

Original article

Serum periostin level in children with bronchial asthma

Background: Periostin is a systemic inflammatory biomarker secreted in large quantities from lung fibroblasts under stimulation by interleukin (IL) -13 and IL-4 activity. Several studies also suggested a relation between serum periostin level and eosinophilic inflammation in asthma. Thus, we sought to determine serum periostin level in children with bronchial asthma in correlation with asthma severity and pulmonary function tests. **Methods:** This controlled cross-sectional study was conducted on 50 children with bronchial asthma and 30 age-matched healthy controls who were recruited from the Children's Hospital, Aswan University, during the period from May 2018 to April 2019. The enrolled patients were subjected to clinical evaluation, pulmonary function testing, complete blood counting, total serum immunoglobulin E (IgE) estimation, and periostin level measurement by ELISA at the time of asthma exacerbation. **Results:** Asthmatic cases had significantly higher serum levels of periostin (113.2 ± 56.17 ng/ml) and total IgE (408.86 ± 287.3 IU/ml) in comparison to controls (52.43 ± 11.15 ng/ml) and (44.8 ± 21.22 IU/ml), respectively; $p < 0.001$. Periostin and total IgE levels were higher in severe than in mild and moderate, and in uncontrolled than well-controlled asthma cases ($p < 0.001$). Serum periostin levels correlated positively with the total IgE, asthma severity, asthma control, and eosinophil count in the asthmatic patients, and negatively with the neutrophil count and all spirometry parameters. Serum periostin had 100 % specificity and 72 % sensitivity at a cut-off level >75 ng/ml in the diagnosis of bronchial asthma. **Conclusion:** Serum periostin could be a useful biomarker in diagnosing bronchial asthma in association with asthma severity.

Keyword: bronchial asthma, periostin, IgE, pulmonary function

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INTRODUCTION

Asthma is a common heterogeneous long-term inflammatory disease in the respiratory tract. Variable and recurrent symptoms, reversible obstruction of airflow, and bronchospasm are characteristic. Classic symptoms include coughing, wheezing, shortness of breath, and chest tightness.¹ Estimating lung functions by spirometry remains a more objective method for diagnosing, tracking, and controlling the disease.² There are numbers of serum biomarkers that could be measured to help our understanding of the asthma pathophysiology, periostin is one of them.³

Periostin is a subepithelial extracellular matrix protein. It is deposited on the thickened basement membrane, suggesting that it is a component of subepithelial fibrosis. Based on the expression of IL-13 and IL-4, asthma patients are classified into "Th2-high" and "Th2-low" groups. Th2-eosinophilic inflammation has been considered to be the dominant inflammatory pattern in asthma. Fahy and colleagues showed that periostin can be a

surrogate biomarker of T-helper 2 mediated immune responses that is simple to measure in serum or sputum.⁴⁻⁶ Also, Nair and Kraft,⁷ serum periostin levels are considerably higher in asthmatic individuals with eosinophilic inflammation.

This study was aimed to determine serum periostin level in a group of children with bronchial asthma as a potential marker of clinical severity and predictor of pulmonary function status.

METHODS

Study design and patients

This controlled cross-sectional study included 50 patients, aged 6-16 years, with bronchial asthma who were recruited from the Pulmonology Outpatient Clinic, Paediatric Intensive Care Unit, and Emergency Department at Aswan University hospital, during the period from May 2018 to April 2019. In addition, 30 age-matched healthy children who did not have any history of wheezy chest or any allergic disorders served as the control group.

Children with acute or chronic systemic illness, those with upper or lower respiratory tract

infections, or who had a respiratory disease other than asthma were excluded from the study. In addition, children with upper airway obstruction, those with a history of prematurity (who required prolonged oxygen therapy or assisted ventilation), and children who refused or were unable to perform spirometry technique properly were excluded from the study.

Methodology:

All cases have been subjected to complete history taking, physical examination, anthropometric measurements, and oxygen saturation (SO₂) measurement. Complete blood count (CBC), immunoglobulin E (IgE) serum level, and serum periostin assay were done at the time of asthma exacerbation. For the admitted cases coming with exacerbations or for cases coming to outpatient clinic for follow up visits, sampling was done at the same time of pulmonary function tests, when the patient was able to perform the test to correlate both with the asthma severity and control.

