Sputum pentraxin 3 concentration as a marker of airway inflammation and remodeling in childhood asthma

Hee Seon Lee

Department of Medicine

The Graduate School, Yonsei University

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Directed by Professor Myung Hyun Sohn

The Master's Thesis
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Master of Medical Science

Hee Seon Lee

June 2015

This certifies that the Master's Thesis of
Hee Seon Lee is approved.

Thesis Supervisor : Myung Hyun Sohn

Thesis Committee Member#1 : Young Sam Kim

Thesis Committee Member#2 : Kyung Won Kim

The Graduate School
Yonsei University

June 2015

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor Prof. Myung Hyun Sohn for the continuous support of my study. Also, I would like to thank Prof. Young Sam Kim for his encouragement and insightful comments. I am grateful and indebted to Prof. Kyung Won Kim for her sincere and valuable guidance and encouragement. My sincere thanks always goes to Prof. Kyu-Earn Kim for his support and consideration.

I wish to express my thanks to Dr. Kyung Eun Lee, Jung Yeon Hong, and Mi Na Kim for showing me how to do the experimental work and helping my study. I am also grateful to my friends Min Jung Kim and In Suk Sol for their kind support. I also gratefully acknowledge the help from all the members of Pediatric Allergy Institute.

Finally, I would like to dedicate this paper to my family. I would like to thank my beloved parents, parents-in-law, my dearest husband Sang Yun Lee for always standing by me, and baby to be born soon.

June 2015

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ABSTRACT

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Hee Seon Lee

Department of Medicine
The Graduate School, Yonsei University

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Pentraxin 3 (PTX3) is soluble pattern recognition receptor, and acute phase protein that has emerged as a new serological marker reflecting tissue inflammation and damage under diverse diseases. We determined whether sputum PTX3 levels are elevated in patients with childhood asthma. We also investigated the relationship between sputum PTX3 levels and airway inflammation, pulmonary function, and bronchial hyperresponsiveness in children. A total of 260 children (140 patients with asthma and 120 control subjects) were enrolled in this study. PTX3 levels were measured in sputum supernatants with ELISA. We performed spirometrys and methacholine challenge tests while measuring total eosinophil count, and serum levels of total IgE and ECP in all children. Sputum PTX3 concentration was significantly higher in children with asthma (mean ± SE, 1094.55 ± 224.65 pg/mL) than control subjects (mean ± SE, 177.36 ± 30.00 pg/mL, $p < 0.001$). Children with moderate-to-severe persistent asthma showed significantly higher sputum PTX3 levels (median, 1190.63 pg/mL; IQR, 302.70 to 2619.84 pg/mL) than those with mild persistent asthma (median, 391.72 pg/mL; IQR, 73.77 to 1352.35 pg/mL, $p = 0.044$) and intermittent asthma (median, 188.15 pg/mL; IQR, 80.41 to 745.08 pg/mL, $p = 0.006$). Positive significant correlations were found between sputum PTX3 and bronchodilator response ($r = 0.25$, $p = 0.013$). Sputum PTX3 levels negatively correlated with FEV_1 ($r = -0.30$, $p = 0.001$), FEV_1/FVC ($r = -0.27$, $p = 0.002$), FEF25-75 ($r = -0.392$, $p < 0.001$). Sputum PTX3 levels also showed significant negative correlation with post- bronchodilator (BD) FEV_1 ($r = -0.25$, $p < 0.001$) and post-BD FEV_1/FVC ($r = -0.25$, $p < 0.001$). Our results would

support that PTX3 is involved in the pathogenesis of asthmatic airways. Sputum PTX3 could be a supportive marker reflecting asthmatic airway inflammation and remodeling in childhood asthma.

Key words : Pentraxin 3, induced sputum, asthma, children.

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Department of Medicine
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I. INTRODUCTION

Asthma is the most common chronic airway inflammatory disorder in children, characterized by variable airflow obstruction, airway hyperresponsiveness (AHR), and bronchodilator reversibility. Asthma has come to be recognized as a major worldwide public health issue.[1] In Korea, the prevalence of asthma in children has increased.[2] Asthma have a complex background that may result from the interaction of various host and environmental factors, and numerous inflammatory cells and mediators have been found to be involved.[1,3] However, the underlying pathogenesis of asthma has not yet been fully defined.

