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ORIGINAL ARTICLE

Fraction of exhaled nitric oxide and soluble receptors for advanced glycation end products are negatively correlated in children with recurrent wheezing

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Abstract

Background: The fraction of exhaled nitric oxide (FeNO) and serum levels of the soluble receptor for advanced glycation end products (sRAGE) have been suggested as biomarkers for asthma.

Objective: This study aimed to assess the correlation between FeNO and sRAGE serum levels in children <5 years old with recurrent wheezing.

Method: In total, 88 children with recurrent wheezing were divided into the high-risk group or low-risk group according to their clinical features. The high-risk group included 60 children, 42 male and 18 female, average age 36.7 months (range 32-48.7 months); the low-risk group included 28 children, 20 male and 8 female, average age 38.1 months (range 33-46.2 months).Asthma in high-risk children was treated with aerosol inhalation of Pulmicort respules 1 mg/d for four continuous weeks, while asthma in low-risk children was treated with symptomatic treatment. FeNO, serum sRAGE and eosinophils (EOS) were examined by ELISA and a regular blood cell analyzer.

Results: The serum sRAGE level was 738±191 and 992.4±210 pg/ml and the mean FeNO level was 27.3 and 17.6 ppm, respectively, in the asthma high-risk and low-risk group, showing significant differences between the two groups. In addition, FeNO and sRAGE serum levels were negatively correlated.After the inhalation of Pulmicort respules, FeNO decreased and sRAGE increased, while EOS showed no significant change.

Conclusions: FeNO and sRAGE serum levels are negatively correlated in children with recurrent wheezing.
Further larger scale studies are needed to test the use of FeNO and sRAGE as biomarkers for the prediction of asthma in children.

Key words: FeNO; sRAGE; eosinophil; children; asthma; biomarkers

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Introduction

Wheezing is a common symptom in infants and young children. An epidemiological survey showed that about 50% children have at least one episode of wheezing before the age of five years.[1] Although recurrent wheezing is the most common symptom indicative of asthma, not all children with wheezing are asthmatic. However, there is no gold standard for the diagnosis of asthma in children under five years of age. In the clinical setting, asthma is typically diagnosed by a history of chronic cough and wheezing, spirometric testing that illustrates airflow obstruction and subsequent reversibility following albuterol administration, as well as a response to anti-asthma treatments including inhaled corticosteroids.[2,3] It is important to develop a diagnostic test that can distinguish children who have or will develop asthma from those who have only transient

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symptoms and are more likely to respond to treatment with inhaled corticosteroids. For persistent wheeze in children younger than five years of age, a low dose of inhaled corticosteroids (ICS) is recommended as the preferred controller therapy, with leukotriene modifiers as an alternative.[4]

Airway inflammation is crucially linked with asthma.[5] The main methods to evaluate airway inflammation include sputum analysis, exhaled breath condensate, and the fraction of exhaled nitric oxide (FeNO). FeNO is a non-invasive, easy, and reproducible measurement of eosinophilic airway inflammation, and helps identify the phenotype of asthma.[6] The FeNO level has been shown to be useful in detecting eosinophilic airway inflammation, determining the likelihood of corticosteroid responsiveness, and revealing otherwise unsuspected non-adherence to corticosteroid therapy.[7]

The receptor for advanced glycation end products (RAGE), a multiple ligand receptor, belongs to the cell surface molecule immunoglobulin superfamily. Soluble RAGE (sRAGE), an isoform of RAGE lacking transmembrane and cytosolic domains, acts as a decoy receptor for RAGE ligands in the extracellular compartment and is believed to afford protection against inflammation and cell injury.[8] sRAGE plays an important role in pediatric respiratory diseases, and may be a novel biomarker of inflammation of the respiratory tract.[9]

The fraction of exhaled nitric oxide (FeNO) and serum levels of sRAGE have been suggested as the biomarkers for asthma. However, the correlation between FeNO and serum sRAGE levels in young children with recurrent wheezing is unclear. In this study, we aimed to assess the correlation of FeNO and serum sRAGE levels in children under 5 years of age with recurrent wheezing, and evaluate their potential to distinguish the children with high-risk of asthma.

Methods

Participants

A total of 88 children (age 1-4 years) with recurrent wheezing were recruited from the outpatient department of Huai'an First Hospital, Nanjing Medical University from September 2012 to August 2014. During their visit, their parents provided signed consent and the children's clinical data were collected by reviewing medical records and interviewing the parents. All cases met the criterion of having experienced three or more episodes of acute wheezing diagnosed by a physician. The children were evaluated based on a questionnaire for allergic factors such as eczema, allergic dermatitis, allergic rhinitis, food allergies, or whether their parents had a history of asthma. A total of 60 cases (including 42 male and 18 female subjects) who had any of the above allergic factors were divided into the high-risk asthma group, while the other 28 cases (including 20 male and 8 female subjects) who had none of these allergic factors were placed into the low-risk asthma group. There were no statistically significant differences in the age and gender of the two groups.

