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Comparison of Serum Eosinophil-Derived Neurotoxin Levels between Wheezing and Non-wheezing Groups in Children with Respiratory Tract Infection

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ABSTRACT

Objective: Eosinophil-derived neurotoxin (EDN) is associated with recurrent wheezing episodes after bronchiolitis, childhood asthma, and allergic rhinitis. We investigated if there is a measurable difference between serum EDN levels in children with wheezing and non-wheezing respiratory infections.

Methods: 171 children who visited a university hospital with respiratory infections were enrolled in the study. Subjects were divided into two groups: wheezing (n=46) and non-wheezing (n=125). Serum EDN levels were compared.

Results: Serum EDN levels in the wheezing group were significantly higher than in the non-wheezing group ($P<0.001$). The non-wheezing group was divided into three subgroups: pneumonia, common cold, and tonsillitis. Serum EDN levels in the wheezing group were significantly higher than in the pneumonia, common cold, or tonsillitis subgroups ($P<0.001$). There was no significant difference in serum EDN levels among the pneumonia, common cold, and tonsillitis subgroups.

Conclusions: These findings suggest that elevated serum EDN levels could be a distinctive feature of respiratory infections with wheezing. EDN's utility as a biomarker for wheezing-associated disease should be explored through further study.

INTRODUCTION

It is known that many hemocytes are involved in asthma onset, and several previous studies have reported elevated numbers of activated eosinophils and neutrophils in asthmatic bronchial mucosa, bronchoalveolar lavage fluid, sputum, and blood specimens [1, 2]. Eosinophils and neutrophils contain a number of chemical mediators and when these cells are activated, these granular mediators are released outside the cell, causing an inflammatory response [3].

Eosinophilic inflammation is one of the most important features of asthma, and eosinophils are known to be the primary effector cells involved in asthma development. Airway epithelial damage by activated eosinophils is a very important mechanism in asthma pathogenesis, but serum biomarkers that reflect activation of eosinophils are not well studied. [4].

Eosinophilic granules are attached to the cell membrane and contain strongly positively charged protein substances that cause tissue damage in these granules [5-7]. Major basic protein (MBP) is at the center of the granule, while eosinophilic cationic protein (ECP), eosinophil peroxidase (EPO), and eosinophil-derived neurotoxin (EDN), are found in the substrate of the granule. Among these granular proteins, EDN has been attracting much attention as a useful biomarker for diagnosis and monitoring of asthma, and it is associated with recurrent wheezing, pediatric asthma, allergic rhinitis, and chronic cough in children after bronchiolitis [8]. According to previous studies, eosinophil counts or percentages in blood do not accurately reflect eosinophil activity; they are merely a measure of eosinophil presence in the fluid tested. In contrast, measurement of the eosinophilic granular proteins EDN and ECP has been reported to

accurately reflect a substantial degree of eosinophilic inflammatory response [9, 10].

The purpose of this study was to analyze serum EDN levels in children with wheezing and non-wheezing respiratory tract infection using the recently developed K[®] EDN ELISA Kit.

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METHODS

Subjects

Subjects included 0- to 7-year-old children who visited the outpatient clinic of Xi'an Jiaotong University Shanxi People's Hospital in Xi'an, Shanxi province, China. All surveys were conducted with the approval of the International Review Board of the Jiatong University Shaanxi Provincial People's Hospital (#2016001).

Measurement of serum EDN levels

Nurses collected blood specimens in BD vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), which are serum separation tubes. It was solidified at 25°C for 1 hour and centrifuged for 10 minutes at 1350g at 4°C. Each serum sample was placed in a new plastic tube and stored at -70°C until tested. Serum EDN was measured using a K[®] EDN ELISA Kit recently developed in Korea (SKIMS-BIO Co., Seoul, Korea [11]).

Wheezing definition

When a subject visited the clinic, they were classified into the wheezing group when the physician heard wheezing upon physical examination. If wheezing was not heard, the subject was classified as non-wheezing. Rales were not considered to be wheezing, and any previous wheezing or recurrent wheezing episodes were not taken into consideration.

