

Elevated Saliva Pepsin Concentration as a Risk Factor for Asthma in Children with Allergic Rhinitis: A Preliminary Study

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Objective: This study aimed to explore whether saliva pepsin concentration (SPC) could be regarded as a risk factor for the occurrence and unfavorable control of asthma in children with allergic rhinitis.

Methods: A prospective study was conducted on a group of 20 consecutive children newly diagnosed with allergic rhinitis and asthma (referred to as the asthma group). All these children underwent fractional exhaled nitric oxide (FeNO) measurement, lung function tests, and assessment of asthma control using the 7-item Childhood Asthma Control Test (C-ACT) score. Simultaneously, a control group consisting of 20 children with simple allergic rhinitis, matched for baseline characteristics, was included. SPC measurement was performed in the two groups.

Results: The SPC value was significantly higher in the asthma group than that in the control group (165.0 ± 82.8 ng/mL vs 68.4 ± 34.5 ng/mL) ($P < 0.001$). In the asthma group, SPC was independently associated with FeNO, the ratio of forced expiratory volume in 1 second (FEV1) to forced vital capacity (FVC), and forced expiratory flow at 50% and 75% of FVC (FEF50 and FEF75) (all $P < 0.05$). The severity of nasal symptoms evaluated by the visual analogue scale (N-VAS) was independently associated with FEF75, the maximal mid-expiratory flow (MMEF), and C-ACT score ($P < 0.05$).

Conclusion: Direct pepsin exposure and uncontrolled nasal symptoms may play crucial roles in the pathogenesis and progression of childhood allergic asthma. The SPC value can be considered as a risk factor for asthma in children with allergic rhinitis.

Keywords: saliva pepsin concentration, asthma, allergic rhinitis, gastroesophageal reflux disease

Introduction

Asthma stands as the prevailing chronic respiratory condition among children globally, impacting approximately 14% of youth and adolescents.¹ Poor asthma control is associated with a number of negative effects on children and their families, including reduced quality of life, increased economic costs, and even the risk of death caused by severe attacks.

Gastroesophageal reflux disease (GERD) has been considered as a risk factor for asthma and a trigger for asthma attacks in children.¹⁻³ It has been estimated to be present in 40–80% of children with asthma.⁴ Nevertheless, the precise pathophysiological mechanism by which GERD exacerbates asthma remains elusive. However, certain drugs routinely used for GERD may have the potential to enhance lung volumes in respiratory function tests and alleviate asthma symptoms. This improvement contributes to a more effective subjective control of asthma.³ Notably, pepsin, consistently found in all refluxate, may be a significant contributor, distinguishing itself from other gastric components, such as bile acids. In vitro experiments suggested that pepsin exposure disrupts respiratory epithelium and causes direct contact between submucosal tissue and allergens and pathogens.⁵

Thus, pepsin concentration in upper respiratory secretions could be theoretically an indicator of asthma risk. To our knowledge, no study has yet assessed the role of pepsin concentration in pediatric asthma risk.

Utilizing the Peptest device, a commercially available immune-serologic test facilitating precise measurement of saliva pepsin concentration (SPC), the present study aimed to assess the involvement of pepsin in the pathogenesis and management of childhood asthma.

Methods

Study Subjects

Between August 2022 and September 2022, data of a total of 20 consecutive children who aged 6–12 years old with newly diagnosed allergic rhinitis and asthma at Peking University First Hospital (Beijing, China) were prospectively analyzed (asthma group). The diagnosis of allergic asthma was performed according to the following criteria: (1) typical clinical symptoms, such as cough, wheezing, shortness of breath, exercise intolerance, and reversibility to bronchodilators and/or positive response to bronchial methacholine challenge;⁶ (2) detection of positive results for at least one allergen through either allergen skin prick or serum immunoglobulin E (IgE) test; and (3) exposure to specific allergens (mainly dust mites, pollen, mold, and animal fur) could trigger or exacerbate the abovementioned symptoms. Children who had other chronic lung diseases or acute respiratory infections over the past four weeks were excluded. During the same period, another group of 20 children with simple allergic rhinitis matched for sex, age, body mass index (BMI), and the severity of nasal symptoms evaluated by visual analogue scale (N-VAS) were included as control group (Table 1). None of children in the control group had experienced any of the abovementioned asthma-related symptoms even under the exposure to specific allergens.