SO₂ measurement was done using portable G1B Pulse Oximeter device specialized for measurement of SO₂ saturation while subjects were exposed to ordinary room air.⁸

Spirometry: Pulmonary function testing was done for all cases and controls using a Spirometer device (WinspiroPRO 6.0.2 - mod. c11). The average of three best graphs and readings obtained, technically satisfactory as outlined in the American Thoracic Society (ATS) recommendations, is recorded.⁹ All subjects were evaluated for forced vital capacity (FVC), the forced first-second Expiratory Volume (FEV₁), FEV₁/FVC ratio, Peak Expiratory Flow (PEF), and Forced Expiratory Flow (FEF 75-25%). Results were determined according to the weight, height, and body mass index as a percentage of expected results (percent predicted).¹⁰

Laboratory investigations:

Venous blood samples (7 cc) were withdrawn from all subjects of the study under complete aseptic technique from the antecubital vein. First part of the sample (2 cc) was collected in EDTA tubes for CBC assessment and eosinophilic counting. Second part (5 cc) was collected in sterile plastic tubes, centrifuged and serum was collected and frozen at -80°C for assessment of Periostin and total IgE levels.

Measurement of periostin level: It was done by enzyme-linked immunosorbent assay (ELISA) technique using human Periostin kit, Catalog No: SG-10345, from China. Purified Periostin antibody was adopted to coat microtiter plate, making solid-phase antibody, then periostin was added to wells.

Combined periostin antibody with labelled horseradish peroxidase (HRP) formed antibody-antigen-enzyme-antibody complex. After washing completely, Trimethyl benzidine (TMB) substrate solution was added. TMB substrate became blue colour at HRP enzyme-catalyzed wells that contained periostin. The reaction was terminated by addition of a stop solution and the colour change was measured at a wavelength of 450 nm. The concentration of periostin in the samples was then determined by comparing the Optical density (O.D.) of the samples to the standard curve.¹¹

Measurement of serum Total IgE level: It was done by ELISA using the total IgE kit, Catalog No: MB-20018, from South San Francisco.¹²

Statistical analysis:

The SPSS version 24 was used to conduct statistical analyses. Categorical variables were represented by a percentage, where mean and standard deviation (SD) described continuous variables. Chi-square test was used to compare between categorical variables, while the comparison between continuous variables was made by t-test.

Receiver-operating characteristic (ROC) curve was used to assess the best cut-off value of serum periostin in diagnosis of bronchial asthma via Area under the curve (AUC), sensitivity and specificity. For correlation coefficients, we used Pearson and Spearman tests. A two-tailed p-value <0.05 was considered statistically significant.

RESULTS

Table (1) shows the basic demographic, laboratory and spirometry data of the studied cases and controls. The mean age of asthmatic patients was 10.07 ± 2.19 versus 10.07 ± 2.19 years in controls. Seventy percent of the patients were males and 62 % were from urban areas versus 43.3% and 76.7% of controls, respectively. Our results showed significantly higher mean levels of serum periostin (113.2 ng/ml ± 56.17 SD) and Total serum IgE (408.86 IU/ml ± 287.3) in asthmatic cases vs 52.43 ± 11.15 ng/ml and 44.8 ± 21.22 IU/ml in controls, respectively. The CBC showed significantly higher mean eosinophilic count in cases (0.48 10³/mL ± 0.32) than control (0.13 10³/mL ± 0.05). There was significantly lower oxygen saturation in asthmatic (94.06 % ± 1.74) than in healthy controls (97.5 % ± 1.53). Different spirometer parameters were significantly lower in asthmatic patients than healthy control with significant improvement after bronchodilator inhalation.

Regarding medical history of asthmatic cases, mean age at onset of asthma was 2.64 years ± 2.18

and the disease period was $7.43 \text{ years} \pm 2.75$. All cases were on short acting β_2 agonist (on demand therapy), 80% were on inhaled corticosteroid, while 14% of the cases were on anti-IgE therapy. Eighty-four percent of the cases had family history of allergy, 58% had allergic rhinitis, 50% had allergic conjunctivitis and food allergy and 46% had associated eczema.

According to GINA 2020 classification, 17 (34%) cases had mild asthma, 17 (34%) moderate, and 16 (32%) had severe asthma. Moreover, 38% (19 cases) were well controlled, 32% (16) partially controlled, and 30% (15 cases) had uncontrolled asthma.

