Increased expression of serum periostin and YKL40 in children with severe asthma and asthma exacerbation

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ABSTRACT

Children with severe asthma or acute asthma exacerbation may encounter difficulties in performing pulmonary function tests. In this situation, serum biomarkers can play a great role in evaluation of such patients. The aim of this study was to estimate the serum levels of human chitinase-3-like protein 1 (YKL40) and periostin in a group of Egyptian children with asthma during acute asthma exacerbation and in stable asthmatics compared with healthy control, and to correlate these findings with the severity of asthma. This cross-sectional study enrolled 120 childrenwith asthma with different degrees of asthma severity, according to the Global Initiative for Asthma guidelines, along with 60 age-matched and sex-matched healthy control. A complete blood count and an estimation of serum periostin and YKL40 levels were performed for all cases and control. Individual and mean values of periostin and YKL40 were significantly higher during acute asthma exacerbations, p<0.001. A highly significant relation between serum levels of periostin and YKL40 and asthma severity, p value for each was <0.001. Absolute eosinophil count was significantly correlated with the serum periostin levels in stable asthmatic group (p=0.01) only. There was significantly positive correlation (P<0.001) between both markers in stable asthmatic group. Spearman's correlation coefficient shows a statistically significant positive correlation between both markers and patient's age and duration of asthma, p value for each was 0.001. These findings highlight the importance of periostin and YKL40 as serum biomarkers for assessment of asthma severity and acute asthma exacerbations in children with asthma.

Significance of this study

What is already known about this subject?

- Human chitinase-3-like protein 1 (YKL40) acts as a specific biomarker of neutrophilic activation in asthma.
- Periostin is a biomarker of type 2 inflammation that plays a major role in asthma pathogenesis.
- Periostin and YKL40 serum levels increased in patients with asthma.

What are the new findings?

- This is the first study that measures the serum levels of periostin and YKL40 at the same time in children with asthma both in stable state and during acute asthma exacerbation.
- This is the first study that demonstrated a relationship between both markers in children with stable asthma.
- Absolute eosinophil count was significantly correlated with the serum periostin levels in stable asthmatic state only but not during asthma exacerbation in children.

How might these results change the focus of research or clinical practice?

- These findings may add to the evaluation and monitoring of different asthma phenotypes.

INTRODUCTION

Asthma is a heterogeneous disorder characterized by bronchial obstruction, airway hyper-responsiveness and many phenotypes, with varying degrees of disease severity and level of control.[1]

Many biomarker researches have been performed to improve asthma management, particularly among those suffering from severe and/or uncontrolled asthma.[2] YKL40 (human chitinase-3-like protein 1) is one of these markers that play a role in the regulation of the innate immune responses in inflammatory conditions including asthma. YKL40 is secreted from neutrophils, macrophages and airway epithelial cells of the respiratory tract mucosa. Therefore, it can act as a specific biomarker of granulocyte function and macrophage activation in patients with asthma.[3] YKL40 may cause asthma-associated inflammation through activation of interleukin-13 pathway that enhances airway hyper-reactivity, a key feature of bronchial asthma.[4]

Another important biomarker is the serum periostin, which is a matricellular protein secreted by bronchial fibroblasts and epithelial cells, and is involved in airway remodeling

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and subepithelial fibrosis.[5 6] Several adult studies have confirmed that serum level of periostin increases in type 2 immune response in asthma.[7 8]

It was hypothesized that the serum levels of YKL40 and periostin would increase during acute asthma exacerbation and would correlate with the disease severity. So, we estimated the serum levels of YKL40 and periostin in a group of Egyptian children with asthma during acute asthma exacerbation and in stable asthmatics compared with healthy control and correlated these findings with the severity of asthma.

METHODOLOGY

Study design

The present cross-sectional study enrolled 120 children with asthma with different grades of asthma severity (60 children with stable asthma and 60 children with acute asthma exacerbation); all were recruited from the Allergy Clinic of the Allergy and Pulmonology Unit of Specialized Children's Hospital, Cairo University, Egypt. Asthma was diagnosed according to the Global Initiative for Asthma (GINA) guidelines 2011.[9] Sixty age-matched and sex-matched healthy children, with no history of atopic disease nor chronic upper or lower airway disease, were included as controls, they were subjected to the same investigations as cases. Exclusion criteria of enrollment were preterm delivery, and suspected or proven alternative causes for recurrent wheezing or other respiratory disease.

