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Study of serum YKL-40 in children with bronchial asthma

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KEYWORDSAsthma;
YKL-40;
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Severity**Abstract** *Background:* Serum YKL-40 (chitinase-3-like-1) has recently been found to be either the cause or a biomarker for asthma. This study quantifies serum levels of YKL-40 in asthmatic children and evaluates the relationship between YKL-40 and asthma severity.*Methods:* We quantified serum YKL-40 levels in two groups of asthmatic children (aged between 2 and 12 years). Group 1 (n : 30) in between the attack (stable group) and group 2 (n : 30) during the exacerbation (acute attack group). Serum YKL-40 was measured by enzyme-linked immunosorbent assay (ELISA) Kits (WKEA MED SUPPLIES CORP). We measured blood eosinophils and peak expiratory flow rate for children > 5 years.*Results:* Serum levels of YKL-40 were significantly elevated in the acute asthma group and in the stable asthma group compared to the control group (673.92 ± 24.23 ng/ml, 552.73 ± 57.34 ng/ml, 136.17 ± 53.14) respectively, $P < 0.001$. YKL-40 levels were correlated positively with blood eosinophils in the stable group ($\rho = 0.67$, $P < 0.001$) and in the acute attack group ($\rho = 0.76$, $P < 0.001$). It is inversely correlated with PEFR in the stable asthma group ($\rho = -0.83$, $P < 0.001$) and in the acute asthma group ($\rho = -0.88$, $P < 0.001$).*Conclusions:* Serum YKL-40 protein was found in increased quantities in patients with asthma in whom the protein level correlated positively with the severity of disease so our data concluded that YKL-40 could be a valuable biomarker for asthma diagnosis and prognosis.© 2015 The Authors. Production and hosting by Elsevier B.V. on behalf of The Egyptian Pediatric Association. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).**Introduction**Asthma is a chronic inflammatory disease characterized by recurrent attacks of breathlessness and wheezing, which vary in severity and frequency from person to person.¹ Asthma isbest thought of as an immunologically mediated disease, in which an abnormal immune response to various inhaled environmental agents and irritants provoke a cascade of events that lead to mucus hypersecretion, airway constriction and hyper-responsiveness and ultimately symptoms.²

YKL-40 (chitinase-3-like-1 [CHI3L1]), (human cartilage glycoprotein-39), is a member of the mammalian chitinase-like protein class. It is a 40 kDA heparin binding glycoprotein. The name YKL-40 is derived from the protein's molecular weight and three N-terminus amino acids (tyrosine, lysine

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and leucine).³ YKL-40 binds chitin which is an abundant, naturally occurring polysaccharide composed of N-acetylglucosamine repeats functioning as a major structural polymer in many lower life forms including cell walls of bacteria and fungi but YKL-40 is deficient in chitinase activity. It is produced at sites of inflammation in many cells and is secreted from vascular smooth muscle cells and macrophages.⁴

YKL-40 is synthesized in neutrophil precursors at the myelocyte–metamyelocyte stage; it is stored in specific granules of neutrophils and released from fully activated cells as well as from macrophages, neutrophils, chondrocytes, vascular smooth muscle and cancer cells.⁵ It is suggested that YKL-40 has a role in inflammation and tissue remodeling in human diseases, such as joint injury, liver fibrosis, type 2 diabetes.^{4,3,6} It is established that YKL-40 was increased in the lung and circulation of patients with severe asthma. Thereby, they reasoned that YKL-40 could either be a cause or marker for asthma.^{7,8}

Patients

This case control study was conducted on 90 children at pediatric allergy clinic and emergency department of Benha University Hospital over a period of 1 year from December 2012 to December 2013. Sixty asthmatic children randomly selected took part in the present study. Their ages ranged from 2 to 12 years and were 41 males and 19 females. Thirty apparently normal children of comparable age, sex and socioeconomic status were taken as a control group. Patients with concomitant diseases such as cancer, arthritis, hepatic fibrosis and diabetes were excluded from the study.