Pentraxin 3 (PTX3) is a member of the long pentraxins, a soluble pattern recognition receptor with non-redundant functions in inflammation and innate immunity.[4,5] It is an acute phase protein secreted in response to toll-like receptor (TLR) activation or proinflammatory cytokines.[6,7] Recently, PTX3 has been studied as an emerging marker reflecting tissue injury and inflammation[6,8] under various pathological conditions such as acute respiratory distress syndrome,[9] atherosclerosis,[10] small-vessel vasculitis,[11] rheumatoid arthritis,[12] chronic kidney disease.[13] PTX3 is produced by immune and structural cells in various tissues.[5,6,14] Recently, it has been reported that airflow limitation are associated with lower pulmonary interstitial expression of PTX3 in chronic obstructive pulmonary disease (COPD).[15] In asthma, the evidence was shown that PTX3 expression is increased in allergic asthmatic airways.[5] In addition, it has been

reported that sputum PTX3 levels were elevated in adult asthma[16] and correlated with the airflow limitation of the asthma patients.[17]

We hypothesized that PTX3 could play an important role in childhood asthma, so we examined sputum PTX3 concentration in childhood asthma and its relationship with various asthma indices. The aim of this study was to determine whether PTX3 concentrations in induced sputum were increased in asthmatic children compared to healthy subjects. We also investigated the relationship between sputum PTX3 levels and asthmatic airway inflammation, bronchial hyperresponsiveness and reversibility, and pulmonary function in children.

II. MATERIALS AND METHODS

1. Subjects

A total of 260 children were enrolled in this study. Among the 260 children, 140 were diagnosed with asthma in accordance with American Thoracic Society criteria.[18] Current asthma was defined as recurrent wheezing or coughs in the absence of a cold in the preceding 12 months with a physician's diagnosis, and AHR upon methacholine challenge (PC_{20} ≤16 mg/ml) or at least 12% reversibility of forced expiratory volume in 1 s (FEV_1) after inhalation of β_2 agonist. Among the 140 asthma patients, 72 were diagnosed with intermittent asthma, 35 were mild persistent asthma and 31 were moderate-to-severe persistent asthma.[19] At the time of enrollment, all the asthmatic children did not received a maintenance therapy. Children treated with systemic corticosteroids due to asthma exacerbation in the preceding month were excluded from the study. The control group consisted of 120 children who had visited the hospital for general health workup or vaccination, and had no history of wheezing, recurrent or chronic diseases, infection in the preceding 2 weeks, or hyperresponsiveness to methacholine. Total serum immunoglobulin E (IgE) levels, peripheral blood eosinophil count, and eosinophil cationic protein (ECP) levels were determined at the initiation of the evaluations. A specific IgE test was performed with six allergens common in Korea: Dermatophagoides pteronyssinus, Dermatophagoides farina, egg whites, cow milk, German cockroach, and Alternaria alternata. Atopy was defined as more than 0.7 KUa/L specific IgE to more than one allergen, or 150 IU/ml total IgE. Atopy was also defined as more than one positive skin test

result of 12 common aeroallergens, including two types of house dust mites, cat and dog epithelium, as well as mold and pollen allergens. This study was approved by the Institutional Review Board of Severance Hospital (Seoul, Korea). Written consent for participation was obtained from parents, with verbal assent from children.

2. Spirometry and methacholine challenge test

Spirometry (VIASYS Healthcare, Inc., Conshohocken, PA) was performed, and flow-volume curves were obtained according to American Thoracic Society guidelines before and after bronchodilator (BD) inhalation.[20] A methacholine challenge test was performed according to standardized procedures.[21] Each child inhaled increasing concentrations of methacholine (0.075, 0.15, 0.31, 0.62, 1.25, 2.5, 5, 10, 25, and 50 mg/ml) nebulized by a dosimeter (MB3; Mefar, Brescia, Italy) until FEV_1 reduced by 20% from a post-nebulized saline solution value. The bronchial response was expressed as a provocative concentration of methacholine causing a 20% fall in FEV_1 (PC_{20}; measured in milligrams per milliliter) and was calculated by linear interpolation of the log dose response curve.

