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The chitinase-like protein YKL-40 is not a useful biomarker for severe persistent asthma in children

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ABSTRACT

Background: The chitinase-like protein YKL-40 is thought to play a role in inflammation and tissue remodeling. In adults with severe asthma, YKL-40 is expressed in the airway and YKL-40 levels are elevated in the serum.

Objective: To compare YKL-40 levels in children with severe persistent asthma with those in adults with severe persistent asthma and to determine whether YKL-40 levels correlate with increasing asthma severity in childhood asthma.

Methods: In this prospective, cross-sectional study, 23 adults and 19 children with severe persistent asthma, 23 children with moderate persistent asthma, and 19 children with mild persistent asthma were enrolled. The following data were collected on each patient: spirometry, exhaled nitric oxide, percutaneous skin testing results to aeroallergens, peripheral eosinophils, serum IgE levels, and serum YKL-40 levels.

Results: Compared with adults, children with severe persistent asthma had significantly lower YKL-40 levels, higher values for forced vital capacity and forced expiration volume in 1 second, higher serum IgE levels, and higher exhaled nitric oxide levels. YKL-40 levels did not correlate with increasing asthma severity in the pediatric cohort.

Conclusion: Severe persistent asthma in childhood is not associated with elevated YKL-40 levels, unlike in adults with severe persistent asthma. YKL-40 is not a useful biomarker for asthma severity in childhood asthma.

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Introduction

Chitin is a polymer of β -1,4-N-acetylglucosamine, which is produced by nearly all animals, including crustaceans, fungi, and insects, but not by mammals. Chitinases are evolutionarily conserved hydrolases that enzymatically degrade chitin, which allows for homeostasis in lower life forms and provides a host defense mechanism to protect against chitin-containing organisms.^{1–3} Although mammals do not produce chitin, they express active chitinases, including acidic mammalian chitinase, and nonenzymatic chitinase-like proteins (CLPs). The role of these proteins is not well understood, but increased levels have been described in patients with several human diseases associated with infection, inflammation, and tissue remodeling.^{4–9}

YKL-40 is a CLP that lacks enzymatic activity. The YKL-40 protein is encoded by the chitinase-3-like 1 gene (*CHI3L1*). Single-nucleotide polymorphisms in the promoter region of *CHI3L1* have been associated with elevated YKL-40 levels and have been linked

to atopy, bronchial hyperresponsiveness, and asthma.^{10,11} A study by Chupp et al¹² concluded that YKL-40 is expressed in airway tissue and is elevated in the serum of adults with asthma, with levels increasing with worsening severity. The study also suggested that YKL-40 levels are inversely associated with forced expiratory volume in 1 second (FEV₁) and positively associated with basement membrane thickness. The investigators concluded that elevated serum YKL-40 levels might serve as a biomarker for severe asthma and indicate a pathologically and biologically distinct form of asthma.

Although chitinases and CLPs are elevated in various inflammatory diseases, their role in the pathophysiology of inflammation remains unclear. Emerging research has shown that chitinases facilitate extracellular matrix deposition and enhance the production and release of proinflammatory cytokines.^{13,14} Asthma-related chitinase research initially focused on murine models, in which acidic mammalian chitinase was noted to be induced during T-helper cell type (T_H) 2-mediated inflammation through an interleukin-13-dependent mechanism.¹³ Chitinases act downstream of interleukin-13, and inhibition of active chitinases has been shown to decrease T_H2-mediated inflammation, bronchial hyperresponsiveness, and tissue eosinophilia. YKL-40 is associated with tissue remodeling in certain inflammatory conditions,

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including asthma, where it has been shown to increase the proliferation and migration of bronchial smooth muscle cells.⁹ Proposed mechanisms for tissue remodeling include YKL-40 binding to type I collagen and subsequent formation of collagen fibrils¹⁵ and stimulation of pro-fibrotic and inflammatory mediators.¹⁶

The authors compared YKL-40 levels with measurements of asthma severity and biomarkers of allergic inflammation in a group of children with severe persistent asthma with those in a group of adults with severe persistent asthma. The authors also aimed to determine whether YKL-40 levels are associated with increasing levels of asthma severity in children.

