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Relationship between sputum periostin level and inflammatory asthma phenotypes in Egyptian patients

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ABSTRACT

Introduction: Asthma is a common chronic inflammatory air way disease which poses a high disease burden worldwide. Asthma is a heterogenous disease with various phenotypes and endotypes. Refractory asthma requires new and personalized approaches to manage it effectively. Periostin is a promising biomarker that may help in predicting severity, prognosis and could be a therapeutic target. We aimed in this study to investigate periostin levels in the sputum of asthmatic Egyptian patients of inflammatory phenotypes and its relation to asthma severity.

Subjects and methods: This study included 96 adult asthmatic patients; 48 patients with mild-to-moderate bronchial asthma, 48 patients with severe bronchial asthma according to ATS criteria and 10 healthy controls. All participants were subjected to full history taking and clinical examination; pulmonary function tests; skin prick test; induced-sputum analysis for inflammatory cells and periostin.

Results: Sputum periostin concentrations were significantly higher in patients with asthma than in controls. Sputum periostin is strongly correlated with age and sputum TLC and inversely correlated with FEV1. It is correlated with sputum neutrophil count and sputum eosinophil percentage. Best cut off value for sputum periostin is >528.25 ng/ml to differentiate between mild-to-moderate and severe asthma.

Conclusion: Sputum periostin levels provide a satisfying diagnostic accuracy in severe asthmatic with persistent airflow limitation than mild-to-moderate asthmatic adults. There is a modest positive correlation between sputum periostin and sputum eosinophilia

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Introduction

Asthma is a common chronic inflammatory disease that affects the airways. Both asthma prevalence and incidence have been increasing worldwide recently, mainly because of a wide range of environmental and lifestyle risk factors, in addition to the genetic background (1). It was estimated in 2016 to affect around 339.4 million people worldwide (2). It represents a high disease burden including exacerbations; chronic morbidity and death as it is estimated to cause 346,000 deaths worldwide annually. Thus, it is considered a critical field of research for better understanding and optimal management of the disease. It is now realized to be heterogeneous rather than as a single disease due to the various underlying disease processes and the dynamic genetic environment interactions.

This understanding led to the emergence of the concept of asthma phenotypes and endotypes (3).

Despite the continuous efforts to achieve asthma control and reduce future complications, a group of patients, with estimated prevalence of 5–10% of all asthmatics, remains unable to reach that goal (4). This group is best referred to as “refractory asthma”. Phenotyping asthma has paved the road to better categorization; prediction of disease severity, prognosis, and response to treatment and helped identify more personalized treatments (5).

Biomarkers seem a promising tool that may aid a more precise subtyping of asthma and more targeted therapies. Periostin is one of the biomarkers currently investigated. It is a matricellular protein that is expressed by airway epithelial cells and lung fibroblast. It is a downstream molecule of interleukin (IL)-4 and

IL-13, which are signature cytokines of type 2 immune responses (6). Recent studies have suggested a role of periostin in the pathogenesis, treatment, and phenotyping of asthma (7–11). It was found to promote subepithelial fibrosis, prolong Th2/eosinophilic inflammation, aggravate airway remodeling and associated with increased number of eosinophils in the airways. Another study concluded that serum periostin level correlates modestly with other biomarkers of type-2 inflammation, such as blood eosinophilic count, serum IgE, and FeNO, thus can be considered as a comparable or complementary biomarker (11). Nevertheless, serum periostin is subjected to some confounding factors which may interfere with its accurate reflection of the level of production in the airways such as bone turnover in children (12). Moreover, a large population-based cohort study did not find any differences in median serum periostin levels between different groups (13).

Therefore, we aimed to investigate periostin levels in the sputum of asthmatic Egyptian patients of different inflammatory phenotypes and its relation to asthma severity.