3. Sputum induction and processing

Sputum induction and processing were performed as previously described by Yoshikawa et al.[22] All children were instructed to wash their mouths thoroughly with water. They then inhaled a 3% saline solution nebulized in an ultrasonic nebulizer (NE-U12; Omron Co., Tokyo, Japan) at maximum output at room temperature. The children were encouraged to cough deeply at 3-min intervals thereafter. After sputum induction, spirometry was repeated. If FEV_1 had fallen, the child was required to wait until it returned to baseline values. Sputum samples were kept at 4°C for no more than 2 hrs before further processing. A portion of the samples was diluted with a phosphate-buffered saline solution containing 10 mmol/L of dithiothreitol (WAKO Pure Chemical Industries Ltd, Osaka, Japan) for cell count and was gently vortexed at room temperature for 20 min. After centrifugation at 400g for 10 min, the cell pellet was resuspended. We performed a sputum viability determination with the trypan blue exclusion method to ensure adequate viability. Total cell counts were performed with a haemocytometer, and slides were prepared with cytospin (Cytospin3; Shandon, Tokyo, Japan) and stained with

May–Gru¨nwald–Giemsa stain) for differential cell counts. Differential cell counts were performed by two observers who were blind to clinical details and who counted 400 nonsquamous cells.

4. Measurement of blood eosinophils, serum total IgE and ECP, and sputum PTX3

Eosinophils were counted automatically (NE-8000 system; Sysmex; Kobe, Japan) in peripheral blood, while serum total IgE and ECP levels were measured (CAP system; Pharmacia-Upjohn; Uppsala, Sweden). Sputum PTX3 was individually detected with enzyme-linked immunosorbent assay kits (R&D Systems; Minneapolis, MN) according to the instructions of the manufacturer.

5. Statistical analysis

Numerical variables were expressed as the mean and standard error (SE). Normal distribution was determined by Kolmogorov-Smirnov test. Numerical parameters with nonnormal distribution were presented as median and interquartile range (IQR). Statistical comparison of values between groups was made by the Mann-Whitney U-test. The correlation between sputum PTX3 concentrations and numerical parameters (blood eosinophil count; serum total IgE and ECP levels; sputum eosinophil count; and lung function parameters) was determined using Spearman rank correlation test. All comparisons were made two-sided. A p value < 0.05 was considered statistically significant. Statistical software (SPSS, version 18.0; SPSS Inc; Chicago, IL) was used for all analyses.

III. RESULTS

1. Subject characteristics

The clinical characteristics of the study subjects are summarized in Table 1. There were no significant differences in age and gender between the groups. The percentage of children with atopy was significantly higher in asthma group than those in control group ($P < 0.001$). Pulmonary function parameters, including FEV_1 ($P < 0.001$), percentage change in FEV_1 after BD therapy ($P < 0.001$), FEV_1/FVC ($P < 0.001$), and forced expiratory flow midexpiratory phase ($FEF_{25–75}$) [$P < 0.001$], showed significantly lower levels in children

with asthma than in control subjects. The percentage of eosinophils in induced sputum was significantly higher in children with asthma than in control subjects ($P < 0.001$). The blood eosinophil count, serum ECP, and serum total IgE levels were increased in children with asthma compared with those in control subjects ($P < 0.001$).

Table 1. Subject characteristics

Characteristics	Asthma (n=140)	Control (n=120)
Age, yr	8.1 (6.6-10.3)	9.3 (7.3-11.4)
Sex, M (%)	95 (66)	77 (61)
Atopy, with (%)	97 (69)*	56 (47)
FEV_1, % pred	96.6 ± 16.5*	105.2 ± 12.2
Change in FEV_1, %	7.2 ± 8.1*	2.3 ± 4.1
FEV_1/FVC, %	84.4 ± 9.1*	89.1 ± 5.8
FEF_{25-75}, % pred	78.7 ± 27.9*	99.1 ± 23.6
Total IgE, IU/mL	274 (104-684)*	113 (47-296)
Blood eosinophil, uL	370 (190-710)*	190 (110-350)
Serum ECP, ug/L	25.1 (13.7-44.2)*	17.0 (9.0-31.2)
Sputum eosinophil, %	4 (1-15)*	0 (0-1)

Values are expressed as number (percentage), mean ± SD, or median (Interquatile range) *$P < 0.001$ compared to control subjects.