Methods

The National Jewish Health institutional review board (Denver, Colorado) approved this prospective, cross-sectional study. Informed consent was obtained from each subject or subject's parent or guardian. Assent also was obtained from each school-age subject. Sixty-one children 5 to 17 years old were enrolled from the pediatric allergy and asthma clinic from August 2009 to July 2010. Children were eligible for entry into the study if they carried a physician diagnosis of mild, moderate, or severe persistent asthma according to the National Institutes of Health National Heart, Lung, and Blood Institute Expert Panel Report 3,¹⁷ if they consented and assented (if necessary) to be in the study, and if they were able to successfully perform spirometry and exhaled nitric oxide (eNO) measurements. Subjects were excluded if they had an upper or lower respiratory infection or had required oral corticosteroids within the past 4 weeks. Other exclusion criteria included a history of preterm birth (<36 weeks of gestational age) or any other form of lung disease.

Twenty-three adults undergoing a clinical pharmacology consultation at National Jewish Health for severe persistent asthma also were enrolled into the study from August 2009 to July 2010. Inclusion criteria included a physician diagnosis of severe persistent asthma according to the Expert Panel Report 3. Exclusion criteria included a history of current or past cigarette smoking, a diagnosis of vocal cord dysfunction, or any other form of lung disease.

All subjects underwent spirometry using Jaeger MasterScreen Pneumo running JLab 5.20 software (Erich Jaeger, Inc, Wurzburg, Germany). Those undergoing β -agonist testing received 180 μ g of albuterol through a metered-dose inhaler and holding device with repeat spirometry 15 minutes afterward. The eNO levels were measured using the Niox Mino (Aerocrine, Solna, Sweden) and the technique recommended by the American Thoracic Society guidelines.¹⁸ Blood was obtained at the time of inclusion into the study to measure circulating eosinophils, with samples processed and counted using a Nebauer hemocytometer, and serum was obtained for measurement of YKL-40 and IgE levels using commercially available enzyme-linked immunosorbent kits (for YKL-40: Quidel, San Diego, California; for IgE: Pharmacia, Uppsala, Sweden). Skin prick testing was performed to 25 common standardized aeroallergens. A test result was considered positive if the skin test resulted in a mean wheal diameter of at least 3 mm larger than a saline control.

Statistical Analysis

P values were derived to compare adults with severe persistent asthma with children with severe persistent asthma from χ^2 tests for categorical variables, Fisher exact test for categorical variables with small expected numbers, analysis of variance for normally distributed continuous variables, or Wilcoxon rank-sum test for variables that were not normally distributed. *P* values also were calculated to compare characteristics in children with different asthma severities using the same methods, except the Kruskal-Wallis test was used for variables that were not normally distributed. Variables such as

serum YKL-40, serum IgE, eNO, and peripheral eosinophils were logarithmically transformed before statistical analysis to allow symmetry of their distributions. Simple associations were assessed using Spearman rank-correlation analysis.

Results

Comparison of Children and Adults with Severe Persistent Asthma

Adults with severe persistent asthma were more likely to be female compared with children with severe persistent asthma. There was no significant difference between the 2 groups in prescribed inhaled corticosteroid dosage, although a larger percentage of adults required chronically administered oral corticosteroids compared with the children studied. The 2 groups were similar in asthma morbidity, as measured by asthma exacerbations in the past year and lifetime hospitalizations for asthma. In addition, the 2 groups required rescue albuterol multiple times per week to treat their symptoms (Table 1).

Significant differences between adults and children with severe persistent asthma were noted in forced vital capacity (FVC; 72.5% of predicted in adults vs 94.7% of predicted in children, $P < .001$) and FEV₁ (55.1% of predicted in adults vs 73.1% of predicted in children, $P = .004$), whereas the FEV₁/FVC ratio and forced expiratory flow between 25% and 75% were similarly impaired in the adults and children studied.

A larger percentage of children than adults were atopic, based on having at least 1 positive skin test reaction to an aeroallergen (68% of adults vs 88% children), but the difference was not statistically significant. The group of children was found to have higher serum IgE levels (362 kU/L in children vs 91 kU/L in adults, $P = .01$) and higher eNO levels (35.5 ppb in children vs 23.5 ppb in adults, $P = .05$) compared with the adult cohort. Serum YKL-40 levels were significantly lower in children compared with adults with severe persistent asthma (26.2 ng/mL in children vs 62.1 ng/mL in adults, $P < .001$).

