



Serum Periostin Level in Children with Bronchial Asthma: A Comparative Study

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ABSTRACT

Background: Assessment of pulmonary function is important for diagnosis and monitoring of bronchial asthma. The role of biomarkers in assessment of bronchial asthma is an area of increasing interest. However, debate continues on the optimal parameters to be included and the role biomarkers play in the clinical assessment of asthma.

Aim: To evaluate the role of serum periostin level for assessment of children with bronchial asthma.

Method: this comparative study included 60 asthmatics and 30 healthy children as a control group; age and sex were matched. They were classified according to severity and level of asthma control. We investigated the correlation between serum periostin level, spirometer parameters and their relation to severity of bronchial asthma and the level of control in children. Regression analysis was done to determine risk factors for impaired pulmonary function in children with asthma.

Results: serum periostin was significantly higher in asthmatics than controls and in patients with uncontrolled compared to controlled asthma. It was significantly higher in those with severe and moderate asthma than those with mild asthma. Significant negative correlation between serum periostin level and pulmonary function parameters were detected. A logistic regression analysis showed that serum periostin level was predictor of impaired forced expiratory volume 1 (FEV1) in asthmatic children with 72.22% sensitivity and 88.1% specificity.

Conclusion: Serum periostin is a promising biomarker of treatment response and disease progression that could be used for optimization of drug doses and prediction of impaired pulmonary function in children with bronchial asthma.

Keywords- periostin, children, spirometer, bronchial asthma.

INTRODUCTION

Asthma is a heterogeneous disorder characterized by acute episodic exacerbations against a background of chronic persistent inflammation associated with structural changes that may produce persistent symptoms and impaired pulmonary

function. Assessment of asthma severity and level of control have become the key element in asthma management. Proper asthma treatment has been adjusted mainly on the basis of symptoms and pulmonary function¹.

Airway inflammation and remodeling contribute to irreversible airflow obstruction and airway hyperresponsiveness, and have been associated with increased disease severity and impaired pulmonary function².

Biomarker can be used to monitor the disease progression, select optimal therapy and act as predictor for exacerbation risk and long term outcomes. Biomarkers have the advantages of being objective, quantitative, and patient-independent to avoid variation among patients in describing asthma symptoms, using rescue medication and controller therapy³.

Periostin is an extracellular matrix protein that contributes to subepithelial fibrosis. It interacts with multiple signaling cascades to modulate the expression of genes that regulate cellular interactions within the extracellular matrix. It is easily released from inflamed tissues into the circulation in response to Th2-type immune responses. Furthermore, its basal serum level is relatively low (~50 ng/ml) compared to other extracellular matrix proteins. Periostin biomarker is easy to collect and measure either in serum or sputum, neither invasive nor expensive. Additionally, its level is stable with little variability which is required for a reliable biomarker⁴.

In contrast to adult asthma, the usefulness of periostin in childhood asthma is still under discussion. In the current study, we aim to evaluate the role of serum periostin in assessment of children with bronchial asthma in relation to pulmonary function test.

PATIENTS AND METHODS

Study design

A case control study was conducted at outpatient clinic of pediatric and chest department of Al-Zahraa, El Hussein and Bab El Sharia University Hospitals, Cairo, Egypt during the period between April 2015 to March 2016. The study was approved by the local Ethics Committee. Informed written parental consent was obtained prior to enrollment in the study.

Study population

A sample of 90 candidates was consecutively enrolled: 60 children with bronchial asthma and 30 healthy non asthmatic children of matched age and sex as controls. Their age ranged between 6 - 15 years. Children who had upper or lower respiratory tract infections, respiratory diseases other than asthma, acute or chronic systemic illness (e.g. cardiac, renal, hepatic diseases), upper airway obstruction, thoracic cage malformation, children who refuse or unable to perform spirometer technique properly were excluded from the study. Control group included healthy children who did not have any history of wheezy chest or any allergic disorders. They fulfilled the same exclusion criteria as patient group.