Asthmatic subjects were divided into two subgroups: stable group (n : 30) was recruited during their scheduled clinic visit. Their symptoms and pulmonary functions were stable and divided into intermittent, mild persistent, moderate persistent and severe persistent. Acute attack group (n : 30) was divided into mild attack, moderate attack and severe attack according to GINA (Global initiative for asthma) criteria.⁹

Methods

The study protocol received approval of the research ethics committee of the pediatric department at Benha University. All candidates were subjected to: detailed medical history taking, Clinical examination, peak expiratory flow rate (PEFR) by peak flow meter. Chest X-ray to exclude chest diseases other than asthma and Laboratory investigation included: complete blood count (CBC) with eosinophilic count and assay of serum YKL-40 level.

Assay of serum YKL-40 level: serum samples for YKL-40 (ng/ml) were centrifuged for 10 min at 3000 rpm and stored at -20°C in Eppendorf vials. Human YKL-40 enzyme-linked immunosorbent assay (ELISA) kit (WKEA MED SUPPLIES CORP, Changchun 130012 China) was used for the serum YKL-40 level measurements. Assay range: 5–200 ng/ml.

Statistical analysis

The collected data were summarized as mean \pm SD and range for quantitative data and proportions for qualitative data.

Differences between the study groups regarding the studied parameters were tested using the Chi-squared (χ^2) test to compare proportions, the Mann–Whitney test to compare two groups and the Kruskal–Wallis test to compare more than two groups. Spearman correlation coefficient (ρ ; ρ) was used to assess the correlation between serum levels of YKL-40 and some parameters. After the calculation of each of the test statistics, the corresponding distribution tables were consulted to get the “ P ” (probability value). Statistical significance was accepted at P value < 0.05 . The cut off value of serum YKL-40 for screening of asthma and the corresponding sensitivity, specificity were calculated. All statistical analyses were carried out in STATA/SE version 11.0 for Windows.

Results

Demographic and laboratory data for the study subjects are demonstrated in [Tables 1 and 2](#). The mean serum YKL-40 level of the control group was found to be (136.17 ± 53.14) ng/ml. Mean serum YKL-40 level of the patients in stable asthma group was (552.73 ± 57.34) ng/ml while the mean serum of YKL-40 in patients of the acute asthma group was (673.92 ± 24.23) ng/ml respectively. There was a significant difference between serum YKL-40 levels of the stable asthma group and the acute asthma group $P < 0.001$. [Fig. 1](#) shows differences in the serum level of YKL-40 between the study groups.

Correlations between serum YKL-40 and other variables are demonstrated in [Table 3](#). There was a significant positive correlation between serum YKL-40 and blood eosinophils in both asthmatic groups ($\rho = 0.67$, $P < 0.001$) & ($\rho = 0.76$, $P < 0.001$) respectively. Also, there was a significant negative correlation between PEFR and serum YKL-40 in both asthmatic groups ($\rho = -0.83$, $P < 0.001$) & ($\rho = -0.88$, $P < 0.001$) respectively. [Fig. 2](#) shows Sensitivity and specificity curves and cut-off level of YKL-40 for diagnosis and screening of asthma. The best cut off value of YKL-40 for diagnosis and screening of asthma was 216.8 ng/ml.

Discussion

Chitinases are among one of the recently described proteins suggested to play a pivotal role in T-helper 2 (Th2)-mediated inflammation and allergic diseases such as asthma.¹⁰ Circulating YKL-40 levels regulated by Polymorphisms in the CHI3L1 (chitinase 3-like 1-proteins) gene. Regulatory SNPs in CHI3L1 were associated with asthma, atopy, and immune-mediated diseases.¹¹ In asthma, YKL-40 are secreted from macrophages and airway epithelial cells with IL-13 related mechanism. Th-2 associated inflammatory response due to allergen exposure, which results in airway hyperresponsiveness and smooth muscle contraction, plays a role in tissue remodeling.¹²

The sex distribution in our study among asthmatic patients revealed male predominance (68.33%) than females (31.67%) with a male to female ratio of 2.1:1 but the difference was statistically insignificant. This finding is supported by studies done by Khaldi et al.¹³ and Awad¹⁴ who reported that was no sex difference in pediatric asthma. Anupama et al.¹⁵ showed a significant male predominance. Concerning residence distribution, in the present study, 70% of asthmatic children were from urban areas while, 30% were from rural areas with a

Table 1 Demographic data of study groups.