Table 1

Comparison of severe persistent asthma in children and adults

Variable	Adults (n = 23)	Children (n = 19)	<i>P</i> value
Age (y)	48.3 $\pm$ 2.0	12.2 $\pm$ 2.1	<.001
Female subjects, %	64	50	.37
Asthma duration (y)	28.1 $\pm$ 2.8	9.1 $\pm$ 3.1	<.001
BMI (kg/m ²)	30.3 $\pm$ 2.0	24.5 $\pm$ 2.1	.05
Inhaled corticosteroid dose (μ g/d fluticasone equivalent)	959 $\pm$ 89	812 $\pm$ 93	.26
Subjects requiring chronic oral corticosteroids, %	43	21	.14
Asthma morbidity			
Albuterol doses/wk, n	11.5 $\pm$ 2.3	13.2 $\pm$ 2.1	.58
Exacerbations/y, n	6.2 $\pm$ 1.1	6.3 $\pm$ 1.0	.92
Lifetime hospitalizations, n	12.1 $\pm$ 5.0	13.8 $\pm$ 4.7	.81
Lung function			
FEV ₁ , % predicted	55.1 $\pm$ 4.0	73.1 $\pm$ 4.2	.004
FVC, % predicted	72.5 $\pm$ 3.4	94.7 $\pm$ 3.5	<.001
FEV ₁ /FVC, %	61.5 $\pm$ 3.1	67.3 $\pm$ 3.3	.21
FEF _{25–75} , % predicted	41.6 $\pm$ 7.7	46.5 $\pm$ 6.4	.63
β -Agonist reversibility, % change	22.0 $\pm$ 4.7	24.0 $\pm$ 4.8	.76
Measurement of atopy and inflammation			
Subjects with positive skin prick test results, %	68	88	.14
Serum IgE (kU/L), median (IQR)	91 (24–291)	362 (215–895)	.01
Circulating eosinophils/mm ³ , median (IQR)	150 (100–300)	200 (0–700)	.14
eNO (ppb), median (IQR)	23.5 (12.8–41.8)	35.5 (28–68.8)	.05
Serum YKL-40 (ng/mL), median (IQR)	62.1 (42–142)	26.2 (17–43)	<.001

Abbreviations: BMI, body mass index; eNO, exhaled nitric oxide; FEF_{25–75}, forced expiratory flow between 25% and 75%; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; IQR, interquartile range.

Comparison of Children with Mild, Moderate, and Severe Persistent Asthma

The 3 groups of children with various asthma severities were similar in age, sex, and asthma duration (Table 2). There was a trend toward increasing body mass index with increasing asthma severity, although this did not reach statistical significance (19.6 kg/m² in mild persistent vs 23.4 kg/m² in moderate persistent vs 24.5 kg/m² in severe persistent, $P = .07$). As expected, the daily inhaled corticosteroid dose increased with increasing levels of severity (177 µg/d in mild persistent vs 380 µg/d in moderate persistent vs 812 µg/d in severe persistent, $P < .001$). There also were significant differences in FVC, FEV₁, FEV₁/FVC, and forced expiratory flow between 25% and 75%, with the greatest differences noted between children with severe persistent asthma, in whom the lowest lung function parameters were noted, and those with mild and moderate persistent asthma.

The 3 groups of children were similar for percentages with positive skin prick test reactions, serum IgE, and circulating eosinophils. Children with severe persistent asthma had significantly elevated eNO levels, required β-agonist therapy more frequently, and had a larger number of asthma exacerbations per year and a larger number of lifetime hospitalizations compared with children with mild and moderate persistent asthma. Although subjects with severe persistent asthma had the lowest serum YKL-40 levels, there was no difference in YKL-40 levels among the 3 groups (35.2 ng/mL in mild persistent vs 29.5 ng/mL in moderate persistent vs 26.2 ng/mL in severe persistent, $P = .12$).

Associations between YKL-40 levels and lung function, biomarkers of atopy and inflammation, and disease activity were evaluated using simple linear regression analysis. When data from all children were analyzed, no significant relations between measurements of asthma morbidity, measures of lung function, or biomarkers of atopy and inflammation and YKL-40 levels were noted.