Asthma exacerbation was defined according to GINA, 2011 as recent worsening of symptoms (shortness of breath, cough, increased chest activity and wheezing) with increase in the frequency of reliever medication use (inhaled short-acting β2 agonists (SABA)), the severity of asthma exacerbations was graded and managed as per the guidelines in GINA, 2011.[9] Accordingly, mild and moderate exacerbation cases were sent to be managed in the triage while severe cases were sent to the emergency ICU.

Ethical considerations

Informed verbal parental consent was obtained from all the study participants. The study design fulfilled the Revised Helsinki Declaration of Bioethics.

Methods

All patients were subjected to complete history taking with special attention to the onset of symptoms, the duration of the disease, the severity of asthma, history of associated allergic manifestations, food allergy and family history of asthma.

The ongoing long-term asthma control medications were recorded for all cases, and all were not stopped prior to enrollment. These medications included SABA, long-acting β2 agonist, inhaled corticosteroids (ICS), mast cell stabilizer or leukotriene antagonist.

Laboratory methods

Complete blood count and estimation of serum levels of YKL40 and periostin were performed for all cases and control.

Complete blood count was done on Cell-dyn3700 (Abbott Diagnostic, USA).

Serum YKL40 levels were measured using human chitinase-3-like protein 1 (YKL-40/CHI3L1) ELISA kit supplied by NOVA (Bioneovan, Beijing, China).[10]

Periostin serum level was measured by ELISA supplied by EIAab (Wuhan EIAab Science, China).[11]

Statistical analysis

Data were analyzed using the statistical package SPSS V.24. Data were presented as mean, SD, median, minimum and maximum for quantitative data and as number and percentage for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney U tests. Correlations between quantitative variables were done using Spearman's correlation coefficient. P values <0.05 were considered as statistically significant.

RESULTS

One hundred twenty children with asthma were enrolled in the present study. Their age ranged from 3 to 11.5 years with a mean age of 7.67±2.40 years. Seventy six of them were males (63.3%) and forty four were females (36.7%). Included patients had different degrees of asthma severity. Sixty healthy controls, who were matched for age and sex, were included with mean age of 7.68±2.48, of them 56.7% were males and 43.3% were females. Our study detected eosinophilia in 64.2% of asthmatic children. There were significant increase in the serum levels of periostin and YKL40 in children with asthma ($p<0.001$) compared with healthy controls. Basic demographic, clinical and laboratory characteristics of the study participants are shown in table 1.

The relation between different asthma phenotypes and serum levels of YKL40 and periostin in all asthmatic children showed a highly significant relation between the degree of asthma severity and both biomarkers levels (YKL40 and periostin), where the p value for each was <0.001 (table 2 and figures 1 and 2, respectively). Also, a highly significant relation was found between acute asthma exacerbation and both markers levels (p value for each was <0.001). Non-significant relations were found between other variables and the measured biomarkers, as demonstrated in table 2.

Individual and mean values of YKL40 and periostin were significantly higher during acute asthma exacerbations; p value for each was <0.001, as are shown in table 3 and figures 3 and 4, respectively.

On studying the relation between the absolute eosinophil count, serum levels of periostin and YKL40 in asthma subgroups and control, the absolute eosinophil count significantly correlate with the serum periostin levels in stable asthma subgroup only ($p=0.01$), as are shown in table 4.

Also, on studying the correlations between serum levels of the two measured biomarkers (serum levels of periostin and YKL40) in control and asthma subgroups, there was significantly positive correlation between the levels of both biomarkers in stable asthmatic subgroup only ($p<0.001$), but non-significant correlations in acute exacerbations or in control groups ($p=0.43$ and 0.32, respectively), as are demonstrated in table 4.

When Spearman's correlation coefficient was used for studying the correlation between patient variables (clinical and laboratory) and the measured biomarkers in all children with

Table 1 Demographic, clinical and laboratory characteristics of the study participants