Variable		Controls <i>N</i> = 30	Cases <i>N</i> = 60	Test	<i>P</i> -value
Age (years)	Mean ± SD; (range)	5.57 ± 2.50; (2–11)	5.87 ± 3.0; (2–12)	$Z^* = 0.16$	0.87
Sex	Male (%)	17 (56.67)	41 (68.33)	$\chi^2 = 1.19$	0.28
	Female (%)	13 (43.33)	19 (31.67)		
Residence	Urban (%)	13 (43.33)	42 (70.00)	$\chi^2 = 5.98$	0.01 (S)
	Rural (%)	17 (56.67)	18 (30.00)		
Family size	≤4 (%)	12 (40.00)	22 (36.67)	$\chi^2 = 0.09$	0.76
	>4 (%)	18 (60.00)	38 (63.33)		
Parents education	High (%)	17 (56.67)	19 (31.67)	$\chi^2 = 5.21$	0.02 (S)
	Low (%)	13 (43.33)	41 (68.33)		
Family history of atopic diseases	Positive (%)	3 (10.00)	44 (73.33)	$\chi^2 = 32.15$	<0.001 (HS)
	Negative (%)	27 (90.00)	16 (26.67)		
Age at onset of asthma (years)	Mean ± SD; (range)		2.13 ± 1.23; (1–6)		

Z^* – obtained using Mann–Whitney test.

Table 2 Clinical and laboratory data of study groups.

Variable	Mean ± SD; (range)			Z1*	P1	Z2*	P2	Z3*	P3
	Controls <i>N</i> = 30	Asthma group 1 (stable) <i>N</i> = 30	Asthma group 2 (acute) <i>N</i> = 30						
PEFR%	87.18 ± 3.71; (80–95) <i>N</i> = 17	72.33 ± 11.73; (58–89) <i>N</i> = 15	66.87 ± 11.39; (50–82) <i>N</i> = 15	3.53	<0.001 (HS)	4.70	<0.001 (HS)	1.25	0.21
Hb (gm/dl)	12.25 ± 0.84; (9.7–13.5)	10.36 ± 1.26; (8.2–12.3)	10.39 ± 1.77; (7.8–13.5)	5.28	<0.001 (HS)	4.03	<0.001 (HS)	0.13	0.90
Total leukocytic count	6.54 ± 1.75; (4–10)	8.87 ± 3.68; (4–20.3)	9.84 ± 3.05; (4.9–16)	2.72	0.007 (S)	4.30	<0.001 (HS)	1.38	0.17
Eosinophils	204.37 ± 61.88; (90–300)	535.2 ± 85.44; (360–690)	616.67 ± 77.92; (500–790)	6.66	<0.001 (HS)	6.66	<0.001 (HS)	3.58	<0.001 (HS)
Ykl 40 (ng/ml)	136.17 ± 53.14; (36–198.5)	552.73 ± 57.34; (440–680.5)	673.92 ± 24.23; (607.5–714)	6.66	<0.001 (HS)	6.66	<0.001 (HS)	6.10	<0.001 (HS)

Z1 and P1 for comparing controls with asthma group 1; Z2 and P2 for comparing controls with asthma group 2; Z3 and P3 for comparing Asthma group 1 and asthma group 2.

Z^* – obtained using Mann–Whitney test.

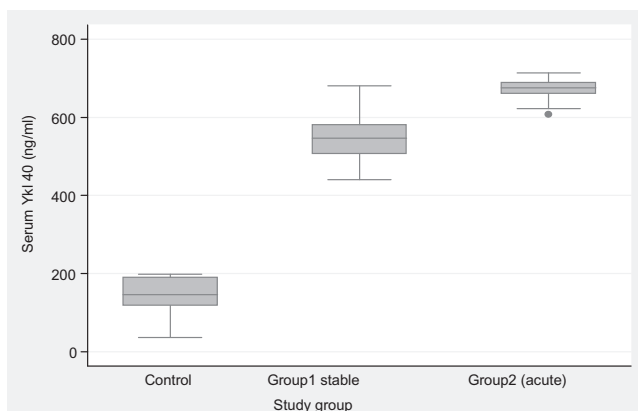


Figure 1 Differences in the serum levels of YKL-40 between the study groups.