Subjects and methods

Subjects

This study is a cross-sectional study which included 96 adult asthmatic patients. They were classified into two groups: group (A) 48 patients with severe bronchial asthma and group (B) comprising 48 patients with mild-to-moderate bronchial asthma, according to International ERS/ATS criteria (14; Box 1). attending the Allergy and Clinical Immunology outpatient clinic, Ain Shams University Hospitals between October 2016 and March 2018. To grade severity, stepwise approach of treatment of GINA (3) was used.

Box 1. Definition of severe asthma for patients aged ≥ 6 years according to ERS/ATS criteria (14).

Asthma which requires treatment with guidelines suggested medications for GINA steps 4–5 asthma (high dose ICS and LABA or leukotriene modifier/theophylline) for the previous year or systemic CS for $\geq 50\%$ of the previous year to prevent it from becoming “uncontrolled” or which remains “uncontrolled” despite this therapy

Uncontrolled asthma defined as at least one of the following:

- 1) Poor symptom control: ACQ consistently ≥ 1.5 , ACT < 20 (or “not well controlled” by NAEPP/GINA guidelines)
- 2) Frequent severe exacerbations: two or more bursts of systemic CS (≥ 3 days each) in the previous year
- 3) Serious exacerbations: at least one hospitalization, ICU stay or mechanical ventilation in the previous year
- 4) Airflow limitation: after appropriate bronchodilator withhold $FEV_1 < 80\%$ predicted (in the face of reduced FEV_1/FVC defined as less than the lower limit of normal)

Controlled asthma that worsens on tapering of these high doses of ICS or systemic CS (or additional biologics)

Abbreviations: GINA = Global Initiative for Asthma; LABA = long-acting β_2 -agonists; CS = corticosteroids; ACQ = Asthma Control Questionnaire; ACT = Asthma Control Test; NAEPP = National Asthma Education and Prevention Program.

The study also included 10 healthy controls of matched age and sex group from the medical staff of our hospital. The study was approved by the local ethical committee. All patients provided written informed consents.

Inclusion criteria: Subjects diagnosed with bronchial asthma according to GINA guidelines (3) aged between 18 and 60 years.

Exclusion criteria: Patients with inflammatory or septic conditions, patients with other pulmonary disorders like bronchiectasis, tumors, occupational lung disorders, etc., patients with associated systemic autoimmune diseases, patients with organ failure, as renal failure, liver cell failure, etc., smokers, pregnant or lactating females, patients older than 60 years and younger than 18 years and patients on immunotherapy before the start of the study.

Methods

All participants were subjected to the following:

History taking and clinical examination. Patients ≥ 18 years old, taking their usual treatment, which was thoroughly recorded and had to have remained unmodified within the last 4 weeks prior to the study. Diagnosis of asthma was done according to GINA 2018 guidelines (3). Full detailed history taking, and thorough clinical examination were done with special emphasis on clinical characteristics of asthma (such as age of onset, asthma exacerbations, symptoms control, and asthma-related medication); coexisting conditions and comorbidities (such as aspirin/non-steroidal anti-inflammatory drugs hypersensitivity, chronic rhinosinusitis, nasal polyposis, gastroesophageal reflux disease, allergic rhinitis, and obesity).

Pulmonary function tests (PFT). Lung functions were measured by a spirometer (Microspiro HI-601 Spiro Analyzers, Chest MI Inc., Japan). PFTs were performed according to ATS/ERS guidelines (15). The set of reference values for all ages used was that of the Global Lung Function Initiative reference equations (16). Bronchial reversibility testing was not included.

Skin prick test (SPT) to common allergens (prepared at the allergen extract unit, Faculty of Medicine, Ain Shams University) such as house dust mite, mixed molts, hay dust, horse hair, mixed pollens, goat

Table 1. Demographic, clinical, and laboratory characteristics of all participants.