Demographic characteristics	Children with asthma n=120	Control n=60	P values*
Age (years)			
Range	7.5 (3–11.5)	8.25 (3–11)	0.831
Mean±SD	7.67±2.40	7.68±2.48	
Gender, n (%)			
Male	76 (63.3)	34 (56.7%)	0.787
Female	44 (36.7)	26 (43.3%)	
Passive smoking n (%)	89 (74.2%)	26 (43.3%)	<0.001
Asthma characteristics			
Asthma subgroups, n (%)			
Stable asthma	60 (50%)	–	–
Acute asthma exacerbation	60 (50%)	–	–
Degree of asthma severity, n (%)			
Intermittent	34 (28.3%)	–	–
Mild persistent	14 (11.7%)	–	–
Moderate persistent	16 (13.3%)	–	–
Severe persistent	56 (46.7%)	–	–
Duration of asthma (month)			
Median (range)	28 (2–96)	–	–
Mean±SD	29.41±25.84	–	–
Associated allergic manifestations, n (%)			
Allergic rhinitis	78 (65%)	–	–
Conjunctivitis	76 (63.3%)	–	–
Eczema	68 (56.7%)	–	–
Food allergy, n (%)	30 (25%)	–	–
Family history of atopy, n (%)	83 (69.2%)	–	
Asthma treatment, n (%)			
Short-acting β agonists	120 (100%)	–	–
Inhaled corticosteroids	86 (71.7%)	–	–
Long-acting β agonists	24 (20%)	–	–
Mast cell stabilizer/ leukotriene antagonist	100 (83.3)	–	–
Serum YKL40 (pg/mL)			
Median (range)	1050 (300–3750)	334 (160–840)	<0.001
Mean±SD	1656.83±1192.04	407.78±185.21	
Serum periostin (ng/mL)			
Median (range)	54 (38–357)	44.4 (36–47.4)	<0.001
Mean±SD	57.78±30.22	43.01±3.46	
Eosinophilia	77 (64.2%)	0 (0%)	<0.001
Anemia	49 (40.8%)	23 (38.3%)	0.747

Data are represented as frequency (%), and quantitative data are presented as median (range), mean±SD.
*P values <0.05 are considered statistically significant.
YKL40, human chitinase-3-like protein 1.

asthma, there was a statistically significant positive correlation between patient's age and serum levels of YKL40 and periostin, also a significant positive correlation between duration of asthma and serum levels of both biomarkers, p value for each was <0.001. Non-significant correlations were found between other variables and the measured biomarkers, as are shown in table 5.

DISCUSSION

Our data from 120 children with asthma show significant increases in the serum levels of YKL40 and periostin, as compared with healthy controls. Both biomarkers correlate with asthma severity. Also, this study recorded a more significant rise in the serum levels YKL40 and periostin during acute asthma exacerbation compared with stable asthma state.

Similar to our finding, Chupp *et al* reported that there are significant increases in the serum levels of YKL40 in asthmatics compared with healthy controls, also, their levels correlate with asthma severity.[12] Despite that, our results were inconsistent with Santos *et al*, who concluded that severe persistent asthma in childhood is not associated with elevated YKL40 levels, unlike in adults with severe persistent asthma.[13]

Furthermore, this study recorded a significant relationship between serum YKL40 levels and acute asthma exacerbation in children (p<0.001), this go in line with a previous report by Specjalski *et al*, who reported that in asthmatics, the level of serum YKL40 was significantly higher in the subgroup with poor control of symptoms and exacerbations compared with stable asthmatics.[14] This also, was supported by Tang *et al*, in their study which showed higher serum YKL40 in patients with more severe or uncontrolled asthma.[15] These studies that have found differences related to asthma severity, and acute asthma exacerbations gave a wider spectrum of severity of YKL40 in patients with asthma.

Although there are several published articles on the role of periostin in asthma and type 2 inflammatory responses in adult, studies on its levels in children with asthma are still few and further researches are required.[6] Our study showed significantly higher periostin levels in children with asthma compared with healthy children. Also, the levels were significantly correlated with asthma severity and acute asthma exacerbation in children. Previous study by Anderson *et al* on children with asthma showed significant increases in periostin levels during early childhood only (145 ng/mL), with non-significant changes at older ages.[16]

We noticed that the mean serum levels of periostin in this work were lower than their levels in a previous study,[17] this could be due to the ELISA technique used. Also, levels in this study may be affected by asthma treatment, where 71.7% patients received ICS, and it has been previously shown that chronic use of ICS causes reduction in serum periostin level.[18]

