

Original article

Serum YKL-40 and assessment of severity of bronchial asthma in Egyptian children

Background: Serum and lung tissue levels of a chitinase-like protein YKL-40 have recently been found to be increased in patients with bronchial asthma. Furthermore, serum YKL-40 levels correlated positively with thickening of the lung sub-epithelial basement membrane, frequency of rescue inhaler use, and deterioration in pulmonary function in European asthmatic subjects. **Objectives:** to assess the role of YKL-40 measurement in evaluating asthma severity, compared to clinical assessment and the related pulmonary function tests. **Methods:** We quantified serum YKL-40 levels in two groups of Egyptian asthmatics: One group with mild to moderate asthma, and one with severe asthma. Serum YKL-40 was measured by enzyme-linked immunosorbent assay (ELISA) kits (Quidel). Clinical scoring of asthma severity by Pediatric Asthma Score (PAS) and pulmonary functions were performed. **Results:** The serum levels of YKL-40 were significantly elevated in severely asthmatic Egyptian children compared with the other group (151ng/ml- 72ng/ml; $p < .05$). YKL-40 levels were correlated positively to PAS ($r = 0.34$, $p < 0.05$), and inversely to FEV_1 ($r = -0.32$, $p < 0.5$). Best cut off value of YKL-40 for asthma prognosis was 90 ng/ml, sensitivity 86.5%, specificity 81%, and diagnostic accuracy of 85%. **Conclusions:** YKL-40 is found in increased quantities in the sera of severe asthmatics, and correlated significantly to PAS and pulmonary function deterioration. YKL-40 is considered a promising biomarker for asthma severity and pulmonary remodeling warranting further study as a potential novel pathway to disease management.

Keywords: YKL-40, Asthma, severity, Egyptian, Children, biomarker.

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INTRODUCTION

The impact of genetic factors on the pathogenesis of allergy and asthma is currently an area of intense investigation. Mammalian chitinases may contribute to the pathogenesis of type 2 helper immune responses^{1,2}. YKL-40 is synthesized in neutrophil precursors at the myelocyte-metamyelocyte stage; it is stored in the specific granules of neutrophils and released from fully activated cells³ as well as from macrophages, neutrophils, chondrocytes, vascular smooth muscle and cancer cells⁴. CHI3L1, the gene that encodes for a chitinase-like protein YKL-40, has recently been shown to be highly heritable and associated with susceptibility to asthma⁵. Hence, Chitinase-like protein YKL-40 (also known as human cartilage glycoprotein 39 [HCgp-39] and chitinase 3-like 1) appears recently, to be important, in the pathogenesis, prediction and follow-up of asthma.

The objective of this study was to assess the role of YKL-40 measurement in evaluating bronchial asthma severity, compared to clinical

assessment and the related pulmonary function tests.

METHODS

This is a cross-sectional observational study.

Study population:

Thirty known asthmatic children were enrolled from Pulmonology Clinic of Children's Hospital, Ain Shams University from October 2008 till May 2009. These Patients were divided into two groups according to the global initiative for asthma⁶, fifteen were mild to moderate persistent asthma and fifteen of them were severe persistent asthma. Another group of 15 healthy age and sex matched subjects was enrolled to represent the control.

We excluded patients with rheumatoid arthritis, inflammatory bowel disease and diabetes mellitus who might have confounders for measured YKL-40.

Tools for Assessment of asthma severity:

In order to fulfill our study objective, we used different tools for severity assessment.

1. Clinical examination of patients was done targeting the assessment of severity of the current state of asthma by Pediatric Asthma Scoring (PAS)⁷.
2. Pulmonary function tests were performed using spirometry and tidal breathing measurements. We considered a typical case of obstructive defect is present when we found normal forced vital capacity (FVC), reduced forced expiratory volume in the first second (FEV₁), and reduced forced expiratory flow more than 25-75% of the FVC with FEV₁/FVC ratio <0.80. Normative values for FEV₁ have been determined for children, based on height, gender, and ethnicity. The guidelines cut-off values of FEV₁ <80% denote severe airway obstruction and <60% of predicted was associated with moderate airway obstruction^{6,8}.
3. YKL-40 was quantitatively measured in patients' sera by ELIZA method (Metra™ YKL-40 EIA Kit-QUIDEL CORPORATION). Serum was collected using standard venipuncture technique. Specimens were collected without anticoagulants and processed to avoid hemolysis. Sera were frozen at ≤ -20°C for safe storage. The test principle depends on; adding a known quantity of purified YKL-40 to serum samples with different levels of endogenous YKL-40. Measuring the optical density (at 405 nm) of the resulting mixture, with plotting the results on the representative standard curve, was done to calculate YKL-40 concentration. YKL-40 assay results are expressed in ng/mL. The minimum detection limit of the YKL-40 assay was 20 ng /mL.