		Cases (n = 96)		Controls (n = 10)		<i>t</i> ^a	<i>p</i> Value
		Mean	SD	Mean	SD		
Age (years)		44.02	12.44	40.90	13.65	0.16	0.87
BMI		23.56	5.85	24.09	6.21	0.88	0.38
FEV ₁ (% predicted)		57.19	17.72	91.3	2.06		<0.001
Sputum TLC (cell/mm ³)		842.58	193.28	108	40.77	31.17	<0.001
Sputum periostin (ng/ml)		577.06	239.98	55.4	35.32	19.38	<0.001
Sputum eosinophils%		5.78	2.3	1.7	0.77	12	<0.001
Sputum neutrophils%		60.81	11.77	48.9	10.46	3.05	0.003
		<i>N</i>	%	<i>N</i>	%	χ^2 ^b	
Gender	Male	50	52.1	6	60	0.17	0.68
	Female	46	47.9	4	40		
Associated allergic disease	None	48	50	–	–		
	Skin allergy	27	28.1	–	–		
	Rhino sinusitis	21	21.9	–	–		
Family history of atopy	Positive	47	49	3	30		
	Negative	49	51	7	70		
Pattern of PFT abnormality	Obstructive	64	68.1	–	–		
	Mixed	30	31.9	–	–		
SPT	Positive	81	84.4	–	–		
	Negative	15	15.6	–	–		

Data are represented in mean $\pm$ standard deviation (SD) or number (percentage).

Abbreviations: FEV₁ = forced expiratory volume in one second; SPT = skin prick test.

^aStudent *t* test.

^bChi square test.

hair, cat hair, cotton dust, tobacco, pigeon, dog hair, cockroach, straw, feather, wool, and latex. All antihistamines were omitted for 24–72 h before testing. After sterilization of the forearm with propyl alcohol swab, a drop of each allergen extract was put on marks 1 cm apart. Additionally, a drop of histamine phosphate and a drop of saline 0.9% were used as positive control and negative control, respectively (17). The immediate response was evaluated after 20 min. A skin test panel was considered valid if the difference in mean wheal diameters between the positive and negative controls was at least 1 mm. A wheal diameter $\geq$ 3 mm more than the negative control was considered positive (18).

Sputum analysis for inflammatory cells and periostin level. Sputum induction was done by nebulized hypertonic saline 3% and salbutamol for maximum 20 min. The sputum was isolated from saliva by forceps. One portion of the sputum was liquefied using ultrasonic waves then underwent ultracentrifugation. The supernatant was used for quantitative measurement of sputum level of periostin using ELISA technique by The Thermo Scientific™ Pierce™ Human Periostin ELISA Kit, Massachusetts, USA. The detection limit is 80 pg/ml. A phosphate-buffered saline solution was prepared with dithiothreitol (DTT), at 0.1% concentrations and was added to an equal volume of sputum to cause mucolysis. The mucus samples were stained with Leishman stain and total and differential cell counts were evaluated. Inflammatory phenotypes of asthma were then defined as eosinophilic if (sputum

eosinophils $\geq$ 3% and sputum neutrophils <61%), neutrophilic if (sputum neutrophils $\geq$ 61% and sputum eosinophils <3%), paucigranulocytic if (sputum neutrophils <61% and sputum eosinophils <3%), or mixed granulocytic if (sputum neutrophils $\geq$ 61% and sputum eosinophils $\geq$ 3%; 19,20).

Statistical methods. Analysis of data was done using SPSS (Statistical Package for Social Science) program version 18. To describe the studied sample, quantitative data were presented as minimum, maximum, mean, and standard deviation. Qualitative data were presented as count and percentage. Student *t* test was used to compare quantitative data between two groups and one-way ANOVA was used when more than two groups were to be compared then post hoc test was used to detect the difference between individual groups. Chi-square test was used to compare qualitative data between different groups, Pearson correlation test was used to compare correlation between different continuous variables, ROC curve was used to measure diagnostic validity, and determine the best cut off value for some variables. *p* Values < 0.05 was considered statistically significant.