2. Comparison of sputum PTX3 levels between subject groups

As shown in Figure 1, sputum PTX3 concentration was significantly higher in children with asthma (mean ± SE, 1094.55 ± 224.65 pg/mL) than control subjects (mean ± SE, 177.36 ± 30.00 pg/mL, $p < 0.001$). Among the asthmatic children, children with atopic asthma showed significantly higher sputum PTX3 levels (median, 533.57 pg/mL; interquartile range [IQR], 96.28 to 1345.87 pg/mL) than those with nonatopic asthma (median, 176.49 pg/mL; IQR, 61.64 to 561.97 pg/mL, $p = 0.015$)[Figure 2]. No significant differences were found between control group with atopy (median, 64.59 pg/mL; IQR, 18.52 to 201.69 pg/mL) and without atopy (median, 50.70 pg/mL; IQR, 23.30 to 304.36 pg/mL, $p = 0.415$).

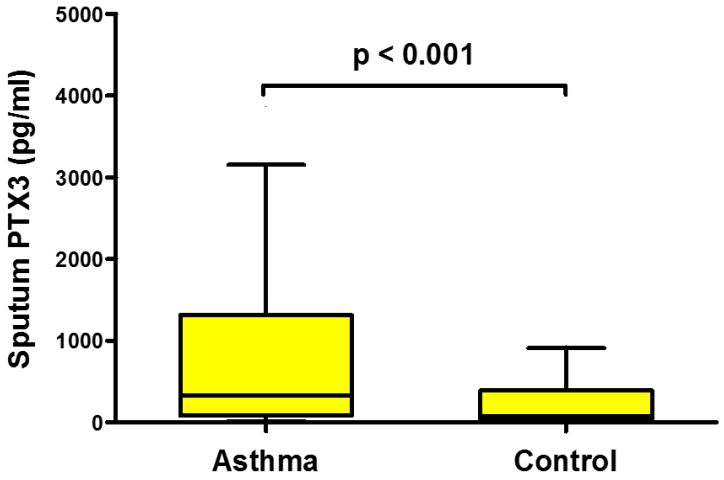

Figure 1. Comparison of sputum PTX3 levels between groups. Asthmatic children had significantly higher levels of sputum PTX3 than the control subjects (P < .001).

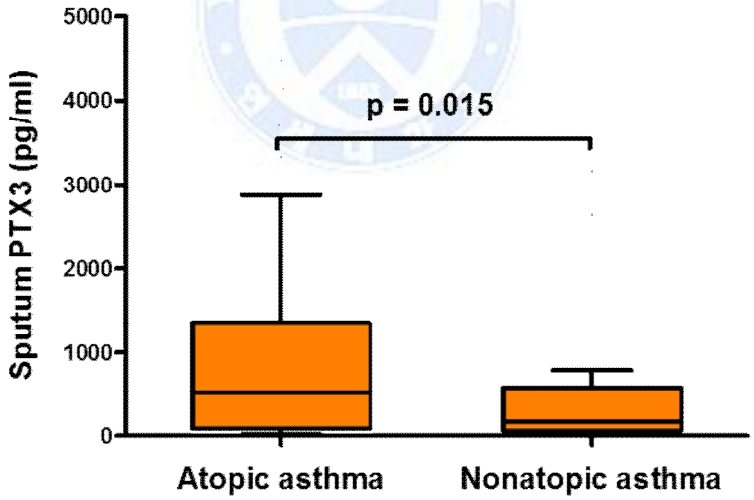

Figure 2. Comparison of sputum PTX3 levels between atopic and nonatopic asthma groups. Children with atopic asthma had significantly higher levels of sputum PTX3 than those with nonatopic asthma (P = 0.015).

3. Sputum PTX3 levels of asthma severity groups

Comparison of sputum PTX3 levels among the asthma subgroups of severity was shown in figure 3. There were no significant differences in age and gender among the groups. Children with moderate-to-severe persistent asthma showed significantly higher sputum PTX3 levels (median, 1190.63 pg/mL; IQR, 302.70 to 2619.84 pg/mL) than those with mild persistent asthma (median, 391.72 pg/mL; IQR, 73.77 to 1352.35 pg/mL, p = 0.044) and intermittent asthma (median, 188.15 pg/mL; IQR, 80.41 to 745.08 pg/mL, p = 0.006). However, the intermittent asthma group and mild persistent asthma groups showed no significant differences (p = 0.27).