Regarding laboratory investigations among cases in relation to asthma severity (table 3), there was a significantly higher serum level of periostin (179.81 ± 46.37) ng/ml and total IgE (660.5 ± 302.76) IU/ml in severe cases than in mild [64.71 ± 12.06] ng/ml, (179.06 ± 98.28) IU/ml] and moderate [99 ± 18.68] ng/ml, (401.82 ± 194.18) IU/ml] asthmatic patients respectively. The blood picture showed significantly higher mean eosinophilic count (0.79 ± 0.36) $10^3/\text{mL}$ in those with more severe than in those with mild-moderate [(0.24 ± 0.09) $10^3/\text{mL}$ – (0.42 ± 0.17) $10^3/\text{mL}$] asthma, and significantly lower mean neutrophilic count (2.09 ± 0.86) $10^3/\text{mL}$ in those with severe than in those with mild-moderate [(3.63 ± 2.44) $10^3/\text{mL}$ – (3.46 ± 2.73) $10^3/\text{mL}$] asthma.

Laboratory investigations among asthmatic cases in relation to asthma control and in comparison to healthy controls are shown in table (4), There was a significantly higher serum level of periostin (172.6 ± 52.96) (ng/ml) and total IgE

(714.8 ± 268.31) (IU/ml) in uncontrolled asthmatics than well-controlled asthmatics [71.58 ± 25.42 (ng/ml), 211.21 ± 142.42 (IU/ml)] and healthy controls [52.43 ± 11.15 (ng/ml), 44.8 ± 21.22 (IU/ml)]. There was significantly higher mean eosinophilic count in uncontrolled (0.76 ± 0.4) ($10^3/\text{mL}$) asthmatic patients than well-controlled asthmatics (0.31 $10^3/\text{mL} \pm 0.15$) and healthy controls (0.13 $10^3/\text{mL} \pm 0.05$).

Table (5) showed that as severity of obstruction increased among asthmatic patients, level of serum periostin increases with highly significant p value among those with severe obstruction (203 ± 33.44) (ng / ml) than those with mild (84.71 ± 31.5) (ng / ml) to moderate obstruction (128.18 ± 49.84) (ng / ml).

Our results showed serum periostin levels correlated positively with total IgE ($r = 0.758$), asthma severity ($r = 0.889$), asthma control ($r = 0.801$) and eosinophilic count ($r = 0.804$) in asthmatic patients, and negatively with neutrophilic count ($r = -0.447$), SO_2 and all spirometer parameters (table 6).

ROC curve for distinguishing between patients with bronchial asthma and controls (Table 7, Fig 1) showed that serum periostin had high specificity 100% and good sensitivity 72% at criterion cut-off level >75 ng/ml. AUC was 0.925.

ROC analysis for distinguishing between patients with severe and those with mild-to-moderate bronchial asthma using serum periostin level (Table 8, Fig 2) showed that serum periostin had high specificity 97.06% and good sensitivity 93.75% at criterion cut-off level >114 ng/ml. AUC was 0.965.

Table 1. Basic demographic, laboratory and spirometry data of studied cases and controls

Parameter	Cases (N=50) Mean \pm SD	Controls (N=30) Mean \pm SD	Test value	P value
Age in years	10.07 \pm 2.19	10.4 \pm 2.93	t= -0.583	0.561
Sex: Male	36 (70%)	13 (43.3%)	X ² = 6.492	0.011
Female	14 (30%)	17 (56.7)		
Residence Rural	19 (38%)	7 (33.3%)	X ² = 1.839	0.175
Urban	31 (62%)	23 (76.7%)		
Periostin (ng / ml)	113.2 \pm 56.17	52.43 \pm 11.15	t= 5.843	<0.001
Total IgE (IU / ml)	408.86 \pm 287.3	44.8 \pm 21.22	t= 6.912	<0.001
WBC ($10^3/\text{mL}$)	7.14 \pm 2.78	6.54 \pm 1.61	t= 1.08	0.283
Neutrophil Count ($10^3/\text{mL}$)	3.08 \pm 2.26	3.07 \pm 1.2	t= 0.008	0.994
Neutrophil (%)	39.87 \pm 12.95	45.59 \pm 9.57	t= -2.087	0.050
Eosinophil Count ($10^3/\text{mL}$)	0.48 \pm 0.32	0.13 \pm 0.05	t= 5.854	<0.001
Eosinophil (%)	7.44 \pm 5.01	1.98 \pm 0.66	t= 5.917	<0.001
Hemoglobin (g/dl)	13.39 \pm 0.81	13.38 \pm 1.3	t= 0.048	0.962
O ₂ Saturation (%)	94.06 \pm 1.74	97.5 \pm 1.53	t= -9.249	<0.001
FVC (% of predicted)				
Pre bronchodilator	89.42 \pm 10.32	98 \pm 6.19	t= -4.124	<0.001
Post-bronchodilator	93.96 \pm 8.79	99.87 \pm 6.35	-3.21	0.002