Statistical analysis :

Results of descriptive statistics were expressed as a fraction of the total population, mean ± SD and median (range). Continuous variables were compared using Student's *t*-test if the variable was normally distributed or Mann Whitney test if the variable was not. The chi-square statistics was used for categorical variables. Spearman correlation test was used to rank different variables against patients' serum YKL-40 positively or inversely. ROC curve (receiver operator characteristic curve), was used for detection of the best cut off value, and validity

of YKL-40 as both a diagnostic and prognostic biomarker of asthma. Results were considered statistically significant with a $p < 0.05$.

The data were coded, entered and processed on computer using statistical package for social sciences (SPSS) software (version 15-USA) by biostatistician.

RESULTS

Serum YKL-40 levels:

Serum YKL-40 was significantly higher in both asthmatic groups (mean 72.3±27 ng/ml in mild to moderate asthmatics, and 151.7±42ng/ml in severe asthmatic children) compared to the controls (mean 35.7ng/ml), ($p < 0.01$ and 0.02 respectively). The best cut off value of YKL-40 for diagnosis and screening of asthma was 45 ng/ ml, with specificity 72% and sensitivity of 89%, positive predictive value (PPV) of 92% and negative predictive value (NPV) of 67% and accuracy of 85%.

Severe cases of persistent asthma showed highly significant increased levels of serum YKL-40 compared to the mild to moderate forms of asthma (mean 151.7 ng/ml and 72.3 ng/ml respectively ; $p < 0.01$).

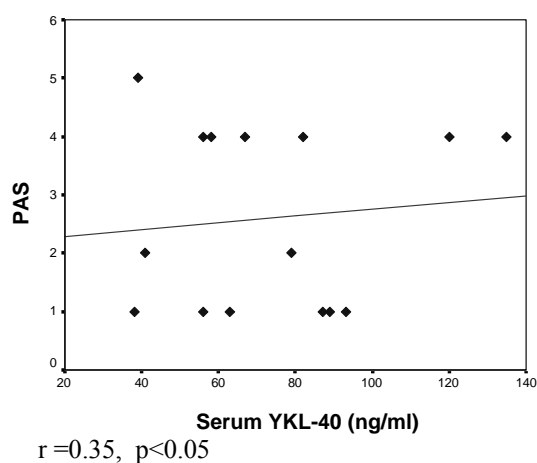
YKL-40 and Assessment of asthma severity:

1- YKL-40 and pediatric clinical scoring of asthma (PAS): correlation of serum YKL-40 and the PAS score showed significant positive correlation between PAS scores and YKL-40 levels with $r = 0.35$ and $p < 0.05$ in both groups of patients (figure 1).

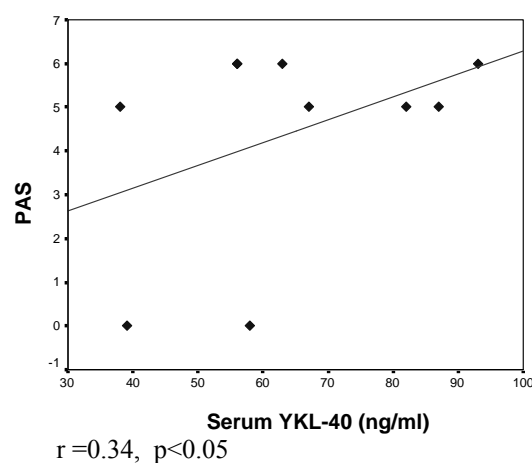
2- YKL-40 and pulmonary function tests: pulmonary function testing showed no significant difference between FEV₁ levels in both groups of asthmatic patients. Serum YKL-40 was inversely correlated to FEV₁. This correlation reached a significant level within the group of severe persistent asthma ($r = -0.32$, $p < 0.05$) but insignificant levels within the group of mild to moderate asthma ($r = -0.12$, $p > 0.05$). The best cutoff value of YKL-40 as a marker for severe asthma in this study was 90 ng/ml, with sensitivity of 86.7% and specificity of 81%, with PPV of 81% and NPP of 43% and accuracy of 85%.

Table 1. Characteristics of the studied groups.