This study recorded a significant difference between cases and controls in passive smoking, which was in agreement with Arshad *et al*,[19] who reported that environmental tobacco smoke is a risk factor for the development and aggravation of asthma symptoms.[19] Nevertheless, a non-significant relation was found between passive smoking and periostin levels in all children with asthma, this was supported by Górska *et al*, who found that smoking status did not influence serum periostin levels or periostin expression in bronchial biopsy.[20] On the other hand, Thomson *et al* detected lower serum periostin levels in asthmatic smokers when compared with never-smokers.[21] Also, a non-significant relation was found between passive smoking and YKL40 levels. Similarly, Naglot *et al* did not observe any statistically significant dependence of YKL40 levels on smoking in their study.[22] But, this was inconsistent with another study conducted by Guerra *et al*, who found that smoking exposure influences the levels of YKL40.[23]

Table 2 The relation between asthma phenotype and the measured serum biomarkers levels

Asthma phenotype	Serum biomarkers YKL40 (pg/mL) Median (range)	P values*	Periostin (ng/mL) Median (range)	P values*
Sex				
Male	1445 (300–3750)	0.89	54 (39–117.9)	0.76
Female	945 (301–3600)		54 (38.4–357)	
Asthma severity				
Intermittent	358.7 (300–800)		46.05 (38.4–55.8)	
Mild persistent	650 (364.5–997)	<0.001	47.4 (42.6–54)	<0.001
Moderate persistent	945 (690–1823)		54 (49.5–57)	
Severe persistent	2979.5 (2040–3750)		60 (51-357)	
Asthma subgroups				
Stable asthma	436.5 (300–1100)	<0.001	48 (38.4–55.8)	<0.001
Asthma exacerbation	2787 (927.5– 3750)		60 (49.5–357)	
Allergic rhinitis	1445 (300–3609)	0.745	54 (38.4–357)	0.305
Allergic conjunctivitis	1050 (300–3705)	0.989	54 (39–108)	0.551
Skin allergy	1050 (300–3600)	0.743	54 (38.4–357)	0.746
Food allergy	1030 (300–3453)	0.837	54(38.4–117.9)	0.998
Family history of asthma	1823 (300–3750)	0.604	54 (38.4–357)	0.253
Passive smoking	1000 (300–3705)	0.199	54 (38.4–117.9)	0.366
Anemia	997 (300–3347)	0.479	54 (40.8–117.9)	0.996

*P values <0.05 are considered statistically significant.
YKL40, human chitinase-3-like protein 1.

Many asthma phenotypes have been identified with different recognizable clusters of demographic, clinical and/or pathophysiological characteristics.[24] In this work, despite the high percentage of children presented with atopy, we found a non-significant association between atopy and serum levels of periostin and YKL40 as demonstrated in table 2. Similar to our finding, Inoue *et al*, in their study on children aged 6–11 years found no correlations between serum periostin levels and various manifestations of allergic disease.[25] Many other studies reported similar findings regarding periostin levels,[21 26] while Li *et al* stated that periostin has been implicated in atopic condition.[27] Also, Naglot *et al* reported a non-significant association between serum YKL40 levels and atopy.[22]

In this study, the mean serum levels of YKL40 (pg/mL) were increased significantly with increasing patient's age and duration of asthma, this was in accordance with Chupp *et al*, who reported a significant positive relationship between the circulating YKL40 levels and the duration of asthma in the three cohorts.[12] Although our study found a significant relation between serum level of periostin and asthma duration among involved children, some of the previous studies recorded a non-significant relations.[28]

The inflammatory response in asthma is highly complex; it includes infiltration of the airways by activated lymphocytes, eosinophils and neutrophils, and it is associated with the release of pro-inflammatory mediators. Increased

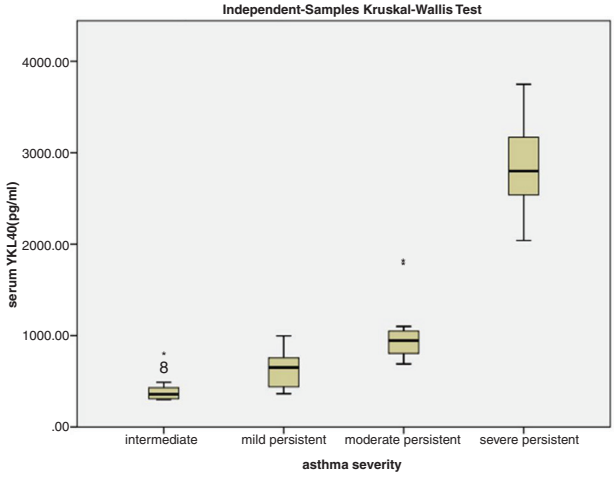

Figure 1 Box-and-whisker plot exhibiting the serum YKL40 (human chitinase-3-like protein 1) levels in children with asthma with different grades of asthma severity.