FEV1 (% of predicted)				
Pre-bronchodilator	77.22 ±14.06	97 ±7.02	t= -7.176	<0.001
Post-bronchodilator	88.68 ±11.26	99.37±7.45	t= -4.622	<0.001
FEV1 / FVC ratio				
Pre-bronchodilator	74.78±7.79	90.97 ±1.79	t= -11.185	<0.001
Post-bronchodilator	82.03±6.45	91.97 ±1.87	t= -8.214	<0.001
PEF (% of Pred.)				
Pre-bronchodilator	71.8 ±13.21	91.97 ±4.17	t= -8.107	<0.001
Post-bronchodilator	84.46 ±10.26	93.4 ±3.77	t= -4.579	<0.001
FEF 25-75 (% of Pred.)				
Pre-bronchodilator	69.38 ±13.44	93.87±4.31	t= -9.663	<0.001
Post-bronchodilator	86.88 ±11.03	95.03±4.69	t= -3.837	<0.001

WBC: white blood cells, O2 Sat.: Oxygen Saturation, FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEF: Peak Expiratory Flow, FEF25-75: Forced Expiratory Flow at 25% to 75% of FVC. Independent-samples T Test and Chi-square test were used. P-value is test of significance between cases and healthy control groups.

Table 2. Medical history of studied cases

Personal history	Cases (N=50)
Age of onset of asthma (year)	
Range	0.33-8
Mean ± SD	2.64 ± 2.18
Median (IQ)	2 (1 - 3)
Duration of asthma (year)	
Range	2-14
Mean ± SD	7.43 ± 2.75
Median (IQ)	7.5 (5-9.2)
Therapeutic history	
SABA (oral or inhaler)	50 (100 %)
ICS	40 (80 %)
LABA	16 (32 %)
Mast cell stabilizers / LTRA	28 (56 %)
Anti-IGE	7 (14 %)
Nasal Steroids	12 (24 %)
Family history of allergy	42 (84%)
Associated Allergic rhinitis	29 (58%)
Associated Allergic conjunctivitis	25 (50%)
Associated Eczema	23 (46%)
Associated Food allergy	25 (50%)
Asthma severity	
Mild	17 (34%)
Moderate	17 (34%)
Severe	16 (32%)
Asthma control	
Well controlled	19 (38%)
Partially controlled	16 (32%)
Uncontrolled	15 (30%)

ICS: inhaled corticosteroids, LABA: long-acting beta2 agonist, LTRA leukotrienes receptor antagonists, SABA: short-acting beta2 agonist.

Table 3. Laboratory investigations among cases (in relation to asthma severity)