Methods

Clinical history and examination:

All the studied children were subjected to a detailed history-taking and thorough clinical examination. History included: demographic data, presenting symptoms, the age of onset, the duration of disease, triggering factors, frequency and severity of acute exacerbation, presence of night symptoms, persistent symptoms in between the attacks, limitation of physical activity, history of other allergies, frequency of rescue medication, type of controller therapy, response to medication, current medications, previous hospitalization due to asthma exacerbation, symptoms suggestive of other system affection.

Chest examinations were done with recording of auscultation findings and signs of respiratory distress. Physical growth was assessed for all children by determining the weight, height, body mass index (BMI). BMI was calculated as weight (in kilograms) divided by the square of height (in meters). All the recorded measures were plotted against the Egyptian growth reference data⁵ to determine the centile and standard deviation. Weight and height were expressed in terms of Z-score which is a quantitative measure of the deviation of a specific percentile from the main values of age-specific population. Z-scores above

the population mean expressed as a positive value and those below the population mean expressed as a negative value⁶.

According to GINA 2015 guideline⁷; diagnosis of bronchial asthma was based on the symptoms of bronchospasm (cough, dyspnea and wheeze) that vary over time and intensity. Together with improvement in response to bronchodilators (increase in FEV1 of >12% predicted). Asthma severity was evaluated by revise treatment step to control symptoms and exacerbations patients and was categorized into: mild, moderate and severe asthma. The level of asthma control was evaluated according to the frequency of symptoms, nocturnal symptoms, need of reliever medication and physical activity limitation. Patients were categorized into controlled, partly controlled or uncontrolled asthma. All the studied asthmatic children were evaluated in between attacks where pulmonary function evaluation by spirometry was done and blood sampling was taken during the same visit.

Spirometry:

All the studied children were evaluated for the pulmonary functions using Spirometry (medi soft micro 5000). Children were encouraged to blow as vigorously as possible. Spirometric-indices were calculated using best out of 3 technically-satisfactory performances in accordance to the recommendations of American thoracic society⁸. Forced vital capacity (FVC), forced expiratory volume in the first second (FEV₁), FEV₁/FVC ratio, Peak expiratory flow (PEF) and maximal midexpiratory flow (MMEF75/25) were measured and recorded using a spirometer by a trained, experienced chest physicians. The results were calculated as percentage of predicted (% predicted) according to patient weight and height.

The following activities were avoided prior to spirometric study: Performing vigorous exercise within 30 minutes, wearing clothes that restrict full chest and abdominal expansion, eating large meal within 2 hours, inhaled short-acting bronchodilators, long-acting β -agonists were withheld for 6 hours and 12 hours respectively.

Laboratory investigations

Sample preparation: Serum coagulation at room temperature for 10-20min, centrifuge at speed of 2000-3000 rpm for 20 min. Remove supernatant, if precipitation appeared, centrifuge again.

Sample Collection: All blood samples were collected and processed within 2 hours and stored at -20 °C until assayed.

Assay procedure: Before the assay, the standard was diluted 5 times by pipetting 50ul standard dilution in each tube. Pipette 100ul standard (135ng/ml) in the first tube. And take out 100ul from the first tube into the second. Pipette 50ul from the second tube to the third tube and produce dilution series. Repeat each of the concentration to get the mean value of each well. The blank wells were set separately (blank comparison wells don't add sample and HRP-Conjugate reagent). Samples were also diluted 5 times by pipetting sample dilution 40ul to testing sample well, then add testing sample 10ul (sample final dilution is 5-fold), pipette sample to wells. Incubate for 30 min at 37°C. Wash solution was diluted 30-fold with distilled water. All samples were washed 5times by washing solution then we add 50ul from HRP enzyme to each well except blank. Then incubate again for 30min at 37C then all samples were washed again 5times by washing solution. Chromogen solution (100ul) was added to each well. Stop solution (50ul) then added to each well to stop the reaction (the blue color change to yellow).The blank well is considered as zero and the absorbance was read at 450nm at 15min from adding stop solution