SPC was measured in both groups of children. In addition, fractional exhaled nitric oxide (FeNO) measurement, lung function tests, and subjective assessment of asthma control were performed for all children in the asthma group on the day of saliva collection. This study was performed followed the principle of the Declaration of Helsinki and the study protocol was approved by the Ethics Committee of Peking University First Hospital (Approval No. 2021–250), and all children's caregivers had signed informed consent forms.

Measurement of SPC

All saliva samples from children were collected in the morning upon waking and standing, prior to having breakfast or brushing their teeth. The children's caregivers were instructed to collect saliva into a tube containing 0.5 mL of 0.01 M citric acid. The samples were refrigerated at 4 °C, and the SPC was evaluated using the Peptest device (RD Biomed Ltd., London, UK) according to the standardized procedure that has been previously described.^{7,8} The result was considered to be valid when a blue line appeared under the letter C (control) of the Peptest device at 15 min after applying the sample. The apparition of a blue line under the letter T (test) was indicative of a positive result. The SPC

Table 1 The Comparison of Baseline Information Between Asthma and Control Groups

| | Asthma Group | Control Group | P |
|--------------------------|--------------|---------------|-------|
| | n = 20 | n = 20 | |
| Sex | | | 1.000 |
| Boy | 17 | 16 | |
| Girl | 3 | 4 | |
| Age (years old) | 9.5±1.5 | 8.6±2.0 | 0.114 |
| BMI (kg/m ²) | 19.5±2.3 | 19.8±1.6 | 0.602 |
| N-VAS | 5.4±1.6 | 5.2±1.7 | 0.778 |

Abbreviations: BMI, body mass index; N-VAS, the severity of nasal symptoms evaluated by visual analogue scale.

was also accurately measured using the PEP-test Cube, which is an electronic lateral flow device that can detect pepsin down to 16 ng/mL. The test was considered positive when the pepsin level reached ≥ 16 ng/mL.

FeNO, Lung Function Test, and Assessment of Asthma Control

FeNO measurement (NIOX MINO device, Circassia, Stockholm, Sweden) was conducted according to the ATS guidelines.⁹ Lung function test was performed using spirometry (Spirolab II, Medical International Research, Rome, Italy) as described previously.¹⁰ The ratio of forced expiratory volume in 1 second (FEV1) to forced vital capacity (FVC) (FEV1/FVC), forced expiratory flow at 50% and 75% of FVC (FEF50 and FEF75), and the maximal mid-expiratory flow (MMEF) were recorded and used in the analyses. The subjective asthma control was evaluated by the 7-item Childhood Asthma Control Test (C-ACT).^{11,12} The Chinese version of this questionnaire has been validated in previous research.¹³ Asthma was regarded controlled if the C-ACT score was greater than 19 points and classified as uncontrolled if it was 19 points or lower.

Statistical Analysis

The statistical analysis was carried out using SPSS 20.0 software (IBM, Armonk, NY, USA). Continuous variables were expressed as mean \pm standard deviation. The unpaired Student's *t*-test was utilized to compare continuous variables between two groups. The Fisher's exact test was used to compare categorical data between two groups. The Pearson correlation test was employed to identify significant associations. The linear regression analysis was used to identify independent significant associations. $P < 0.05$ was considered statistically significant.

Results

As illustrated in Figure 1, the overall SPC in the asthma group was 165.0 ± 82.8 (range, 25.0–301.2) ng/mL, which was significantly higher than that in the control group (68.4 ± 34.5 ng/mL; range, 16.5–150.6 ng/mL) ($P < 0.001$). According to the definition of pathological pepsin level of ≥ 75 ng/mL,¹⁴ the proportion of children with proximal reflux in the asthma group was 90% (18/20), which was also significantly higher than that in the control group (45% (9/20)) ($P = 0.006$).

The FeNO values, lung function test results, and the C-ACT scores of 20 children in the asthma group and their correlations with SPC values are presented in Table 2. It was revealed that the SPC was significantly correlated with FeNO, FEV1/FVC, FEF50, FEF75, MMEF, and C-ACT score (all $P < 0.05$).

