > 300 cells/μL, fractioned exhaled nitric oxide (FeNO) > 35 ppb (in children), total IgE > 100 IU/mL, and allergy. [9,10] Type 2 airway inflammation is characterized by an

eosinophilic infiltrate closely associated with asthma exacerbations. [11,12] Therefore, abating eosinophilic inflammation could be an ideal therapeutic strategy for asthmatic patients. The identification of different phenotypes in pediatric asthma, with distinct clinical presentation, pathophysiologic mechanisms and response to therapeutic interventions, has led to the recognition of underlying molecular pathways (endotypes) and related specific indicators (biomarkers).

A biomarker is "a defined characteristic that is measured as an indicator of a normal biological process, pathological process or response to an exposure or intervention, including therapeutic interventions". [13] On time diagnosis, management of the symptoms and proper clinical decision are main factors in preventing the unpleasant consequences of disease. [14] Diagnosis, determination of diseases severity and patients responses to treatment are basically done according to the patient history, physical exams and some paraclinical measures including spirometry. [15,16] Also, many of the diagnostic tests do not show the main pathology clearly. According to the inflammatory nature of asthma, many immune system cells play a role in this disorder. [2] Investigation of these cells. and their products known as inflammatory biomarkers might be helpful in diagnosis and assessment of diseases severity and response to treatment. [17] In this regard, due to this fact that asthma is an allergic disease as type I sensitivity dispersion and considering the role of Eosinophil and Immunoglobulin E (IgE) in increase of this sensitivity and numerous reports on enhancement of Eosinophil and IgE in the patients with asthma [17]; these factors can be specified as asthma biomarkers. Recently, Eosinophil is the most available biomarker for management of asthma. [18] Eosinophil Cationic Protein (ECP) has been investigated and is one of the most important cation granular proteins secreted from activated Eosinophil and nowadays, it is one of the important biomarkers used for evaluation of Eosinophil activity in asthma. [19] Other biomarker is Periostin as a secreted extracellular matrix protein that plays a key role in increase of inflammation in allergic diseases. This protein leads to increase of Eosinophil in the tissue during inflammation and allergic processes which is associated with the epithelial fibroses process in asthma. [20]

Considering all the mentioned issues and this fact that the children asthma is mostly allergic type, examining the immune system factors and prediction of the disease stage in this age group seems essential. So measuring the levels of IgE, Eosinophil, Periostin, and eosinophil cationic protein (ECP) in blood of children with asthma can be helpful in evaluation of response to treatment. Thus, in order to investigate the role of biomarkers, this study examines the changes of serum levels of IgE, eosinophils, Priestin and ECP which may occur as a result of treatment in children with asthma.

Material & Methods

The present study was conducted for the duration of 1 year and children were referred to Upgraded Department of Pediatrics, Patna Medical College and Hospital, Patna, Bihar, India were examined. 50 children were included in the study.

Inclusion Criteria

Children aged 4 to 16 year with primary diagnosis of asthma. In order to receive the same interventions, children with moderate persistent asthma entered the study.

Methodology

The children demographic characterization and informations about their disease were documented in the questionnaire. For measuring the before treatment biomarkers serum levels, peripheral venous blood was withdrawn. Then, the children were treated for six months under the drug therapy of moderate persistent asthma according to Kendig and Chernick's protocol. Therefore, all of the children received same intervention with medications of short-acting beta-agonist (SABA), inhaled corticosteroid (ICS), long-acting beta-agonist (LABA), leukotriene receptor antagonist (LTRA) and Methylxanthines Dosages of these medications arranged based on child's age. After six months treatment period, the blood sampling was done for second time for measuring the after-treatment biomarkers serum levels.

The blood samples were collected in two tubes, one including EDTA anticoagulant (2 ml) and other without anticoagulant (3 ml). The tubes with EDTA were examined radially for complete blood count (CBC) for measuring the blood counts. The samples without anticoagulant were centrifuged for ten minutes and the serum samples were removed. The serum samples were divided into small vials (Ellicott) and transferred into freezer -70 degrees Celsius before conducting the test. Thus, the ECP, Periostin and IgE biomarkers were measured by Enzyme linked immunosorbent assay (ELISA) in the serum samples.

Statistical Analysis

After collecting the data, the statistical analysis was done using software SPSS version 16. The descriptive statistics including frequency, percentage; mean and standard deviation (SD) were used for describing the patients' characteristics and their serum levels. For evaluating correlation between demographic data and serum levels, the

independent t test and Pearson correlation coefficient were used. P-value less than 0.05 were considered significant statistically.

Results

Table 1: Mean age of the studied children

Age (month)	Mean (SD)	Min	Max	Number
Male	75.85 (28.72)	26	154	35
Female	74.86 (22.81)	46	115	15
Total	76..34 (27.1)	26	15440	50

According to the results, 70% of the children were male and 30% were female. Table.1 summarizes the mean and standard deviation of the children age.