	Mild (n=17)	Moderate (n=17)	Severe (n=16)	P1/t	P2/t	P3/t
	Mean \pm SD	Mean \pm SD	Mean \pm SD			
Periostin (ng/ml)	64.71 \pm 12.06	99 \pm 18.68	179.81 \pm 46.37	<0.001 t= -6.358	<0.001 t= -9.627	<0.001 t= -6.64
Total IgE (IU/ml)	179.06 \pm 98.28	401.82 \pm 194.18	660.5 \pm 302.76	<0.001 t= -4.22	<0.001 t= -6.067	0.006 t= -2.94
WBC (10 ³ /mL)	7.64 \pm 2.73	7.54 \pm 3.52	6.2 \pm 1.63	0.926 t= 0.094	0.075 t= 1.856	0.174 t= 1.39
Neutrophil Count (10 ³ /mL)	3.63 \pm 2.44	3.46 \pm 2.73	2.09 \pm 0.86	0.854 t= 0.185	0.023 t= 2.388	0.063 t= 1.976
Neutrophil (%)	44.51 \pm 13.37	41.46 \pm 13.12	33.25 \pm 9.98	0.507 t= 0.671	0.010 t= 2.727	0.051 t= 2.03
Eosinophil Count (10 ³ /mL)	0.24 \pm 0.09	0.42 \pm 0.17	0.79 \pm 0.36	<0.001 t= -3.763	<0.001 t= -6.125	0.001 t= -3.836
Eosinophil (%)	3.34 \pm 1.49	6.26 \pm 2.31	13.04 \pm 4.46	<0.001 t= -4.385	<0.001 t= -8.484	<0.001 t= -5.532
Hemoglobin (g/dl)	13.25 \pm 0.77	13.32 \pm 0.75	13.61 \pm 0.9	0.772 t= -0.292	0.226 t= -1.234	0.337 t= -0.976
O2 Sat. (%)	95.41 \pm 0.6	94.35 \pm 0.7	92.31 \pm 1.89	<0.001 t= 4.667	<0.001 t= 6.42	<0.001 t= -6.64

WBC: white blood cells, O2 Sat.: Oxygen Saturation, Independent-samples T test was used. P1: P-value of test of significance between mild and moderate groups. P2: P-value of test of significance between mild and severe groups. P3: P-value of test of significance between moderate and severe groups.

Table 4. Laboratory results among patients with different degrees of asthma control versus healthy controls

	Well controlled (n=19)	Partly controlled (n=16)	Uncontrolled (n=15)	Healthy controls (n=30)	P1	P2	P3	P4
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD				
Periostin (ng / ml)	71.58 \pm 25.42	106.94 \pm 33.87	172.6 \pm 52.96	52.43 \pm 11.15	<0.00 t= 3.627	0.002 t= -3.439	<0.001 t= -7.334	<0.001 t= -4.14
Total IgE (IU / ml)	211.21 \pm 142.42	356.75 \pm 179.25	714.8 \pm 268.31	44.8 \pm 21.22	<0.001 t= 6.327	0.014 t= -2.624	<0.001 t= -7.039	<0.001 t= -4.34
WBC (10 ³ /mL)	7.73 \pm 2.58	7.45 \pm 2.81	6.08 \pm 2.87	6.54 \pm 1.61	0.084 t= 1.796	0.761 t= 0.307	0.093 t= 1.738	0.191 t= 1.338
Neutrophil Count (10 ³ /mL)	3.74 \pm 2.26	3.16 \pm 2.23	2.15 \pm 2.1	3.07 \pm 1.2	0.184 t= 1.348	0.451 t= 0.762	0.042 t= 2.116	0.206 t= 1.294
Neutrophil (%)	45.78 \pm 11.64	40.16 \pm 11.33	32.06 \pm 12.75	45.59 \pm 9.57	0.951 t= -0.791	0.158 t= 1.444	0.003 t= 3.238	0.073 t= 1.866
Eosinophil Count (10 ³ /mL)	0.31 \pm 0.15	0.41 \pm 0.21	0.76 \pm 0.4	0.13 \pm 0.05	<0.001 t= 5.977	0.121 t= -1.6.02	<0.001 t= -4.514	0.007 t= -3.02
Eosinophil (%)	3.99 \pm 1.95	6.29 \pm 3.23	13.01 \pm 4.57	1.98 \pm 0.66	<0.001 t= 5.227	0.020 t= -2.489	<0.001 t= -7.783	<0.001 t= -4.701
Hemoglobin (g/dl)	13.15 \pm 0.71	13.71 \pm 0.97	13.35 \pm 0.65	13.4 \pm 1.3	0.486 t= -0.70	0.067 t= -1.911	0.386 t= -0.879	0.241 t= 1.199
O2 Sat. (%)	95.16 \pm 0.90	94.38 \pm 0.81	92.33 \pm 1.99	97.5 \pm 1.53	<0.001 t= -6.05	<0.001 t= 3.69	<0.001 t= 5.535	<0.001 t= 3.792

Independent-samples T Test were used. WBC: white blood cells, O2 Sat.: Oxygen Saturation, P1: P-value of test of significance between well-controlled and healthy control groups. P2: P-value of test of significance between well and partly controlled groups. P3: P-value of test of significance between well and uncontrolled groups. P4: P-value of test of significance between partly and uncontrolled groups.