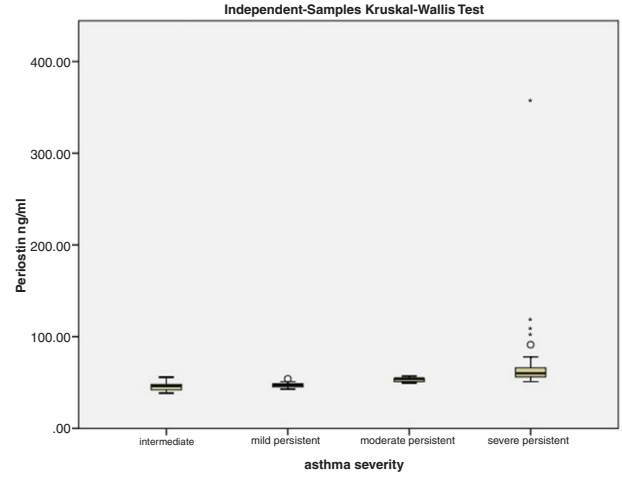

Figure 2 Box-and-whisker plot exhibiting the serum periostin levels in children with asthma with different grades of asthma severity.

Table 3 Comparing the mean levels of serum YKL40 and periostin in control and asthma subgroups

Biomarkers	Group			
	Acute asthma exacerbation n=60	Stable asthma n=60	Controls n=60	P values*
YKL40 (pg/mL)				
Median (range)	2787 (927.5–3750)	436.5 (300–1100)	334 (160–840)	<0.001
Mean±SD	2767.33±548.58	546.55±248.41	407.78±185.21	
Periostin (ng/mL)				
Median (range)	60 (49.5–357)	48 (38.4–55.8)	44.4 (36–47.4)	<0.001
Mean±SD	67.88±40.17	47.68±4.47	43.01±3.46	

*P values <0.05 are considered statistically significant.
YKL40, human chitinase-3-like protein 1.

blood eosinophils in children with asthma often correlate with greater asthma severity.[29] Similarly, our study shows increased absolute eosinophilic count among children with asthma. But what is interesting is that it is significantly correlated with the serum periostin levels in stable asthmatic group (p=0.01) only, which can be explained by its relation to the underlying eosinophilic inflammation among stable group, and this was masked by increased neutrophils, rather than eosinophils, with acute asthma exacerbations precipitated by infections, this was supported by Monteseirín who linked increased neutrophils to the development of severe acute asthma exacerbation.[30] Inoue *et al* found moderate relationships between periostin and blood eosinophils.[31] Jia *et al* in their study on severe asthmatics found a significant relation between increased serum periostin level and airway eosinophilia.[8]

On the other hand, Habernau *et al* and Konradsen *et al* reported that no correlations were found between serum periostin and blood eosinophils in children with severe or uncontrolled asthma,[17 32] what was agreed with Inoue *et al* findings in school-aged children.[25] Moreover, Jayaram *et al* showed that patients may experience an exacerbation of asthma without an increase in eosinophilic inflammation.[33]

An interesting finding in this work was the significantly positive correlation (p<0.001) between periostin and YKL40 in stable asthmatic group, while non-significant correlations between both markers in acute exacerbations and control group.

All the previously mentioned studies had comparable results to our work in that both markers are increased in patients with asthma and thus can be used as an inflammatory marker in asthma, also, reliable biomarkers of asthma severity and acute exacerbations in children. However, further multicenter studies with larger number of children with asthma are needed to explore the associations of these markers with the severity of exacerbations and with the patient's clinical outcomes related to pediatric ICU admission and mechanical ventilation.

In conclusion, serum periostin and YKL40 levels were significantly increased in relation to asthma severity in children, and more significant increase in their levels was observed in those with acute asthma exacerbation compared with stable asthmatics. These findings highlight