Variable	Control (n=15)	Mild-Moderate asthma (n=15)	Severe asthma (n=15)
Age (yrs) mean±SD	7.7±4.7	6.6±4.9	3.3±2.7
Gender (male); n (%)	10 (67%)	9 (60%)	8 (50%)
PAS score			
0-4 (normal)	-	-	-
5-7 (mild attack)	-	5	-
8-11(moderate)	-	10	5
11-15 (severe attack)	-	-	10
Serum YKL-40 (ng/ml) mean±SD	35.7±11	72±27	151±42
Pulmonary function test:			
Normal	-	1(6.7%)	5(33%)
Mild obstruction	-	8(53%)	11(73%)
Moderate obstruction	-	2(13%)	2(13%)
Severe obstruction	-	-	1(7%)



(a)



(b)

Figure 1. The correlation between PAS and serum levels of YKL-40 in patients with mild to moderate asthma (a) and severe asthma (b).**DISCUSSION**

Serum 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. This gene represents a novel asthma susceptibility gene. Variations in CHI3L1 have been associated with asthma risk and progression⁹. We tried in this study to answer the question of whether measuring serum YKL-40 can be useful in the diagnosis of asthma and assessment of its severity, so can replace a more sophisticated diagnostic and prognostic tool like PFT in our country. We found a significant

increase in serum YKL-40 in asthmatic patients; this can be explained by the release of IL-13 from Th2 cells in the initial phases of asthma, which potently and selectively induces the expression of chitinase in airway epithelial cells and alveolar macrophages. Chitinase expression in epithelial cells stimulates the release of macrophage inflammatory protein (MIP)-1 which especially recruits monocytes, eotaxin and eotaxin-2 which specifically recruit eosinophils, and neutrophils into the airways resulting in airway inflammation and directly evoke smooth muscle contraction

manifested as airway hyper-responsiveness and asthma⁵.

Chupp et al.¹ quantified serum YKL-40 levels in three cohorts of patients with asthma and showed that serum levels of YKL-40 were increased in patients with asthma (median, 97.7 ng/ml) compared with healthy persons. Ober et al.¹⁰ performed a genome-wide association study of serum YKL-40 levels in asthmatic patients and tested them for an association between an implicated Single-Nucleotide Polymorphism (SNP) in the promoter region of CHI3L1 gene and serum YKL-40 levels in asthma. They found that serum levels of YKL-40 were 15% higher in those with asthma than in controls. Similar to our finding presented follow-up data demonstrating significantly higher levels of YKL-40 in both serum and BAL fluid of asthmatic patients after they were challenged with various allergens¹¹.

Ober and Chupp⁹ performed a cross-sectional analysis of samples from an established cohort of asthmatic subjects and controls from the Yale Center for Asthma and Airways Disease and showed that YKL-40 levels were significantly higher in the serum of asthmatic subjects compared with non-asthmatic subjects.

Serum YKL-40 was significantly higher in patients with severe asthma compared to those with mild to moderate asthma (best cut-off value was 90 ng/ml). These levels were significantly positively correlated to the corresponding PAS score and the degree of airway obstruction by PFT.

This is consistent with Bask et al.¹² who showed that the value of commonly used pulmonary function tests in short- and long-term evaluations of childhood asthma remains controversial. They recommended that other parameters may be more valuable for this purpose.

Moreover, there is a general finding that most asthmatic children have FEV₁ values in the normal range. So if the diagnosis or the management of asthma greatly relies on FEV₁ measurements, there will be a risk of under-diagnosing or under-treating asthmatic children¹³.

Some researchers explain the significant rise of YKL-40 in severe asthmatics by the pathophysiological role of YKL-40 in asthma as it is Th2- cytokine IL-13 dependent¹¹. Shuhui et al.⁵ revealed that YKL-40 might have a protective role to the lung through attenuating airway inflammation and airway hyper-responsiveness. Persistently elevated serum YKL-40 level was a significant marker of antigen-driven inflammation and remodeling in the asthmatic airway.

Though the mechanism of this marked increase in severe asthmatics is not well known in the literature, yet YKL-40 was found in serum before and 24 hours after segmental allergen challenge in 13 patients with allergic asthma, YKL-40 concentrations were significantly elevated in serum before challenge ($p = 0.01$) and even more elevated ($p = 0.003$) 24 hours after allergen¹¹. Moreover, serum YKL-40 levels were significantly correlated to the degree of alveolar membrane thickening in severe asthmatic patients after taking lung biopsies¹. Ober et al.¹⁰ showed that serum YKL-40 levels were inversely correlated with FEV₁ ($p = 0.02$) but not with forced vital capacity (FVC) ($p = 0.16$), the FEV₁: FVC ratio ($p = 0.98$), or forced expiratory flow between 25% and 75% of the FVC (FEF₂₅₋₇₅) ($p = 0.41$).

In conclusion, serum YKL-40 protein was found in increased quantities in asthmatic patients and these levels correlated positively with the severity of the disease. YKL-40 proved to be a simple and a novel useful biomarker for asthma severity assessment that can overcome the difficulties associated with PFTs in children.

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