Table 5. Periostin level in relation to degree of obstruction among asthmatic patients

	No or mild obstruction (best/predicted FEV1% > 70%) (n=31)	Moderate obstruction (best/predicted FEV1% 60 -70%) (n=11)	Severe obstruction (best/predicted FEV1% < 60%) (n=8)	P1/t	P2/t	P3/t
	Mean ± SD	Mean ± SD	Mean ± SD			
Periostin	84.71±31.5	128.18±49.84	203±33.44	0.001 t= -3.353	<0.001 t= -9.025	<0.001 t= -3.913

FEV1: Forced Expiratory Volume in 1 second

Independent-samples T Test was used.

P1: P-value of test of significance between no or mild and moderate obstruction groups.

P2: P-value of test of significance between no or mild and severe obstruction groups.

P3: P-value of test of significance between moderate and severe obstruction groups.

Table 6. Correlation of periostin level to other factors among cases and controls

	Periostin (ng/ml)			
	Cases (n=50)		Healthy controls (n=30)	
	r	p	r	p
Age in years	0.095	0.511	0.011	0.954
Age of onset of asthma in years	-0.093	0.520		
Duration of disease (year)	0.230	0.108		
Family history of allergy	0.049	0.734		
NEU count	-0.447**	0.001	0.006	0.973
NEU percent	-0.457**	0.001	-0.075	0.696
ANC (Cells / mm ³)	-0.478**	0.000	-0.047	0.806
EOS count	0.804**	0.000	-0.117	0.538
EOS percent	0.849**	0.000	-0.033	0.864
AEC (Cells/mm ³)	0.791**	0.000	-0.081	0.670
Total IgE (IU / ml)	0.758**	0.000	0.145	0.443
SO2 %	-0.875**	0.000	-0.363*	0.048
FEV1 best/ Pred% (Pre-bronchodilator)	-0.761**	0.000	0.075	0.695
FEV1 best/Pred % (Post-bronchodilator)	-0.793**	0.000	0.062	0.745
FVC best/Pred % (Pre-bronchodilator)	-0.494**	0.000	-0.123	0.518
FVC best/Pred % (Post-bronchodilator)	-0.509**	0.000	-0.034	0.859
FEV1 / FVC best/Pred. (pre-bronchodilator)	-0.690**	0.000	-0.203	0.282
FEV1 / FVC best/Pred. (Post-bronchodilator)	-0.625**	0.000	-0.091	0.631
PEF best/Pred % (Pre-bronchodilator)	-0.799**	0.000	-0.264	0.158
PEF best/Pred % (post-bronchodilator)	-0.766**	0.000	-0.245	0.193
FEF 25-75% best/Pred % (Pre-bronchodilator)	-0.740**	0.000	-0.095	0.616
FEF 25-75% best/Pred% (Post-bronchodilator)	-0.548**	0.000	-0.004	0.982

Pearson correlation coefficient was used. P-value less than 0.05 considered statistically significant. NEU: neutrophil. EOS: eosinophil, O2 Sat.: Oxygen Saturation, FVC: Forced Vital Capacity, FEV1: Forced Expiratory Volume in 1 second, PEF: Peak Expiratory Flow, FEF25-75: Forced Expiratory Flow at 25% to 75% of FVC

Table 7. ROC for distinguishing between patients with bronchial asthma and controls

Items	AUC	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy
Periostin (ng/ml)	0.925	>75	72	100	100	68.2	86.00

AUC: area under the curve, PPV: Positive predictive value, NPV: Negative predictive value

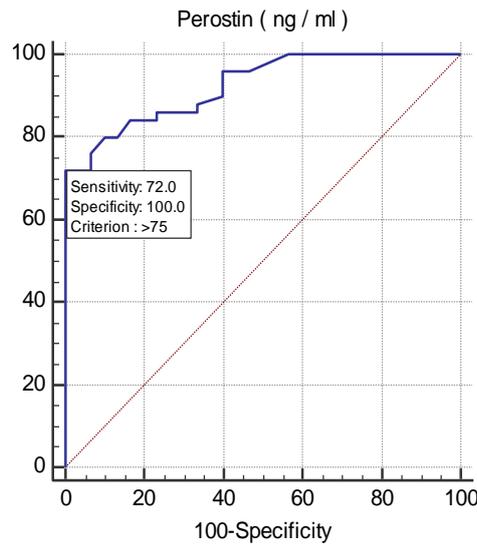


Figure 1. ROC for distinguishing between patients with bronchial asthma and controls using serum periostin level.