Discussion

In this study, the cohort of adults with severe persistent asthma had elevated circulating levels of YKL-40, with values similar to those described in the study by Chupp et al.¹² In contrast, children with severe persistent asthma had significantly lower serum YKL-40 levels than adults with severe persistent asthma, with comparable values to those in children with mild and moderate persistent

asthma. Interestingly, YKL-40 values were highest in children with mild persistent asthma compared with children with moderate and severe persistent asthma, although this difference did not reach statistical significance, likely owing to the relatively small sample. Therefore, this variable should be investigated in larger samples.

Most studies investigating the role of YKL-40 in asthma have evaluated adults rather than children with asthma. A study by Konradsen et al¹⁹ evaluated a group of Swedish children with asthma, including cohorts with severe, therapy-resistant asthma and controlled persistent asthma, and compared them with healthy controls. Their results showed that serum YKL-40 levels were higher in children with severe, therapy-resistant asthma compared with healthy controls. The differences in YKL-40 levels between children with severe, therapy-resistant asthma and children with controlled persistent asthma were not statistically significant. However, after adjustments for the *CHI3L1* promoter single-nucleotide polymorphism, significantly higher levels of YKL-40 were found in children with therapy-resistant asthma compared with children with controlled persistent asthma. Tang et al²⁰ found similar results in an adult population, showing that YKL-40 levels are significantly higher in those with asthma compared with healthy controls, and that YKL-40 levels positively correlate with asthma exacerbations. The present study showed a significant positive correlation between YKL-40 levels and age in participants with asthma across all age groups (data not shown). Although the present study did not find a significant difference among children with different asthma severities, it did not include *CHI3L1* single-nucleotide polymorphism genotyping, which might have led to different outcomes. This study also did not include analysis of children and adults without asthma, another likely limitation.

Chitinases and CLPs have been associated with T_H2-mediated diseases, such as asthma. Studies have shown that chitinases facilitate extracellular matrix deposition¹⁴ and enhance the production and release of proinflammatory cytokines, such as monocyte chemoattractant protein-1 and eotaxin-1.¹³ In murine models, acidic mammalian chitinases are induced during T_H2-mediated inflammation through an interleukin-13–dependent mechanism. Chitinases act downstream of interleukin-13, and inhibition of active chitinases decreases T_H2-mediated inflammation, bronchial hyperresponsiveness, and tissue eosinophilia.²¹ The present cohort of children with severe persistent asthma differed from the adults

Table 2
Comparison of children with mild, moderate, and severe persistent asthma

Variable	Mild (n = 19)	Moderate (n = 23)	Severe (n = 19)	P value
Age (y)	11.2 ± 0.8	12.1 ± 0.7	12.2 ± 2.1	.58
Female subjects, %	42	26	50	.32
Asthma duration (y)	6.8 ± 1.0	7.9 ± 0.8	9 ± 3.1	.33
BMI (kg/m ²)	19.6 ± 1.7	23.4 ± 1.5	24.5 ± 2.1	.07
Inhaled corticosteroid dose (µg/d fluticasone equivalent)	177 ± 68	380 ± 60	812 ± 93	<.001
Asthma morbidity				
Albuterol doses/wk, n	1.6 ± 1.7	4.3 ± 1.3	13.2 ± 2.1	<.001
Exacerbations/y, n	1.5 ± 0.6	3.7 ± 0.5	6.3 ± 1	<.001
Lifetime hospitalizations, n	0.4 ± 1.8	1.5 ± 1.6	13.8 ± 4.7	<.001
Lung function				
FEV ₁ , % predicted	92.8 ± 3.8	89.3 ± 3.5	73.1 ± 4.2	<.001
FVC, % predicted	100 ± 3.7	107.5 ± 3.4	94.7 ± 3.5	.05
FEV ₁ /FVC, %	82.7 ± 2.4	73.0 ± 2.2	67.3 ± 3.3	<.001
FEF _{25–75} , % predicted	75.1 ± 5.5	59.5 ± 5	43.2 ± 5.5	<.001
β-Agonist reversibility, % change	8.1 ± 5.1	15.7 ± 3.9	24 ± 4.8	.05
Measurement of atopy and inflammation				
Subjects with positive skin prick test results, %	62	71	88	.25
Serum IgE (kU/L), median (IQR)	122 (7–620)	322 (53–1,104)	362 (215–895)	.11
Circulating eosinophils/mm ³ , median (IQR)	300 (125–475)	250 (100–400)	300 (0–700)	.14
eNO (ppb), median (IQR)	21.5 (10–31)	25 (15–69)	35.5 (28–68.8)	.01
Serum YKL-40 (ng/mL), median (IQR)	35.2 (29–54)	29.5 (20–42)	26.2 (17–43)	.12