Table 3 Correlation between serum YKL-40, age, age at onset of asthma, eosinophils and PEFR% among patients of group 1 (stable asthma group) and group 2 (acute asthma group).

Variable	Serum YKL-40 (ng/ml)					
	Asthma group 1 (stable asthma)			Asthma group 2 (acute asthma)		
	No	ρ	<i>P</i> -value	No	ρ	<i>P</i> -value
Age (years)	30	0.32	0.09	30	−0.15	0.43
Age of onset (years)	30	0.55	0.002 (S)	30	−0.15	0.42
Eosinophils	30	0.67	<0.001 (HS)	30	0.76	<0.001 (HS)
HB (gm/dl)	30	−0.14	0.45	30	0.02	0.91
PEFR%	15	−0.83	<0.001 (HS)	15	−0.88	<0.001 (HS)

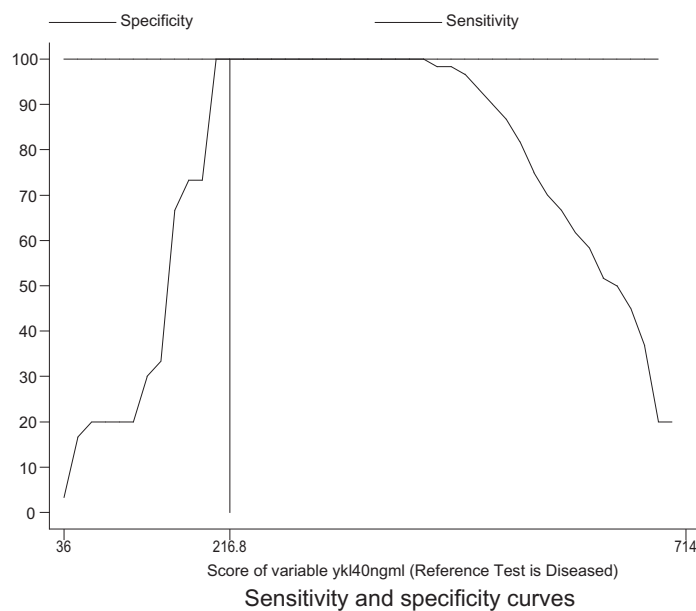


Figure 2 Cut-off level of YKL-40 for diagnosis and screening of asthma.

statistically significant difference ($P = 0.01$). Colilla et al.¹⁶ found that a higher percentage of asthmatic students living in urban areas compared to asthmatic students living in rural areas and they explained that by high air pollution in urban areas. In contrast to this, Morrison et al.¹⁷ reported that asthma prevalence was as high in rural as in urban areas.

From our results, the prevalence of asthmatic children whose family size > 4 (63.33%) was more than children whose family size ≤ 4 (36.67%) but no significant differences were seen among groups. Nafstad et al.¹⁸ detected higher asthma prevalence in larger families. On the other hand Westergaard and Melby¹⁹ have found an inverse relation between asthma prevalence and family size, where children in large families showed a lower prevalence of asthma but there is no biological explanation for this observation.

In this study, the mean age at onset of the disease among asthmatic children was (2.13 ± 1.23) years. Masuda and Fujisawa²⁰ found that the mean age of onset of asthma was 2.8 years. However, Abd el-Fattah et al.²¹ reported that about 50% of asthmatics experienced their first symptoms before 2 years of age. Hossny et al.²² found the mean age of onset of studied groups was one year. This highlights the role of viral infections as inducers of wheezing in infancy.

As regards the laboratory data of the studied children, we found that asthmatic patients had a highly significant decrease in their hemoglobin level than the control group. This agreed with Ramakrishnan and Borade²³ who found that anemic children are 5.75 times more susceptible to develop childhood asthma compared to the non-anemic children. Fida and Kamfar²⁴ detected low values for hematocrit (41.0%), mean corpuscular volume (41.9%), hemoglobin (17.9%), platelets (0.9%), iron (5.1%) and ferritin (12.0%) in asthmatic patients. They concluded that iron deficiency anemia could be considered as a risk factor in asthmatic patients.