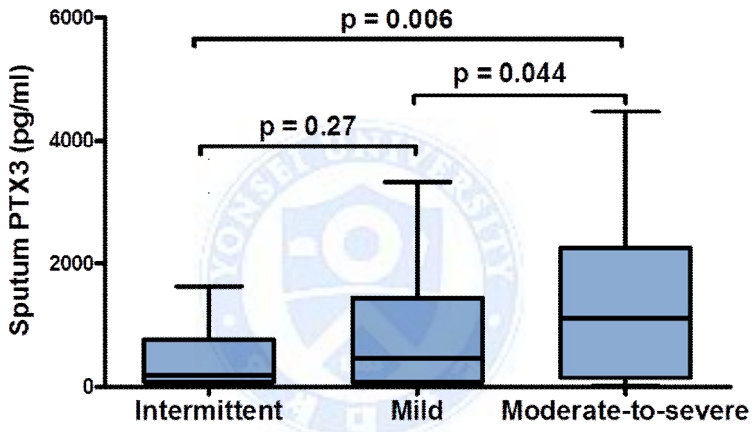

Figure 3. Comparison of sputum PTX3 levels among asthma subgroups of severity. Children with moderate-to-severe persistent asthma showed significantly higher sputum PTX3 levels than those with mild persistent asthma (P = 0.044) and intermittent asthma (P = 0.006). The intermittent asthma group and mild persistent asthma groups were showed no significant difference (p = 0.27)

4. Relationship between sputum PTX3 and allergic inflammation

Serum total IgE were positively correlated with sputum PTX3 concentrations (r = 0.16, p = 0.015)[Figure 4A]. Significant positive correlations were also found between sputum PTX3 levels and serum ECP (r = 0.16, p = 0.024)[Figure 4B], blood eosinophil count (r = 0.15, p = 0.024)[Figure 4C], sputum eosinophil count (r = 0.30, p < 0.001)[Figure 4D].

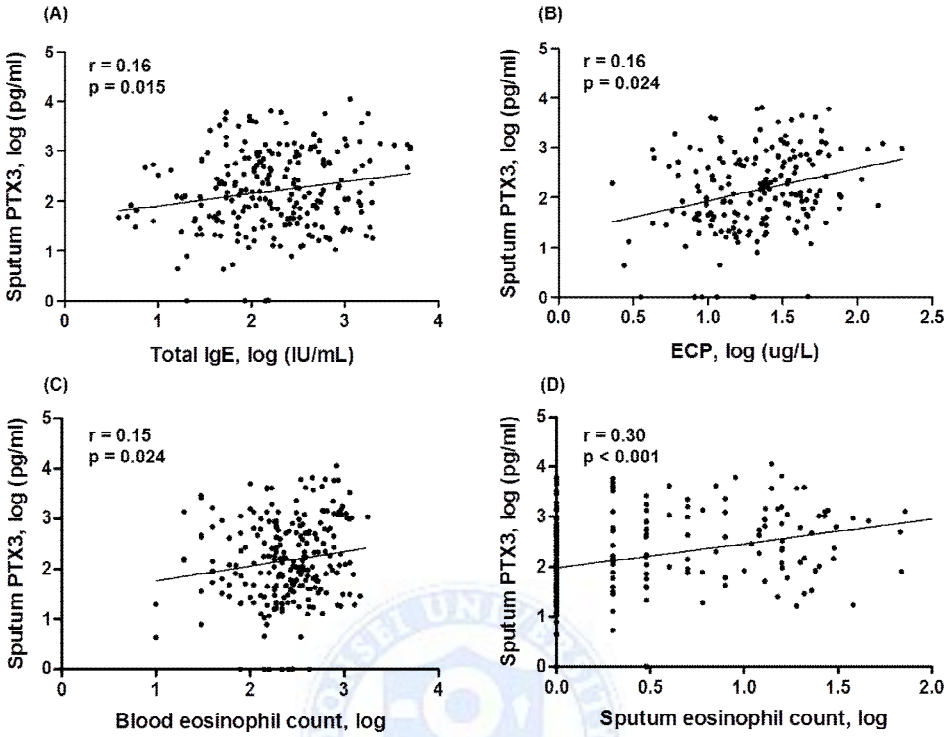

Figure 4. Correlation of sputum PTX3 levels with serum total IgE, ECP, blood and sputum eosinophil. Serum total IgE were positively correlated with sputum PTX3 concentration (A). Significant positive correlations were also found between sputum PTX3 levels and serum ECP (B).