Linear regression analysis, involving sex, age, BMI, N-VAS, and SPC to predict the abovementioned parameters, was conducted. The independent factors associated with the outcomes are presented in Table 3, revealing that SPC was the sole parameter independently correlated with FeNO, FEV1/FVC, and FEF50 (all $P < 0.05$). SPC and N-VAS were both

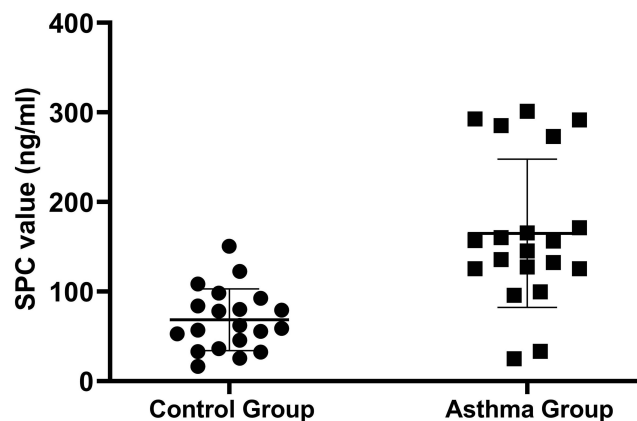


Figure 1 The comparison of SPC values between the asthma group and the control group.

Table 2 FeNO Values, Lung Function Test Results, and C-ACT Scores of 20 Children in the Asthma Group and Their Correlations with SPC Values

| | Asthma Group (n = 20) | Correlation Coefficient | P |
|----------|-----------------------|-------------------------|--------|
| FeNO | 36.6±15.4 | 0.824 | <0.001 |
| FEV1/FVC | 81.6±9.6 | −0.707 | <0.001 |
| FEF50 | 64.5±18.4 | −0.630 | 0.003 |
| FEF75 | 46.1±10.9 | −0.644 | 0.002 |
| MMEF | 57.8±15.8 | −0.542 | 0.013 |
| C-ACT | 23.0±5.1 | −0.734 | <0.001 |

Abbreviations: SPC, saliva pepsin concentration; FeNO, fractional exhaled nitric oxide; FEV1/FVC, the ratio of forced expiratory volume in 1 second to forced vital capacity; FEF50 and FEF75, forced expiratory flow at 50% and 75% of FVC; MMEF, the maximal mid-expiratory flow; C-ACT, the 7-item Childhood Asthma Control Test.

Table 3 Factors Independently Associated with FeNO Value, Lung Function Test Results, and C-ACT Score

| | Parameter | Adjusted R ² | Coefficient | P |
|----------|-----------|-------------------------|-------------|--------|
| FeNO | SPC | 0.679 | 0.153 | <0.001 |
| FEV1/FVC | SPC | 0.500 | −0.082 | <0.001 |
| FEF50 | SPC | 0.397 | −0.140 | 0.003 |
| FEF75 | SPC | 0.539 | −0.150 | <0.001 |
| | N-VAS | | 4.063 | 0.046 |
| MMEF | SPC | 0.515 | −0.229 | <0.001 |
| | N-VAS | | 7.801 | 0.013 |
| C-ACT | N-VAS | 0.603 | −2.413 | <0.001 |

Abbreviations: SPC, saliva pepsin concentration; N-VAS, the severity of nasal symptoms evaluated by visual analogue scale; FeNO, fractional exhaled nitric oxide; FEV1/FVC, the ratio of forced expiratory volume in 1 second to forced vital capacity; FEF50 and FEF75, forced expiratory flow at 50% and 75% of FVC; MMEF, the maximal mid-expiratory flow; C-ACT, the 7-item Childhood Asthma Control Test.

independently associated with FEF75 and MMEF (all $P < 0.05$), while N-VAS was the only parameter that was independently associated with C-ACT score ($P < 0.05$).