Abbreviations: BMI, body mass index; eNO, exhaled nitric oxide; FEF_{25–75}, forced expiratory flow between 25% and 75%; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; IQR, interquartile range.

with severe persistent asthma in that the children had better lung function, were more likely to be atopic, and had higher serum IgE and eNO levels than adults. Given that 43% of the adult cohort was treated with chronic oral corticosteroids, a treatment modality that is known to decrease circulating eosinophils and eNO, a subgroup analysis was performed comparing allergic parameters in adults treated with chronic oral corticosteroids with those who did not require chronic corticosteroid therapy. This analysis showed no significant differences in circulating eosinophil, eNO, serum IgE, and YKL-40 levels between the 2 groups (data not shown). Because eNO is a noninvasive measurement of eosinophilic inflammation,²² the present data showing higher eNO levels in the severe persistent pediatric cohort suggests that children with severe persistent asthma are more likely to have T_H2-mediated airway inflammation compared with adults with severe persistent asthma. Because the adult and pediatric cohorts with severe persistent asthma were characterized by similar rates of atopy, the significantly higher eNO values seen in the pediatric group are likely secondary to distinct T_H2-driven inflammation in this group and not due to differences in rates of atopy between the 2 groups. The present group of children with severe persistent asthma had significantly lower CLP levels than the group of adults with severe persistent asthma. Thus, the present findings stand contrary to the findings of these aforementioned studies^{13,14,18} associating chitinases and CLPs with T_H2-mediated disease. This also might be secondary to the relatively small sample.

The present data suggesting that children with severe persistent asthma are more likely to have T_H2-predominant inflammation compared with adults with severe persistent asthma is in agreement with results from a single-site study from the National Heart, Lung and Blood Institute's Severe Asthma Research Program.²³ In this study, investigators found that children with severe persistent asthma had higher eNO levels and greater allergic sensitization compared with children with mild or moderate persistent asthma. They suggested that severe persistent asthma in childhood is characterized by a distinct phenotype, and unlike in adults with severe persistent asthma, in whom many distinct phenotypes exist, children with severe persistent asthma are characterized by T_H2-mediated inflammation.

In the present cohort of adults with severe persistent asthma, in whom YKL-40 levels were elevated, YKL-40 might serve as a T_H1-driven marker of inflammation. YKL-40 levels are elevated in several T_H1-mediated inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease.^{6,7} YKL-40 levels also are positively correlated with C-reactive protein, a prototypic marker of T_H1-driven inflammation in patients with ulcerative colitis.²⁴ It has become increasingly clear that severe asthma in adults is a heterogeneous disease.^{25–27} Among the best-described phenotypes of severe persistent asthma in adults is one characterized by airway neutrophilia. Wenzel et al²⁶ were among the first to describe this phenotype of severe asthma. In their sentinel study, subjects with refractory asthma could be separated into those with airway eosinophilia and those without airway eosinophilia, but in the 2 phenotypes, airway neutrophilia was present. A large cross-sectional study from the European Network for Understanding Mechanisms of Severe Asthma also found that patients with severe asthma had airway neutrophilia, whereas no differences in the number of sputum eosinophils, eNO levels, or amount of circulating eosinophils were noted in those with severe persistent asthma compared with those with mild or moderate persistent asthma.²⁷

In summary, the present data suggest that serum YKL-40 is neither a marker of severe persistent asthma in children, as seen in adult asthma, nor is it associated with other measurements of asthma severity, lung function, or biomarkers of atopy and eosinophil-mediated inflammation in children. In addition, the pathobiology of severe persistent asthma was distinct in children compared with adults, because this cohort of children with asthma displayed significantly lower YKL-40 levels than the adults with

severe persistent asthma, and additional parameters point toward the predominance of T_H2-driven inflammation in childhood asthma. This may suggest an association of YKL-40 levels with a T_H1-driven asthma phenotype in adults. Further studies using larger samples and *CHI3L1* genotyping are necessary to confirm the role of YKL-40 in various asthma phenotypes in adults and children.

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