5. Correlation of sputum PTX3 levels with pulmonary function and BDR

Sputum PTX3 concentrations negatively correlated with FEV_1 ($r = -0.30$, $p = 0.001$)[Figure 5A], FEV_1/FVC ($r = -0.27$, $p = 0.002$)[Figure 5B], FEF_{25-75} ($r = -0.392$, $p < 0.001$)[Figure 5C]. Significant positive correlations were found between sputum PTX3 levels and BDR ($r = 0.25$, $p = 0.013$)[Figure 5D].

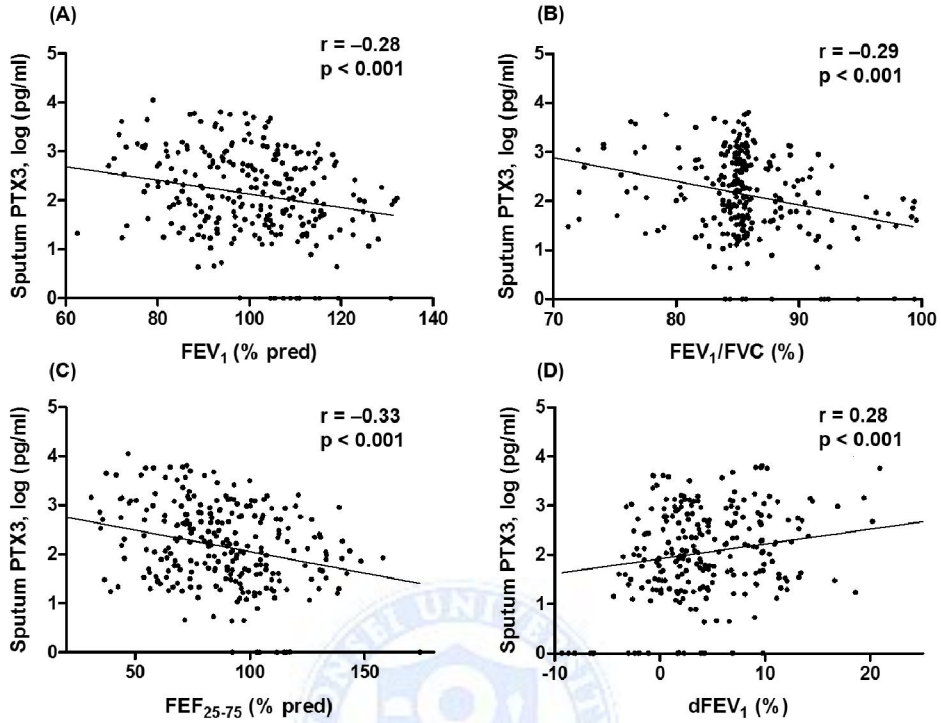

Figure 5. Correlation of sputum PTX3 with pulmonary function and BDR. Sputum PTX3 concentrations negatively correlated with FEV_1 (A), FEV_1/FVC (B), FEF_{25-75} (C). Significant positive correlations were found between sputum PTX3 levels and BDR (D).

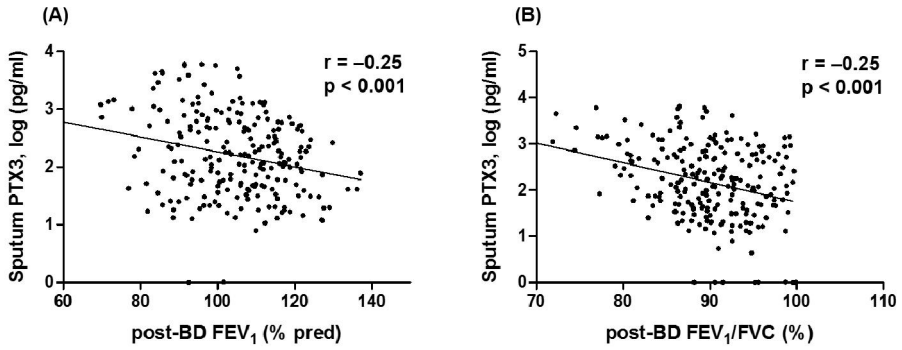

Figure 6. Correlation of sputum PTX3 levels with post-bronchodilator (BD) FEV_1 and FEV_1/FVC. Sputum PTX3 levels showed significant negative correlation with post-BD FEV_1 (A) and post-BD FEV_1/FVC (B).