Results

Table 1 summarizes the demographic and clinical characteristics of all participants. The mean age of patients was 44 ± 12 years old, 52% were males and 48% were females, sputum periostin was markedly elevated in comparison to the controls (*p* values <0.001),

Table 2. Comparison between group A (severe asthma) and B (mild-to-moderate asthma) regarding demographic, clinical, and laboratory data.

		Group A (n = 48)		Group B (n = 48)		Test value	p Value
		Mean	SD	Mean	SD		
Age (years)		50.37	6.77	37.67	13.59	5.80 ^a	<0.001
BMI		24.09	6.21	23.04	5.49	0.88 ^a	0.38
Duration of asthma (years)		29.21	10.36	21.62	12.95	3.17 ^a	0.002
FEV ₁ (% predicted)		40.85	6.10	73.52	7.24	23.91 ^a	<0.001
Sputum TLC (cell/mm ³)		907.71	175.68	777.46	189.69	3.49 ^a	0.001
Sputum periostin (ng/ml)		744.60	174.09	409.52	169.59	9.55 ^a	<0.001
Sputum eosinophil %		5.16	2.21	6.39	2.28	2.68 ^a	0.01
Sputum neutrophil %		60.08	12.94	61.53	10.57	0.60 ^a	0.55
		N	%	N	%		
Gender	Male	26	54.2	24	50.0	0.1 ^b	0.68
	Female	22	45.8	24	50.0		
Associated allergic disease	None	24	50.0	24	50.0	0.09 ^b	0.96
	Skin allergy	13	27.1	14	29.2		
	Rhinosinusitis	11	22.9	10	20.8		
Family history of atopy	Negative	26	54.2	23	47.9	0.38 ^b	0.54
	Positive	22	45.8	25	52.1		
Pattern of PFT abnormality	Obstructive	28	59.6	36	76.6	3.13 ^b	0.08
	Mixed	19	40.4	11	23.4		
Severity of asthma according to GINA	Step I	0	0	8	16.7	114.6 ^c	<0.001
	Step II	0	0	22	37.5		
	Step III	0	0	18	45.8		
	Step IV	8	16.7	0	0		
	Step V	32	66.7	0	0		
	Step VI	8	16.7	0	0		
SPT	Negative	9	18.8	6	12.5	0.71 ^a	0.40
	Positive	39	81.3	42	87.5		
Inflammatory Asthma phenotype	Eosinophilic	24	50.0	29	60.4	5.96 ^c	0.12
	Neutrophilic	4	8.3	1	2.1		
	Mixed granulocytic	16	33.3	18	37.5		
	Paucigranulocytic	4	8.3	0	0		
Mono/polysensitized	Monosensitized	9	23.1	7	16.7	0.52 ^b	0.47
	Polysensitized	30	76.9	35	83.3		

Data are represented in mean $\pm$ standard deviation (SD) or number (percentage).

Abbreviations: FEV₁ = forced expiratory volume in one second; SPT = skin prick test.

^aStudent *t* test.

^bChi Square test.

^cFisher Exact.

50% of cases had associated allergic diseases; namely skin allergy and rhinosinusitis. About 49% of them had a positive family history, 84% had a positive SPT, and 68% had obstructive PFT pattern while 32% had mixed pattern. On comparing patients; those with severe asthma (group A) were older and had longer duration of asthma (*p* values 0.002) than those with mild-to-moderate asthma (group B). Group A had significantly higher levels of sputum periostin (744.6 ± 174 ng/ml; *p* values < 0.001), higher sputum total leucocytic count (907.7 ± 175 cells/mm³, *p* values < 0.001), and the eosinophilic phenotype was significantly more prevalent than in group B (*p* values 0.01). Sputum neutrophilia was comparable in both groups (Table 2).