Statistical analysis

Because the EDN data in the wheezing group did not show a normal distribution, comparisons between the wheezing and non-wheezing groups were analyzed using the Mann-Whitney test. The non-wheezing group was divided into three subgroups (pneumonia, common cold and tonsillitis), and the wheezing group was also included for a total of the four groups. EDN levels among these four groups were compared using the Kruskal-Wallis test. The Kruskal-Wallis test was also used to compare EDN levels by age in both the wheezing and non-wheezing groups. In the Kruskal-Wallis test, the Mann-Whitney test was performed in pairs of two groups for the post-test. Comparisons of categorical data between the two groups were analyzed using the Fisher's exact test. All statistical analyses were performed using SPSS ver. 17.0 (SPSS Inc., Chicago, IL, USA), and when *P*-values less than 0.05 were considered to be statistically significant.

RESULTS

Subject characteristics

171 people were included in the study, with 46 in the wheezing group and 125 in the non-wheezing group. The non-wheezing group consisted of 30 patients with pneumonia, 61 patients with a common cold, and 34 patients with tonsillitis. The mean age of the wheezing group was 42.3 months, which was significantly higher than the mean age of 22.2 months in the non-wheezing group. The sex ratio was not different between the two groups. A previous history of atopic dermatitis diagnosis was significantly higher in patients with wheezing than in those without wheezing (10.5% vs 0.8%). There was no difference in the frequency of food allergy diagnosis, parental asthma diagnosis, pet ownership, or house dust mite-specific antibody positivity between the two groups (Table 1).

Comparison of EDN levels by age group in the wheezing and non-wheezing groups

As there was a significant difference in age distribution between the wheezing and non-wheezing groups, each group was further divided into three groups according to age: 0 to < 1 year, 1 to < 3 years, and 3 to < 7 years of age. In the wheezing group, there was no difference in EDN values between the age groups 1 to < 3 years old and 3 to < 7 years old (Fig. 1), while in the non-wheezing group there were no significant differences in EDN levels among all three age groups (Fig. 2).

Comparison of EDN levels between wheezing and non-wheezing groups

The mean value of EDN in the wheezing group was 48.8 ± 71.4 ng/ml, which was significantly higher than the 13.3 ± 5.1 ng of EDN in the non-wheezing group ($P < 0.001$) (Fig. 3). When EDN values were compared among the 4 groups (the three non-wheezing groups plus the wheezing group), the mean EDN value in the wheezing group was significantly higher than the 15.1 ± 5.5 ng/ml in the pneumonia group, the 12.4 ± 4.9 ng/ml in the common cold group, and the 13.4 ± 4.7 ng/ml in the tonsillitis group ($P < 0.001$). There was no significant difference in EDN levels among the pneumonia, common cold, and tonsillitis groups (Fig. 4).

DISCUSSION

In this study, serum EDN levels were compared in wheezing and non-wheezing children aged 0 to 7 years at the outpatient department of a Chinese university hospital. Serum EDN levels were significantly higher in the wheezing group than in the non-wheezing group. When the EDN levels of the four groups (one wheezing group and three non-wheezing groups with pneumonia, common cold, or tonsillitis) were compared, serum EDN levels were significantly higher in the wheezing group than in the three non-wheezing groups, and there was no significant difference among the pneumonia, common cold, and tonsillitis groups.

The association of disease severity with EDN levels in children with recurrent wheezing or asthma has been previously reported [10,12-14]. It has been reported that EDN levels in infants with wheezing after bronchiolitis due to RSV are significantly higher than in healthy controls, [12] and serum EDN levels in asthmatic children significantly increased as the asthma severity increased. It has been concluded that EDN levels reflect asthma severity more accurately than ECP and eosinophil counts [10]. In adult asthmatics positive for specific antibodies to house dust mites, EDN levels were significantly inversely associated with FEV1 on a pulmonary function test, while eosinophil counts were not [13]. Other studies in adult asthmatics have also shown that EDN has a significant inverse association with FEV1 and FEV1/FVC on asthma symptoms and pulmonary function tests [14]. These results suggest that serum EDN levels are elevated in recurrent wheezing, asthma, and aggravation of disease, and that EDN more accurately reflects the disease state and eosinophil activity compared to other biomarkers such as eosinophil count. This indicates that EDN may be an important

biomarker for predicting recurrent wheezing and asthma. It is not yet clear whether EDN is directly involved in bronchial inflammation, asthma exacerbation, airway hyperresponsiveness, or airway remodeling. However, Kim et al. [15] recently reported that EDN is involved in airway inflammation and airway hyperresponsiveness. The role of EDN as a biomarker has already been reported in several studies, and EDN is known to reflect the active state of eosinophils accurately [12].