As regards TLC, there was significant difference between stable asthma and controls ($P = 0.007$) and highly significant difference between acute the asthma group and controls

($P < 0.001$). Mann and Chung²⁵ reported that the circulating neutrophil in severe asthma shows evidence of activation, as measured by the increased expression of the adhesion molecules, CD11b and CD35 and are not inhibited by oral corticosteroids. So, they may be important in the pathogenesis of severe asthma Kikuchi et al.²⁶ suggested that neutrophils that have migrated to IL-8 may subsequently cause eosinophils to accumulate in the asthmatic airways.

Our results showed a highly significant increase in eosinophilic count among asthmatic children in the stable group and acute asthma group compared to the control group ($P < 0.001$). Our results were in agreement with those of Simpson et al.²⁷ who found that at most about one-half of asthma cases appear to be due to allergic mechanisms. Bousquet and Vignola²⁸ have shown that the number of eosinophils in peripheral blood and in bronchial lavage from subjects with asthma is associated with more severe disease. In contrast, Jayaram et al.²⁹ had a study which showed that patients may have severe and persistent asthma in the absence of eosinophilic inflammation, and they may experience an exacerbation of asthma without an increase in eosinophilic inflammation.

In our study, we found that asthmatic children had a highly significant decrease in PEF_R% level when compared to the controls ($P < 0.001$). In agreement, Stout et al.³⁰ found a decrease in the FEV₁/forced vital capacity ratio as asthma severity increased. O'Byrne et al.³¹ showed that severe asthma exacerbation may result in an accelerated loss of pulmonary function. Patients who frequently experienced asthma exacerbation showed a greater annual decline in FEV₁ than those with infrequent exacerbations.

In this study, we found that the serum YKL-40 level was higher in the acute asthma group than patients in stable asthma group and the control group. Kuepper et al.⁸ measured YKL-40 levels in serum before and 24 h after a segmental allergen challenge in 13 patients with allergic asthma, YKL-40 concentrations were significantly elevated in the serum before challenge ($P = 0.01$) and even more elevated ($P = 0.003$)

24 h after allergen. Chupp et al.⁷ quantified serum YKL-40 levels in three cohorts of patients with asthma and showed that circulating levels of YKL-40 were increased in patients with asthma, as compared with healthy persons (median, 97.7 ng per milliliter). Duru et al.³² found a significant difference between serum YKL-40 levels of well controlled asthma patients and the acute exacerbation group ($P < 0.0001$). Konradsen et al.³³ reported that serum YKL-40 levels were significantly higher in children with therapy-resistant asthma than in healthy children ($P = 0.03$). Also, Specjalski et al.³⁴ revealed that in asthmatics, the level was significantly higher in the subgroup with poor control of symptoms and exacerbations compared to stable asthmatics ($P < 0.001$).

In our study, serum YKL-40 showed a highly significant positive correlation with eosinophilic count in the stable asthma group and in acute asthma. This agreed with Tang et al.³⁵ and Lee et al.³⁶ who reported that there were positive correlations between serum levels of YKL-40 and peripheral blood eosinophils. In our study, serum YKL-40 levels showed a highly significant negative correlation with PEFr in stable asthma cases and in acute asthma cases. Otsuka et al.³⁷ found that sputum YKL-40 levels were negatively correlated with pre- and post-bronchodilator % FEV₁ ($r = -0.47$ and -0.42 , respectively; $P < 0.01$) and forced mid-expiratory flow ($r = -0.48$ and -0.46 , respectively, $P < 0.01$). Also, Saba et al.³⁸ showed a negative correlation with different spirometric indices in such a way that it was significant with FEV₁/FVC and FEF_{25-75%}.

Conclusion

In conclusion, Circulating YKL-40 protein was found in increased quantities in patients with asthma in whom the protein level correlated positively with the severity of disease so our data concluded that YKL-40 could be a valuable biomarker for asthma diagnosis and prognosis.

Recommendations

Although our data suggest that YKL-40 either participates in the pathogenesis of asthma or is a biomarker of severity, prospective studies will be required to evaluate the potential role of serum YKL-40 levels in the management of asthma and asthma research. Further studies to investigate genetic polymorphism of YKL-40 in the Egyptian population are suggested to be performed.

Conflict of interest

None.

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