Sputum periostin level was positively correlated with age, duration of asthma, sputum total leucocytic count (TLC), sputum eosinophilic percentage (*r* 0.27, *p* values 0.007), and asthma severity (*p* values < 0.001). It was negatively correlated with FEV₁; but it was not correlated with gender, BMI, and sputum neutrophilic

percentage (Table 3). Using receiver operating characteristic curve (ROC) analysis of the validity of sputum periostin for differentiation between severe asthma and mild-to-moderate asthma. the cutoff value of sputum periostin was found to be ≥ 528.25 ng/ml, with a test sensitivity of 95.8% (95% CI, 86.5–97.6) and specificity of 77.1% with a positive predictive value of 80.7% and a negative predictive value of 94.9% (Figure 1; Table 4).

Discussion

To our knowledge, there are only two recent studies (21,22) beside ours that compared periostin levels in sputum of asthmatic patients to that of healthy controls. A lot of studies were conducted lately on serum periostin in asthma (9,23–26) but more recently, sputum periostin in asthmatics caught the scientific attention to explore its potentials as a biomarker and a companion diagnostic (21–23,27).

Table 3. Correlation between sputum periostin level and patients' demographic, clinical, and laboratory data.

		Sputum periostin (ng/ml)		Test value	p Value
		Mean	SD		
Gender	Male	573.62	257.45	0.15 ^a	0.89
	Female	580.80	222.21		
Severity of asthma according to GINA	Step I	410.31 ^a	173.39	78.75 ^b	<0.001
	Step II	381.02 ^a	149.76		
	Step III	444.01 ^a	192.67		
	Step IV	727.89 ^b	146.17		
	Step V	745.66 ^b	181.54		
	Step VI	757.10 ^b	188.84		
Inflammatory asthma phenotype	Neutrophilic	548.75 ^a	218.88	3.02 ^b	0.03
	Mixed granulocytic	797.80 ^b	210.39		
	Paucigranulocytic	562.58 ^a	261.91		
	Eosinophilic	799.40 ^b	126.39		
Age in years				0.407 ^c	<0.001
BMI				0.053 ^c	0.607
Duration of asthma (years)				0.200 ^c	0.051
FEV ₁ (% predicted)				-0.761 ^c	<0.001
Sputum TLC (cells/mm ³)				0.420 ^c	<0.001
Sputum neutrophil %				0.152 ^c	0.140
Sputum eosinophil %				0.274 ^c	0.007

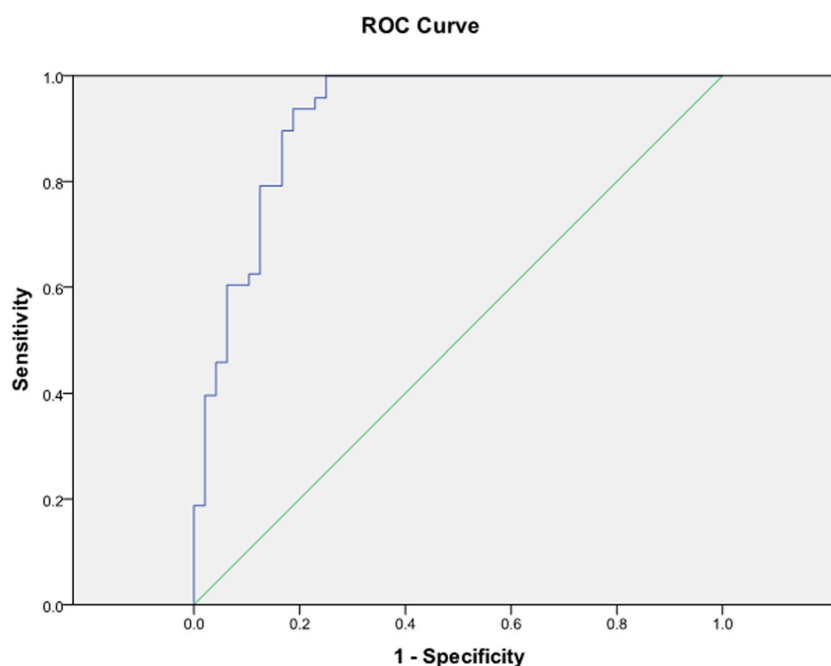
Data are represented in mean $\pm$ standard deviation (SD).