As shown in Figure 6, sputum PTX3 levels also showed significant negative correlation with post-BD FEV_1 ($r = -0.25$, $p < 0.001$)[Figure 6A] and post-BD FEV_1/FVC ($r = -0.25$, $p < 0.001$)[Figure 6B]. No significant correlation was observed between sputum PTX3 levels and PC_{20} in methacholine challenge test.

IV. DISCUSSION

PTX3, the first long pentraxin discovered, is an acute phase protein expressed locally in response to a various inflammatory responses.[7] PTX3 is involved in innate immunity, inflammatory response, and female fertility.[23-25] Furthermore, it has been suggested to be one of the inflammatory mediators related to lung injury.[14,26] However, very little is known about the expression of PTX3 and its role in asthma. Recently, it has been demonstrated that PTX3 immunoreactivity was increased in bronchial tissues of allergic asthmatics compared to healthy controls, and mainly localized in the smooth muscle bundle.[5] The authors provided the first evidence that PTX3 expression is increased in asthmatic airways.[5] In this study, we found that sputum PTX3 concentration was significantly elevated in asthmatic children compared to control subjects. Results of the present study correspond with that of earlier study. Furthermore, because bronchial biopsy is a comparatively invasive procedure, especially in children, we suggest sputum induction as an alternative method for the quantitation of PTX3 in this study.

Another finding in our study is the correlation between sputum PTX3 and allergic inflammation. Children with atopic asthma showed higher sputum PTX3 levels than those with nonatopic asthma. Serum total IgE was positively correlated with sputum PTX3 levels. It is well known that IgE-dependent mast cell activation plays an important role in allergic airway inflammation.[27] It has been shown that there is a direct relationship between level of serum IgE and likelihood of wheeze and likelihood of reduced lung function.[28] Furthermore, current study found a significant positive correlation of sputum PTX3 levels with ECP, blood and sputum eosinophils. This result indicate that PTX3 involves in eosinophilic inflammation, one of the most important features in asthmatic airway. Previously it has been demonstrated that PTX3 enhanced CCL11/eotaxin-1 release in human airway smooth muscle cells (HASMC), chemokine that plays an important role in eosinophilic airway inflammation and remodeling.[5,29,30] Therefore, we may support the previous finding that sputum

PTX3 may play an important role in airway inflammation of atopic asthma.

There are few previous studies that assessed the correlation between PTX3 levels and lung function parameters. Recent report has been shown that sputum PTX3 levels correlated with the airflow limitation in 29 asthmatic adults.[17] In COPD patients, PTX3 expression in induced sputum did not have any correlation with FEV_1.[15] In current study, sputum PTX3 levels showed significant negative correlation with FEV_1, FEV_1/FVC, FEF_{25-75}, and positive correlations were found significantly between sputum PTX3 and BDR. The correlation between sputum PTX3 and BDR was demonstrated for the first time in this study. Sputum PTX3 is associated with critical characteristics of asthmatic airways including airflow limitation and airway reversibility. Our results can support the findings of earlier studies that PTX3 plays an important role in the pathogenesis of asthma.

Although asthma has been considered as a condition of reversible airflow obstruction, many asthmatic patients have evidence of residual airway obstruction.[31] Subjects with mild asthma have pulmonary function that is relatively well preserved. On the other hand, children with more severe asthma appear to have persistent airflow obstruction and failure to respond to bronchodilator.[32] In this study, children with more severe asthma showed higher sputum PTX3 concentration. Especially, patients with moderate-to-severe persistent asthma showed significantly higher PTX3 levels compared to those with intermittent and mild persistent asthma. Recent studies have suggested an association between disease severity and airway remodeling characterized as structural changes.[32-36] Airway remodeling has been observed in bronchial biopsies of both adults and children with asthma.[36] However, the invasiveness of these diagnostic procedures limits the use of these methods for clinical routine in most asthma patients, especially in children.[37] Indirect methods to detect features of airway remodeling include evaluation of airway wall thickness by imaging studies, measurement of cytokine and cell profiles, or assessment of lung function.[38] Loss of reversibility of airway obstruction is a typical feature of airway remodeling.[38] Therefore, the low post-BD FEV_1/FVC ratio is useful as a marker of airway remodeling.[38] In this study, sputum PTX3 concentrations were highly correlated with post-BD FEV_1 and post-BD FEV_1/FVC. PTX3 was previously shown to play an important role may play a role in tissue remodeling at sites of inflammation.[39] Previous in vitro study[5] has demonstrated that HASMC are one of the major sources of PTX3 in the airways.