Table 8. ROC for distinguishing between patients with severe and with mild-to-moderate bronchial asthma using serum periostin level.

Items	AUC	Cut-off	Sensitivity	Specificity	PPV	NPV	Accuracy
Periostin (ng / ml)	0.965	>114	93.75	97.06	93.7	97.1	95.41

AUC: area under the curve, PPV: Positive predictive value, NPV: Negative predictive value

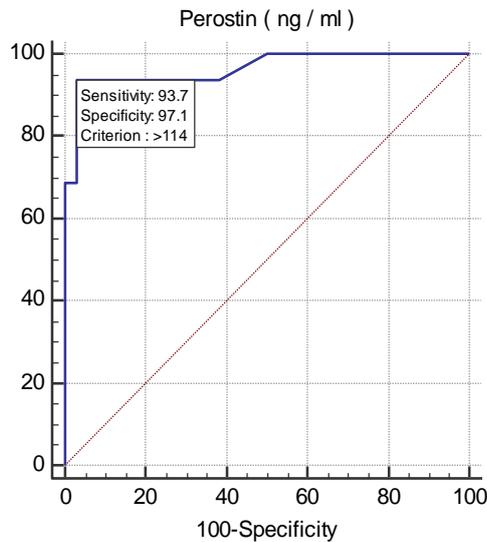


Figure 2. ROC for distinguishing between patients with severe and with mild-to-moderate bronchial asthma using serum periostin level.

DISCUSSION

The rapid increase and application of molecular techniques to the field of allergic airway diseases and asthma had led to the emergence of biomarkers helping in diagnosis and treatment of different types of diseases.¹³

Periostin is a candidate systemic biomarker of IL-13 and IL-4 activity; it is secreted in large amounts from lung fibroblasts upon stimulation.¹⁴ It also plays a significant role in eosinophil

accumulation. In patients with severe asthma who were uncontrolled despite receiving maximum inhaled corticosteroid treatment, a high periostin level was found to be the strongest single predictor of airway eosinophilia.¹⁵

In our study, periostin level was significantly higher in asthmatic patients than in the control group (p-value <0.001). Our results agree with those of Inoue et al,¹⁶ who investigated 28 asthmatic patients and 27 healthy controls, aged 6-

16 years in Japan. They reported that periostin levels were significantly higher in the asthmatic group (even in mild asthma) compared to controls (p-value =0.012), denoting a possible role for periostin as a helpful marker in asthma diagnosis, making periostin a possible valuable biomarker for diagnosing asthma in children who are unable or unwilling to undertake a lung function test.

Periostin stimulates bronchial muscle hypertrophy and enhances the expression of contractile proteins, increasing bronchospasm, which is a common symptom of asthma attacks.¹⁷ Periostin is deposited on the thickened basement membrane, implying that it is a component of bronchial asthma subepithelial fibrosis.¹⁸ It is also involved in the regulation of mucus secretion by goblet cells of the respiratory bronchial epithelium, by inhibition Gob5 (putative calcium-activated chloride channel involved in the regulation of mucus production) expression and by binding to integrins $\alpha 4$ and $\beta 1/2$ and induction of activation of intracellular pathways resulting in the reduction of the expression of transcription factors, such as NF- κ B, Sp1, and AP-1.¹⁹

In our study, periostin level was significantly higher in patients with severe asthma than those with moderate (p-value <0.001) and mild asthma (p-value <0.001), and in patients with uncontrolled than those with partly (p-value <0.001) and well controlled asthma (p-value <0.001). In severe asthma, periostin was suggested to be a mediator that prolongs bronchial mucosa Th2 cell and eosinophil inflammation and aggravates bronchial wall remodeling.²⁰ Mansur et al²¹ reported in their study that included 115 asthmatic patients, that serum periostin was higher in uncontrolled asthmatics than those with well controlled asthma (p-value=0.014). However, a study by Licari et al,²² that included 107 asthmatic children from Genoa, Italy, observed that serum periostin was not related to the level of asthma control and did not correlate with blood eosinophils and considered it ineffective in clinical practice to assess asthma control in children with allergic asthma. The difference between our study and theirs might be related to different sample size and population characteristics concerning asthma severity, control, atopic status and the ongoing inhaled and/or systemic asthma treatment.