Abbreviations: FEV₁ = forced expiratory volume in one second.

^aStudent t test.

^bOne Way ANOVA test [(a,b) *post hoc* test].

^cPearson correlation coefficient.

**Figure 1.** ROC curve for validity of sputum periostin for differentiation between severe asthma and mild-to-moderate asthma.**Table 4.** ROC curve analysis for the value of serum classification in classification of subjects into those with severe asthma and with mild-to-moderate asthma.

Area under the ROC	0.921, $p < 0.001$ (95% CI, 86.5–97.6)
Best cut off value; ng/ml	≥ 528.25
Sensitivity; %	95.8
Specificity; %	77.1
Positive Predictive value; %	80.7
Negative Predictive value; %	94.9

Abbreviations: ROC = receiver operating characteristic; CI = Confidence interval.

Although measurement of serum periostin is more feasible in clinical practice, information from the airways gives us a closer look on the pathogenesis of severe asthma without the confounding effect of other parts of the body secreting periostin in the bloodstream.

On comparing our findings to other studies, we found that, as regards the sputum periostin levels, Carpagnano et al. found similar results to ours as they

were able to detect higher periostin levels in the exhaled breath condensate (EBC) and in induced sputum of patients with severe asthma compared with patients with mild-to-moderate asthma and healthy control subjects, respectively (23). In contrast, Wardzyńska et al. did not observe any statistically significant differences in periostin in EBC of asthmatics in comparison to healthy subjects. Moreover, they failed to find any correlation between EBC periostin and other asthma parameters (24).

In our study, sputum periostin level had a strong positive correlation with age, duration of asthma, and sputum TLC. On the other hand, it did not correlate with gender, BMI, nor sputum neutrophilic percentage. There was a modest positive correlation between sputum periostin and sputum eosinophilia (p values 0.007; Table 3). This could be explained by the fact that all the patients included in this study, especially those with severe asthma, were on inhaled corticosteroids (ICS) in high doses which could have caused a decrease in airway eosinophils and hence sputum eosinophils as demonstrated by Cowan and colleagues (28). Bobolea et al. similarly stated that “periostin levels in sputum are associated with persistent airflow limitation in asthma patients with airway eosinophilia despite treatment with high-dose inhaled corticosteroids” (21).

Simpson et al. found a statistically significant correlation between both serum and sputum periostin and the sputum eosinophil proportions. However, they concluded that the strength of the association was relatively modest such that neither sputum nor serum periostin levels could sufficiently predict the presence of an eosinophilic inflammatory subtype. Sputum periostin levels were also higher in patients with persistent airflow limitations compared to those without airflow limitations (29).

In contrast to our study, Gorska et al. did not find any correlations between periostin levels and asthma inflammatory phenotype in mild-to-moderate asthma. It is worth noting that the participants in this study, unlike ours, were not on ICS for 6 weeks before the enrollment to the study (30).

As we can see, limited and controversial data are available till date and larger scale studies are needed to further explore the potential role of sputum periostin as a biomarker in asthma.

Limitations of the study

Mean dose of ICS used by the different groups of patients and its correlation with sputum periostin level should have been assessed.

Conclusion

Identifying inflammatory asthma phenotypes is now a well-recognized way to better management. As such, there is an unmet need for surrogate biomarkers to help clinical decision making in asthma.

Sputum periostin levels provide a relatively satisfying diagnostic accuracy in severe asthmatics more than in mild-to-moderate asthmatic adults. Moreover, there is a modest positive correlation between sputum periostin and sputum eosinophilia; thus, a surrogate biomarker to severe eosinophilic asthma and a therapeutic target. More studies are needed exploring the potential role of periostin levels in bronchial fluids.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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