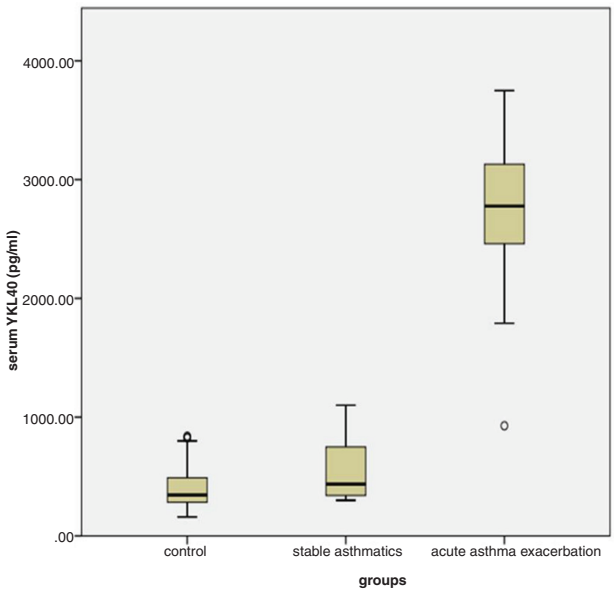

Figure 3 Box-and-whisker plot exhibiting the serum YKL40 (human chitinase-3-like protein 1) levels in control and asthma subgroups.

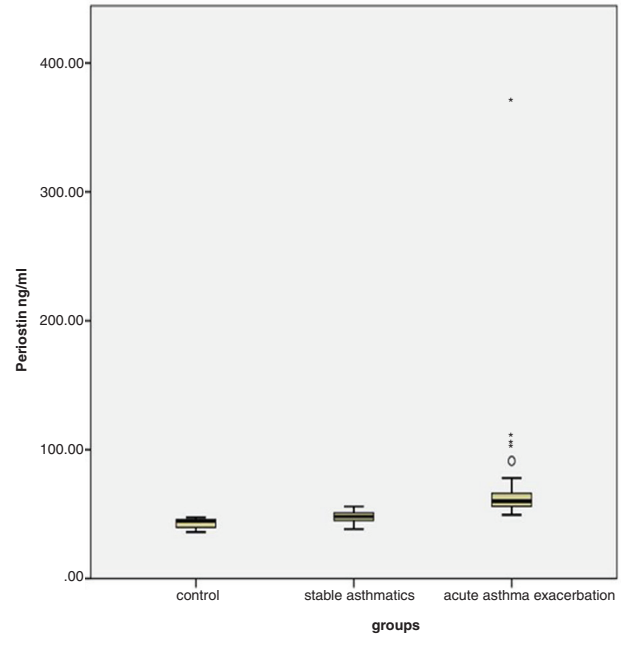

Figure 4 Box-and-whisker plot exhibiting the serum periostin levels in control and asthma subgroups.

Table 4 Correlations between the measured parameters in control and asthma subgroups

Measured parameters		Groups: Control YKL40 (pg/mL)	Control: Periostin (ng/mL)	Stable asthmatics: YKL40 (pg/mL)	Stable asthmatics: Periostin (ng/mL)	Acute asthma exacerbation: YKL40 (pg/mL)	Acute asthma exacerbation: Periostin (ng/mL)
AEC (cells/mm)	Correlation coefficient	0.063	−0.128	0.055	0.325	−0.087	0.119
	P values*	0.634	0.332	0.677	0.011	0.508	0.365
Serum YKL40 (pg/mL)	Correlation coefficient	1.000	−0.104	1.000	0.606	1.000	0.129
	P values*	–	0.431	–	<0.001	–	0.326
Serum periostin (ng/mL)	Correlation coefficient	−0.104	1.000	0.606	1.000	0.129	1.000
	P values*	0.431	–	<0.001	–	0.326	–

*P values <0.05 are considered statistically significant.
AEC, absolute eosinophilic count; YKL40, human chitinase-3-like protein 1.

Table 5 Correlation between clinical and laboratory patient variables and the measured biomarkers in all children with asthma

Variables	Markers: Serum YKL40 Correlation coefficient	Serum YKL40 P values*	Serum periostin Correlation coefficient	Serum periostin P values*
Age	0.451	<0.001	0.403	<0.001
Respiratory rate	0.102	0.26	−0.023	0.8
Duration of asthma	0.386	<0.001	0.403	<0.001
Total leukocytic count	0.108	0.24	0.122	0.18
Absolute eosinophilic count	0.012	0.8	0.15	0.09

*P values <0.05 are considered statistically significant.

the importance of periostin and YKL40 as serum biomarkers for assessment of asthma severity and acute asthma exacerbations in children with asthma.

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Competing interests None declared.

Patient consent Parental/guardian consent obtained.

Ethics approval This study was approved by the Scientific Ethics Committee of the Faculty of Medicine of Cairo University, Egypt.

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