On comparing between well controlled asthmatics and healthy controls, our study showed that patients with well controlled asthma had significantly higher periostin, total IgE and eosinophilic count (p-value <0.001 for each). These results conform with Inoue et al.¹⁶ who reported

significantly higher periostin (p-value=0.012), total IgE (p-value <0.001), and eosinophilic count (p-value <0.001) in well controlled asthmatics than the healthy controls. Elevated periostin level has been linked to eosinophilia and Th2-mediated inflammation. Activated Th2 cells can produce several inflammatory cytokines, such as IL-4 and IL-13, which belong to the main activators of periostin gene expression in the bronchial wall in asthmatic disease.²³ Biopsies of the bronchial mucosa of individuals with allergic bronchial asthma have previously revealed eosinophil infiltration, along with mast cells and T lymphocytes, primarily Th2 cells.¹⁷

Our study also revealed significant positive correlation between serum periostin and eosinophil counts (p-value=0.000), but negative correlation with neutrophil counts (p-value=0.000) among asthmatic patients, further supporting the link between serum periostin and eosinophilic asthma. Elhady et al²⁴ study that enrolled 60 asthmatic children and 30 healthy controls, in Egypt, revealed significant positive correlation between periostin and eosinophils (p-value <0.001) and insignificant positive correlation between periostin and neutrophil (p-value=0.666) in their asthmatic children.

In our study, significant positive correlation between serum periostin and total IgE (p-value=0.000) in asthmatic children was demonstrated. An association between total serum IgE and an Fc ϵ RI α (high-affinity receptor for the Fc region of immunoglobulin E) polymorphism had been previously noted; the importance of this polymorphism relies in the enhanced facilitated antigen presentation by antigen-presenting cells that favours Th2-skewed immunity.²⁵

Our study revealed significant positive correlation between periostin level and asthma severity (P-value=0.000), and significant correlation with asthma control (P-value=0.000). This agrees with Elhady et al²⁴ who reported that serum periostin level was significantly correlated with asthma severity (p-value <0.001), and level of asthma control (p-value <0.001) as well, thus adding to the value of serum periostin as a valuable biomarker, not in only in asthma diagnosis, but also in evaluation in asthma severity and prediction of control as well. Further studies are needed to validate these assumptions.

Results of our study revealed significant negative correlation between serum periostin level and parameters of pulmonary function test (p-value=0.000). Moreover, periostin level was significantly correlated to the severity of

obstruction (p-value=0.000) in asthmatic patients. This is in line with Geng et al,²⁶ whose study included 63 asthmatic children and 30 healthy controls. They observed that serum periostin levels in the exacerbation and remission stages were negatively correlated small airway function (p-value<0.005). Same findings were reported as well in a large population based study by James et al., 2017 who emphasized the presence of a significant association between serum periostin, pulmonary function tests and exhaled nitric oxide as well as markers of type II inflammation among asthmatics.²⁷ However our results disagree with those of Inoue et al¹⁶ who reported that serum periostin was not correlated with either PEF% or FEV1% in children with asthma, but their study did not include children with severe or uncontrolled asthma.

In conclusion, our study could provide more evidence that serum periostin level represents a useful biomarker in the diagnosis of bronchial asthma and can be a useful indicator of asthma severity and treatment response as well. Wider scale longitudinal studies are warranted to validate our findings and to investigate the value of this biomarker in monitoring asthma management and treatment response in different asthma phenotypes and with different asthma treatment modalities.

Study limitation: Our study was limited by several factors including the small sample size and the lack of determination of the atopic status of our patients. In addition, the cases and controls were not matched by gender, which may have influenced the interpretation of the results. Also, the cross-sectional design of our study is another important limiting point as we did not track changes in serum periostin levels over time and their relation to asthma treatment.

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