Statistical Analysis

Statistical analysis of the results was done using statistical package for social sciences (SPSS version 20.0; Chicago, IL, USA). Differences between groups were analyzed with one-way analysis of variance (ANOVA) test. Correlations were tested between serum level of periostien, absolute esinophilic count and spirometric parameters using Spearman correlation test. Liner and logistic regression analysis was done to determine the

predictors of impaired FEV1 in asthmatic children. Receiver operating characteristic curves (ROC) were used to identify sensitivity, specificity and determine optimal cut-off points of periostien serum level for prediction of impaired FEV1 (<60%) in asthmatic children. P-value < 0.05 was considered to be significant.

RESULTS

This case control study included 60 children with bronchial asthma (34 males and 26 females) aged 10.82 ± 2.35 years old. The age of onset ranged between 4 months and 5 years with a mean duration of illness of 8.70 ± 3.43 years. Twenty seven of them (45%) had history of other allergic disorder. Most of them (70%) had positive family history of bronchial asthma. Forty five of them (75%) received controller therapy either inhaled steroid or leukotriene antagonist. Twenty one of them had mild asthma, 22 had moderate asthma and 17 had severe asthma. Regarding the level of asthma control; 19 had controlled symptoms in the previous 6 months, 23 of them had poorly controlled asthma and 18 of them had moderately controlled asthma. Serum periostin level was significantly higher in children with moderate and severe asthma than those with mild asthma and healthy controls. Additionally, its level was significantly higher in children with uncontrolled asthma in comparison to those with controlled asthma as demonstrated in table 1.

Pearson correlation analysis shows that the serum level of periostin had significant negative correlation with the spirometric parameters (FVC %, FEV1%, FEV1/FVC ratio, PEF, MMEF75/25) and positive correlation with absolute eosinophil count (AEC) in asthmatic children as demonstrated in Table 2.

Linear regression analysis demonstrated that there was highly statistical significant association between FEV 1, serum periostien level, eosinophil % and AEC while no statistical significant association was found with neutrophil% or absolute neutrophil count (ANC) as shown in table 3. However, logistic regression analysis demonstrated

that serum periostien level was the only predictor for impaired FEV1 (<60% of predicted) with p-value of 0.001 and odds ratio 1.019 with 95% CI (1.008 – 1.30) as shown in table 4.

At cut off point of 75ng/ml serum periostien level had 72.22% sensitivity and 88.1% specificity for prediction of impaired FEV1 (<60% of predicted) in children with bronchial asthma as shown by ROC curve (figure 1).

Table (1): Comparison of serum periostien level between asthmatic children and healthy controls in relation to asthma severity and level of asthma control

Variables	Mild asthma (n=21) Mean±SD	Moderate asthma (n=22) Mean±SD	Severe asthma (n=17) Mean±SD	Healthy controls (n=30) Mean±SD
	Serum periostien (ng/ml)	59.723 ± 4.085	74.673 ± 44.801	181.704 ± 83.063
ANOVA test				
f	9.864			
P-value	<0.001*			
Variables	Controlled asthma (n=19) Mean±SD	Partially controlled asthma (n=18) Mean±SD	Uncontrolled asthma (n=23) Mean±SD	Healthy controls (n=30) Mean±SD
	Serum periostien (ng/ml)	58.725 ± 4.598	105.573 ± 68.971	129.124 ± 90.666
ANOVA test				
f	9.864			
P-value	<0.001*			

*significant

Table (2): Correlation between serum periostien level, AEC and spirometric findings in asthmatic children

	Serum periostien level	
	r	p-value
AEC (cell/mm ³)	0.497	<0.0001*
ANC (cell/mm ³)	0.046	0.666
FEV1 (% of predicted)	-0.552	<0.0001*
FVC (% of predicted)	-0.572	<0.0001*
FEV1/FVC ratio	-0.541	<0.0001*
PEF (% of predicted)	-0.464	<0.0001*
MMEF75/25(% of predicted)	-0.480	<0.0001*