Eosinophils play an important role in the allergic airway inflammatory response in asthmatic patients. Several studies have reported that eosinophils are involved in changes in immune response, airway hyperresponsiveness, and airway remodeling in patients with asthma [15]. In patients with recurrent wheezing or asthma, eosinophils are activated by infection or allergic reactions, and the activated eosinophils secrete eosinophil degranulation proteins such as EDN [7]. Therefore, serum EDN levels indirectly indicate the degree of eosinophilic bronchial inflammation. In allergic diseases such as asthma, EDN and ECP are biomarkers that reflect eosinophilic inflammation, and EDN is superior to ECP in test reproducibility [8]. It is known that as EDN has a weak electric charge compared to ECP, it does not adhere well to the walls of measuring instruments or other cell surfaces [16].

To date, asthma-related studies have referred to symptoms, pulmonary function, and frequency of symptom relief in assessing whether or not the disease is well controlled in patients with recurrent wheezing or asthma [17]. These items, however, do not accurately reflect the degree of eosinophilic inflammation of the airway, [18] especially in younger children, and pulmonary function tests are further limited because it is difficult to secure patient cooperation [8]. In addition, in order to perform accurate

pulmonary function tests in children, a skilled workforce is needed, which makes it burdensome to perform pulmonary function tests for screening purposes. In recent clinical trials, the degree of inflammation of the airway has been evaluated through FeNO measurement, but the association is not yet clear [19].

Other methods, such as eosinophil counts, have been previously performed to assess the degree of eosinophilia, but the association was reported to be weak [9, 10]. Because eosinophil activity is highly associated with eosinophil concentration and eosinophil secretion level [9], as reported in our asthma allergy center, EDN measurements can be useful in the diagnosis of recurrent wheezing and asthma and in monitoring the worsening of the disease, and similar results have been confirmed in studies of foreign children, which makes this study more meaningful.

In this study, although the number of the subjects was small, the incidence of atopic dermatitis was significantly higher in patients with wheezing than in those without wheezing, which can be explained by the allergic march theory that the incidence of atopic dermatitis before 1 year of age was associated with asthma and allergic rhinitis. [20] These results are also thought to support the hypothesis of this study that the wheezing group has a higher possibility of asthma morbidity and exacerbation.

In the present study, recurrent wheezing and asthma were used interchangeably, but since the definition of asthma was based only on the physician's stethoscopic findings at the time of the examination, it didn't take into account history of previous wheezing or its recurrence. The physician did not objectively diagnose asthma according to airway hyperresponsiveness through bronchial provocation tests, which is a major limitation of this study. Moreover, this study didn't confirm the history of previous recurrences of

wheezing in the non-wheezing group, and there is difference in the age distribution of the wheezing group and non-wheezing group, which is another limitation of this study. Although there was a difference in age distribution between the wheezing and non-wheezing group, statistical analysis showed that the difference in age-group EDN values was not significant, which suggests that a difference in age distribution may have a non-significant effect on EDN level. In addition, the reason the age distribution of the wheezing group was significantly higher than that of non-wheezing group may be mainly because wheezing children are more likely to have actual asthma rather than bronchiolitis or transient wheezing due to respiratory virus infection, which means they are relatively older.

Our study of Chinese children found that serum EDN levels were significantly higher in those with wheeze than in those without wheeze, which may suggest an association between higher EDN levels and wheezing. These results will need to be confirmed through additional studies, including possibly extrapolating the results to children from other countries.

CONCLUSION

Our findings suggest that elevated serum EDN levels may be a distinctive feature of respiratory infections with wheezing. Its usefulness as a potential biomarker should be evaluated through large-scale studies.

Declaration of Interest

The authors declare that they have no conflicts of interest.