Table 2: The comparison of the biomarkers in asthmatic children before and after the treatment

Biomarkers	Test	Mean	Std. deviation	t-test	P-value
ECP (pg/mL)	Before	48.8036	42.08153	7.697	0.000
	After	42.5810	39.04652		
POSTN (pg/mL)	Before	58.8912	39.81358	3.968	0.000
	After	49.9615	32.63296		
Eos (%)	Before	5.1502	3.06827	2.817	0.007
	After	4.0848	1.58587		
Eos Count(×1/µL)	Before	580.7620	376.82635	5.355	0.000
	After	316.7404	140.18024		
Serum total IgE (IU/ML)	Before	342.332	198.235	6.221	0.000
	After	136.870	101.312		

In order to investigate the role of biomarkers in the evaluation of the treatment and management of asthma, the results of the analysis showed that serum levels of Periostin, ECP, IgE and number and percentage of eosinophil decreased after six months of treatment. Based on the results of pairedt-test, there was a significant difference between the mean biomarkers studied in beginning of the study and the six months later.

Table 3: Comparison of the blood cell in asthmatic children before and after the treatment

Blood Cells	Test	Mean	Std. deviation	t-test	P- value
Hb (g/dl)	Before	13.1000	1.10778	1.208	0.232
	After	13.0111	.93419		
PLT ($\times10^3$/µL)	Before	318.42	57.93	2.011	0.079
	After	306.74	43.71		
ESR (min)	Before	9.0370	7.71415	2.480	0.016
	After	6.5926	4.16870		
WBC (×1/µL)	Before	11348.8889	4047.41924	6.995	0.000
	After	8150.3704	2789.23061		

Comparison of mean blood cells before and after treatment showed a significant difference in white blood cells and erythrocyte sedimentation rate. In addition to the main findings, regarding the relationship between the demographic variables and the biomarkers levels, result of independent t-test showed that there was no significant relationship between gender and serum biomarkers in pre-treatment and post- treatment measurements (P>0.05). Also, based on Pearson test results, there was no significant relationship between age and serum biomarkers in pre-treatment and post-treatment measurements (P>0.05).

Discussion

It is estimated that asthma which has affected about 300 million people all over the world will be increased to 400 million people in 2025. [21] In addition, asthma is the most common chronic disease in the children which its incidence is estimated 5-10 percent. [22,23] In this disorder, the airways chronic inflammation results in limitations of extensive and varied reversible airflow, which cause various symptoms. [22,24] Genetic and environmental factors influence these disorders which their mechanisms have not been identified completely. [25] These factors can influence the disease severity, patient response to treatment and symptoms such as recurrent wheezing, shortness of breath, and cough. [26,27] The mortality of asthma in the world is 0-0.7 in 100 children which can be prevented. [22]

According to the results, 70% of the children were male and 30% was female. In order to investigate the role of biomarkers in the evaluation of the treatment and management of asthma, the results of the analysis showed that serum levels of Periostin, ECP, IgE and number and percentage of eosinophil decreased after six months of treatment. Based on the results of pairedt-test, there was a significant difference between the mean

biomarkers studied in beginning of the study and the six months later. Comparison of mean blood cells before and after treatment showed a significant difference in white blood cells and erythrocyte sedimentation rate. In addition to the main findings, regarding the relationship between the demographic variables and the biomarkers levels, result of independent t- test showed that there was no significant relationship between gender and serum biomarkers in pre-treatment and post- treatment measurements (P>0.05). Also, based on Pearson test results, there was no significant relationship between age and serum biomarkers in pre-treatment and post-treatment measurements (P>0.05). Based on the findings, serum levels of Periostin decreased significantly after 6 months treatment. This finding was similar to the results of related studies. Izuhara et al. and Parulekar et al. showed that the serum levels of Periostin can show asthma prognosis and how to respond to treatment. [28,29] The study by Inoue et al. confirmed the higher serum levels of Periostin in children with asthma than in healthy individual. [30] It can be said that serum Periostin is an important biomarker for asthma due to two main reasons. First, this protein is easily released from the inflamed tissue into the bloodstream. Therefore, its serum level indicates the amount of its production from inflamed tissue and the extent of inflammation. Secondly, the serum levels of this protein are physiologically (normal) very low (about 50 ng / ml). [31]

In another study, James and colleagues showed that there was a significant correlation in Periostin in the patients with asthma and inflammatory factors and the lung activity, but there was no significant difference between the two groups of asthma and healthy control in Periostin level. [32] Based on the findings of the current study and the results of other studies, it can be concluded that the ECP biomarker is more useful for assessing the severity of asthma and the response to treatment. A systematic study indicated that ECP biomarker is also increased in other atopic diseases, but this biomarker is not suitable for diagnosis and due to the increase in ECP in airway inflammation, it is beneficial for evaluating the progress of treatment and control of the disease. [33] According to the findings of the current study, the number and percentage of eosinophil and serum IgE levels had a significant relationship before and after asthma treatment. In two studies conducting on the children aged between 1 and 12 years old [34] and adults [35], the eosinophil and IgE values were compared in both healthy and patient groups.

Conclusion

The research results showed the ECP, Eosinophil, Periostin and IgE serum levels were reduced in the children with asthma at the end of the treatment period. Thus, in this study, the role of selective biomarkers in management of the children with asthma was confirmed. The physicians are able to change the disease symptoms from the subjective state to objective and measurable state reliably by measuring these biomarkers serum level.

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