It has been shown that PTX3 has the ability to inhibit FGF2-induced migration of HASMC at least in vitro and can upregulate CCL11/ eotaxin-1 release. So, it has been suggested that PTX3 may potentially contribute to airway inflammation and subsequent remodeling in allergic asthma.[5] In the current study, it is suggested that sputum PTX3 would be one of the helpful biomarkers of airway remodeling. The reason why PTX3 is highly elevated in induced sputum of asthmatic children and correlated with lung function remains unclear, further studies will be required to confirm the roles of sputum PTX3 in asthma.

V. CONCLUSION

To our knowledge, this is the first study that evaluated PTX3 in induced sputum of children with asthma. Our results could support that PTX3 is involved in the pathogenesis of asthmatic airway. Sputum PTX3 would be a supportive biomarker reflecting asthmatic airway inflammation and remodeling in childhood asthma.

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ABSTRACT (IN KOREAN)

소아 천식에서 기도염증 및 기도재형성을 반영하는 지표로써 유도객담 내 pentraxin 3 농도의 의의

<지도교수 손명현>

연세대학교 대학원 의학과

이 희 선

Pentraxin 3 (PTX3)는 패턴인식수용체에 속하는 급성기 반응 단백의 일종이며, 조직의 손상 및 염증 반응을 나타내는 표지인자로서의 역할에 대한 연구가 최근 들어 활발해지고 있다. 본 연구에서 저자는 소아천식 환자들의 유도객담을 분석하여 PTX3의 농도가 증가되어 있는지를 확인하고자 하였다. 또한, 유도객담 내 PTX3 농도와 소아의 기도 염증 및 과민성, 폐기능 등과의 연관성을 확인하고자 하였다. 총 260명의 소아들을 대상으로 연구를 진행하였으며 이들 중 천식환자 140명, 대조군 120명이었다. 모든 대상 소아들에게 폐기능검사와 메타콜린 기관지유발검사를 시행하였으며, 혈액을 채취하여 총 호산구 수, 총 IgE 농도, 혈청 호산구 양이온 단백의 농도를 측정하였다. 유도객담 내 PTX3의 농도는 천식환자군이(mean ± SE, 1094.55 ± 224.65 pg/mL) 대조군에 비해(177.36 ± 30.00 pg/mL, $p < 0.001$) 유의하게 높은 수치를 나타내었다. 또한, 중등도 및 중증 지속성 천식 환아들이(median, 1190.63 pg/mL; IQR, 302.70 to 2619.84 pg/mL) 경증 지속성 천식과(median, 391.72 pg/mL; IQR, 73.77 to 1352.35 pg/mL, $p = 0.044$) 간헐성 천식 환아들에 비해(median, 188.15 pg/mL; IQR, 80.41 to 745.08 pg/mL, $p = 0.006$) 유의하게 높은 PTX3 농도를 보였다. 유도객담 내 PTX3 농도는 폐기능검사에서 기관지확장제 반응 결과와 유의한 양의 상관관계를 보였으며($r = 0.25$, $p = 0.013$), FEV_1 ($r = -0.30$, $p = 0.001$), FEV_1/FVC ($r = -0.27$, $p = 0.002$), FEF_{25-75} ($r = -0.392$, $p < 0.001$)와는 유의하게 음의 상관관계를 나타내었다. 또한 기관지확장제 흡입 후

FEV_1 ($r = -0.25$, $p < 0.001$) 및 FEV_1/FVC ($r = -0.25$, $p < 0.001$)와 역시 유의한 음의 상관관계를 보였다. 유도객담 내 PTX3의 농도는 소아천식에서 기도염증 및 기도재형성을 반영하는 지표로써 역할을 기대할 수 있을 것으로 생각된다.

핵심되는 말 : Pentraxin 3, 유도객담, 천식, 소아.