Discussion

GERD has been considered as a possible risk factor for childhood asthma.^{1,2} However, the mechanisms were not fully explored; some attributed it to the direct noxious effects of gastric contents in the tracheobronchial tree, while others demonstrated that reflux into the esophagus might activate a neural reflex arc through the vagus nerve, indirectly leading to asthma-related symptoms. Additionally, a preliminary clinical study has suggested the coexistence of both mechanisms.³ The findings of the present study could provide robust evidence supporting the first mechanism, given that pepsin A is an enzyme exclusively produced by gastric chief cells. Its presence in upper airway secretions may be considered as a marker of reflux.¹⁵ This was consistent with the results of an *in vitro* experiment, which indicated that refluxate exposure could lead to bronchial epithelial damage, barrier leakiness, and/or neutrophil breach of the epithelium, highlighting the crucial role of pepsin in exacerbating asthma.⁵

The aforementioned results also indicated the significance of proximal reflux, also known as laryngopharyngeal reflux (LPR), in exacerbating asthma. LPR is characterized by the reflux of gastric acid and refluxate (containing pepsin) above the upper esophageal sphincter.¹⁶ Despite being considered as an extension of GERD, it frequently manifests without typical GERD symptoms, such as heartburn and regurgitation, posing a challenge for clinicians in making accurate diagnosis and determining an appropriate treatment.¹⁷ Therefore, a portable and non-invasive test is essential to confirm the possibility of reflux and provide evidence for further treatment, including SPC measurement.

Another important finding of the current study was that the SPC value was independently associated with FeNO value and all lung function test variables in the asthma group, and it was significantly correlated with C-ACT score. FeNO represents type 2 airway inflammation, which is closely associated with the occurrence and progression of asthma.¹⁸ FEV1 and FEV1/FVC mainly reflect large airway function, while FEF50, FEF75, and MMEF are traditional indices used to assess peripheral airway obstruction.¹⁹ Therefore, pepsin exposure may exacerbate asthma by inducing airway inflammation and decreasing the patency of both small and large airways.

In the asthma group, a higher N-VAS score was also found to be independently associated with unfavorable pulmonary function and unsatisfactory asthma control. Similarly, Ohta et al reported a close relationship between severe rhinitis and asthma exacerbation.²⁰ Explaining this is not challenging, given the strong correlation between asthma and allergic rhinitis. They share common allergens and inflammatory processes.^{21,22} The clinical significance of this finding emphasizes the importance of controlling rhinitis symptoms.

This study has certain limitations that should be pointed out. Firstly, the sample size was limited, although meaningful results were still obtained. Secondly, the absence of a normal control group comprising children without allergic rhinitis and asthma could enhance the reliability of the results; however, this is planned for the future research. Thirdly, the results lack additional verification, and a study exploring the impact of anti-reflux therapy on the aforementioned parameters could strengthen the conclusions. Additionally, the detection of SPC alone has limitations in diagnosing GERD, as physiological reflux may also lead to pepsin presence in upper airway secretions. On the other hand, GERD with mainly non-acid reflux may also showed a negative result of SPC test. Combining it with the gold standard method for diagnosing GERD in children, dual-probe esophageal 24-h pH monitoring²³ might yield more meaningful results, while this test is invasive and may pose ethical limitations in this study. Lastly, the symptoms of asthma itself, such as cough, may potentially exacerbate GERD, introducing a correlation that cannot be ruled out and acting as a potential confounder.

In conclusion, it was found that the SPC was significantly higher in children with allergic rhinitis and asthma than that in children with simple allergic rhinitis. Moreover, a high SPC value was closely correlated with the increased airway inflammation, unfavorable pulmonary function, and more importantly, poor asthma control. The abovementioned findings indicated a significant role of direct pepsin exposure in the pathogenesis and progression of childhood allergic asthma. Furthermore, it was revealed that the SPC value could be regarded as a potential risk factor for asthma in children with allergic rhinitis.

Abbreviations

GERD, gastroesophageal reflux disease; SPC, saliva pepsin concentration; BMI, body mass index; N-VAS, the severity of nasal symptoms evaluated by visual analogue scale; FeNO, fractional exhaled nitric oxide; FEV1/FVC, the ratio of forced expiratory volume in 1s to forced vital capacity; FEF50 and FEF75, forced expiratory flow at 50% and 75% of FVC; MMEF, the maximal mid-expiratory flow; C-ACT, the 7-item Childhood Asthma Control Test; LPR, laryngopharyngeal reflux.

Data Sharing Statement

The data underlying this article will be shared upon reasonable request to the corresponding author.

Ethics Statement

The study protocol had been proved by the Ethics Committee of Peking University First Hospital with an ID of 2021-250, and all the children's caregivers had provided signed informed consents. No patient-identifying information was accessible during the study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest and report no conflicts of interest for this work.

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