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Table 1. Subject characteristics

	Wheezing (n=46)	Non- wheezing (n=125)	<i>P</i> -value
Age (month)	42.3±20.0	22.2±19.3	<0.001
Gender, male/female	27/19	65/10	0.436
Food allergy history	1/46 (2.2%)	2/125 (1.6%)	1.000
Atopic dermatitis history	5/46 (10.5%)	1/125 (0.8%)	0.006
Parental asthma history	1/46 (2.2%)	0	0.269
Pet ownership	0	2/125 (1.6%)	0.533
Specific IgE, HDM	2/46 (4.3%)	1/125 (0.8%)	0.177
HDM: house dust mite			

Figures Legends

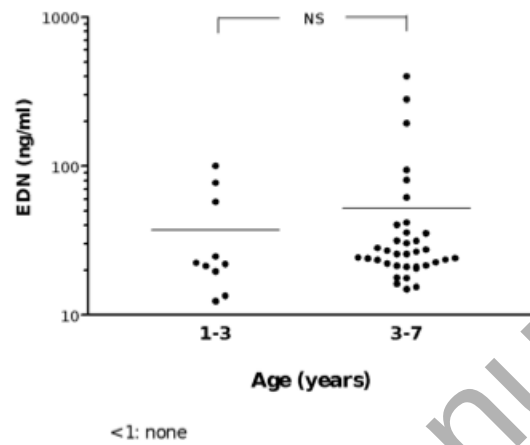


Figure 1. Comparison of serum EDN level among three age groups (<1 year old group, n=0; 1-3 years old group, n=10; 3-7 years old group, n=36) in wheezing group (n=46).

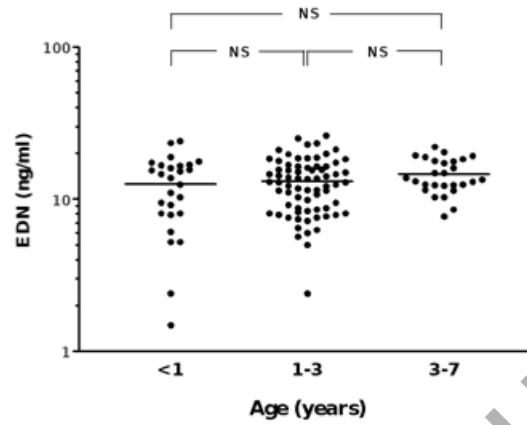


Figure 2. Comparison of serum EDN level among three age groups (<1 year old group, n=27; 1-3 years old group, n=71; 3-7 years old group, n=27) in non-wheezing group (n=125).

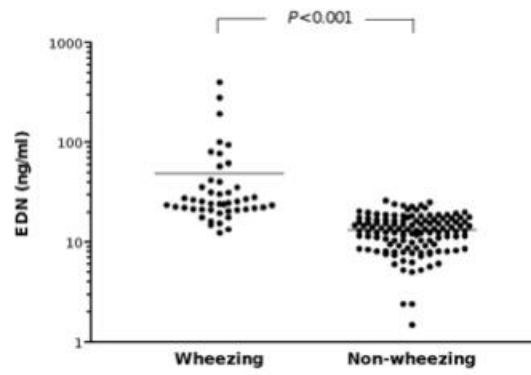


Figure 3. Comparison of serum EDN level between wheezing (n=46) and non-wheezing groups (n=125).

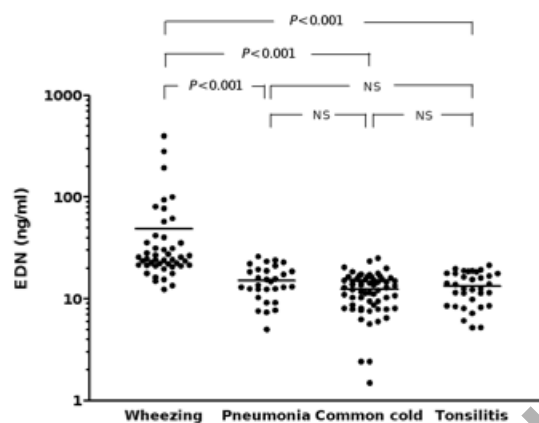


Figure 4. Comparison of serum EDN level among wheezing (n=46), pneumonia group (n=30), common cold group (n=61), and tonsillitis group (n=34).