*significant

AEC: absolute eosinophil count; ANC: absolute neutrophil count; FEV1: forced expiratory volume in 1st second; FVC: forced vital capacity; PEF: peak expiratory flow; MMEF: maximal midexpiratory flow

Table (3): Linear regression analysis for predictors of impaired FEV1 in asthmatic children

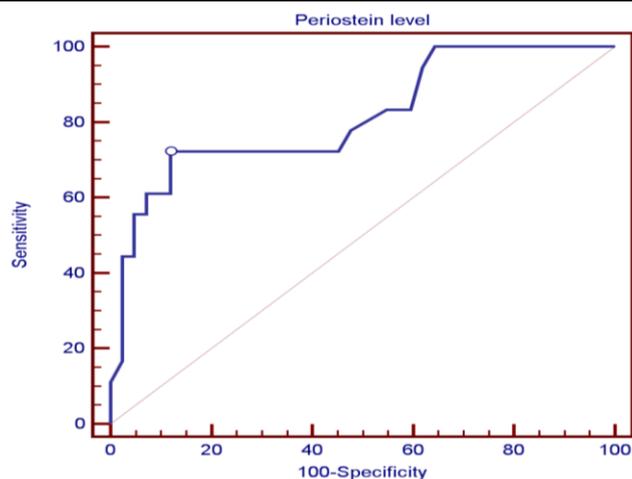
	Unstandardized Coefficients		Standardized Coefficients		t	P-value
	B	Std. Error	Beta			
(Constant)	84.830	3.960			21.422	<0.001*
Serum periostin (pg/ml)	-0.154	0.032	-0.534		-4.804	<0.001*
Esinophil%	-4.143	1.506	-0.340		-2.751	0.008*
AEC(cell/mm ³)	-0.039	0.014	-0.346		-2.810	0.007*
Neutrophil%	0.366	0.458	0.104		0.799	0.427
ANC(cell/mm ³)	-0.002	0.002	-0.110		-0.844	0.402
Age of onset (months)	0.103	0.172	0.078		0.597	0.553
Duration of illness (years)	-0.120	1.132	-0.014		-0.106	0.916

*significant

AEC: absolute eosinophil count; ANC: absolute neutrophilic count; FEV1: forced expiratory volume in 1st second

Table (4): Logistic regression analysis for predictors of impaired FEV1 in asthmatic children

	B	S.E.	Wald	P-value	Odds ratio	95% C.I.for Odds ratio	
						Lower	Upper
Serum periostin (pg/ml)	0.018	0.005	11.647	0.001	1.019	1.008	1.030
Eosinophil%	0.283	0.173	2.685	0.101	1.328	0.946	1.863
AEC(cell/mm ³)	0.002	0.002	2.448	0.118	1.002	0.999	1.005
Neutrophil%	-0.002	0.040	0.004	0.952	0.998	0.923	1.079
ANC(cell/mm ³)	0.000	0.000	0.875	0.349	1.000	1.000	1.000
Age of onset (months)	-0.006	0.018	0.112	0.738	0.994	0.960	1.030
Duration of illness (years)	-0.033	0.116	0.083	0.774	0.967	0.770	1.214
Gender	0.486	0.568	0.729	0.393	1.625	0.533	4.952
Passive smoking	0.788	0.588	1.800	0.180	2.200	0.695	6.962



Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
>75 (pg/ml)	0.813	72.22	88.10	72.2	88.1

AUC: area under curve; +PV: positive predictive value; -PV: negative predictive value

Figure 1: ROC curve of periostin serum level for prediction of impaired FEV1 in asthmatic children

DISCUSSION

Bronchial asthma is a chronic inflammatory airway disease with airway inflammation and remodeling are the main pathological features. Clinicians should individualize the treatment regimens to prevent the development or worsening of airway remodeling¹. Assessment of airway remodeling by analysis of blood and sputum biomarkers as periostin has been developed as a minimally invasive way to bypass tissue biopsy.

Our study revealed that serum periostin level was significantly correlated with asthma severity, level of asthma control, blood eosinophilia and impaired pulmonary function. Experimental studies demonstrated that periostin is linked to severe airway inflammation and hyperresponsiveness⁹. Rupani et al¹⁰ revealed that serum periostin could be a predictor of childhood asthma development. Its level is higher in children who developed asthma than those who did not develop asthma.

Our study findings were in consistent with previous adult studies. Scichilone et al¹¹ reported that serum periostin level can discriminate between subjects with recurrent exacerbations and those in stable conditions. Nair and Kraft¹² demonstrated that serum periostin level is significantly elevated in asthmatic patients with evidence of eosinophilic airway inflammation. Jia et al¹³ found that serum periostin is the single best systemic biomarker of airway luminal and tissue eosinophilia in severe, uncontrolled asthmatics with a positive predicted value of 93 %.

FEV1 is an independent predictor of asthma exacerbations; asthmatic children with a baseline FEV1 <60% predicted have a doubled risk for asthma exacerbations in the subsequent year as compared with children with an FEV1 >80% predicted¹⁴.

Our study reviled a significant negative correlation between serum periostin level and FEV1 in asthmatic children. Kanemitsu et al¹⁵ reported that high serum periostin concentration (≥95 ng/ml) is the unique biomarker, among several serum markers, associated with the greater annual decline in FEV1 (at least 30 ml/year). Baek et al¹⁶ reported

that in children with exercise induced asthma, the maximum decreases in FEV1% after exercise was positively correlated with serum periostin levels. On the other hand, Inoue et al¹⁷ demonstrated that serum periostin have not correlated either with PEFR or FEV1 in children with asthma. However the previous study did not include children with severe or uncontrolled asthma.

In the current study, elevated serum periostin level could identify patients at risk of rapid FEV1 decline and asthma exacerbation despite high doses of ICS. In accordance with our findings, Bobolea et al¹⁸ found that sputum periostin levels are associated with persistent airflow limitation and eosinophilic inflammatory phenotype despite high-dose ICS therapy. There was an inverse correlation between postbronchodilator FEV1 /FVC and periostin levels. These findings are in consistent with previous reports that serum periostin levels may decreased by systemic anti-inflammatory drugs but not ICS¹⁹.

Due to the heterogeneity of the disease, many biomarkers for assessment of children with bronchial asthma are available. However, periostin could signal different aspects in the pathogenesis of bronchial asthma that provide additional information for the diagnosis and management. our findings demonstrated that serum periostin level could be a valuable marker to identify asthma phenotypes, airway remodeling and treatment response.

One of the limitations of the current study is the small number of included patients. Our study was based on cross-sectional analysis, so we could not monitor the changes in serum periostin level through the course of the disease or its response to therapeutic interventions. We included children with bronchial asthma mainly depending on their level of control on treatment over the previous 6 months. Due to the explorative nature of this study, results need to be confirmed in a larger sample of patients including follow up. Another limitation is that serum periostin level could be elevated in many allergic disorders. However if we strictly exclude all children with coincident atopy, this limits the number of available asthmatic children to be

included. Our study gets its strength from the significant correlation between elevated serum periostin level and impaired pulmonary function. The standard assessment of remodeling is obtained by tissue biopsy through bronchoscopy which is costly and sophisticated invasive procedure that needs experts especially for evaluation of distal air way²⁰. Instead air way remodeling was assessed by measuring serum level of periostin which can assess both airway inflammation and remodeling which was correlated with air way obstruction as determined by impaired FEV1.

CONCLUSIONS

Serum periostin is a promising biomarker of treatment response and disease progression that could be used for individualization of treatment and optimization of drug dose in children with bronchial asthma. It is applicable in the clinical setting especially in children who are unable or have difficulty in performing lung function test.

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