All participants were born at term. The exclusion criteria were congenital diseases (cystic fibrosis lung and bronchopulmonary dysplasia), cardiovascular diseases, systemic and local history of hormone application within two weeks, wheezing caused by inhaling foreign materials and autoimmune disease history. The participants who suffered from bronchitis and other respiratory diseases in the past two weeks were also excluded. Total eight children were excluded from the cohort. The study was approved by the ethics committee of the hospital.

Treatment

Pulmicort respules are the standard of care at our institution, and the children from the high-risk asthma group were treated with aerosol inhalation of Pulmicort respules (1 mg/day) for four continuous weeks, while low-risk asthma group received symptomatic treatment such as the use of anti-tussive agents.

Measurements of FeNO

FeNO was measured when the children with wheezing visited the doctor using a Nitric Oxide Monitoring System (NIOX), as described previously.[10] The children were not allowed to eat, drink or participate in heavy exercise before testing. The child's nose was clipped and air was fully expelled from the lungs, then the mouthpiece of the NIOX system was inserted and the child inhaled NO-free air calmly to total lung capacity within 2-3 sec. Then, the child exhaled steadily at a target flow rate of 50 ml/sec. The system automatically calculated the value during the last 3 sec of exhalation. Each child repeated the measurements at least three times to obtain three reliable FeNO values, defined as three values varying by <10% from each other. The means of the three values were then calculated. The children had a 2-min rest between each individual FeNO measurement. FeNO was measured at baseline for both groups and in patients in the high-risk group once again after approximately 4 weeks of Pulmicort inhalation therapy.

sRAGE serum levels

sRAGE serum levels were measured when the children with wheezing visited the doctor. Fasting peripheral venous blood was collected from each child early in the morning and centrifuged at 3000 rpm for 15 min to separate serum. The separated serum was stored at -80°C for subsequent analysis. The sRAGE level in serum samples was measured using a sandwich ELISA kit (R&D, Minneapolis, MN, USA). The intra-assay coefficient of variation was 6%. All samples were measured in triplicate. The sRAGE level was measured at baseline for both groups and only in patients in the high-risk group after approximately 4 weeks of Pulmicort inhalation therapy.

Blood eosinophil (EOS) counts

EOS counts were measured using an automated blood analyzer.

Statistical analysis

The chi-squared was used to analyze categorical data, and the Kruskal-Wallis test or Mann-Whitney U-test was applied to analyze continuous data. Measurement data with a normal distribution were expressed as mean±standard deviation. Correlations between FeNO, sRAGE and EOS were determined

Table 1. Characteristics of two groups

	Asthma high-risk group	Asthma low-risk group	*p*
N	60	28	
Male/Female (%)	42(70)/18(30)	20(71)/8(29)	0.937
Age (months)	36.7(32-48.7)	38.1(33-46.2)	0.726
Weight (kg)	14.56±6.53	14.85±4.87	0.828
Height (cm)	92.3(62-108.7)	89.5(65-102.3)	0.977
Wheezing episodes	3.85±0.94	3.94±1.08	0.676
FeNO (ppm)	27.3(19.8-87.4)	17.6(9.8-26.4)	0.012
EOS (%)	30	20	0.315
sRAGE (pg/mL)	738±191	992.4±210	<0.001

Table 2. FeNO, EOS, and sRAGE changes in high-risk asthma group before and after inhaling budenoside

	Before inhaling	After inhaling	t	*p*
FeNO (ppm)	26.38±14.73	22.23±8.85	2.84	0.006
EOS (%)	3.65±1.48	3.43±0.99	1.69	0.096
sRAGE (pg/mL)	886.55±78.76	949.4=18±54.57	-9.79	<0.001

by Spearman's rank correlation test. $p<0.05$ was accepted as statistically significant.

Results

We found no significant differences in the age, gender, height, weight, wheezing frequency and EOS between the two groups. Conversely, there were significant differences in FeNO and serum sRAGE levels between the two groups (Table 1).

For the correlation analysis, we found no correlation between FeNO and EOS (r=0.233; $p=0.303$, Figure 1). Additionally, sRAGE was not correlated with EOS (r=0.118, $p>0.05$, Figure 2). However, there was a significant negative correlation between serum sRAGE and FeNO (r=-0.766, $p<0.001$, Figure 3). In the high-risk asthma group, sRAGE levels increased significantly while FeNO decreased significantly after Pulmicort therapy, while EOS did not change significantly (Table 2).

Discussion

Children with wheezing are frequently diagnosed with asthmatic bronchitis, asthma, bronchitis, recurrent pneumonia, or recurrent capillary bronchitis.[11] The diagnosis of asthma is very important to employ effective treatment to alleviate the symptoms and prevent mortality. However, novel biomarkers for asthma are seldom reported.

FeNO is a recently developed non-invasive and specific method to measure airway inflammation. The FeNO level is closely related to airway inflammation and has the advantages of high repeatability and accuracy.[12,13] The use of

Figure 1. Spearman's rank correlation test showed that there was no correlation between FeNO and EOS in children with recurrent wheezing.

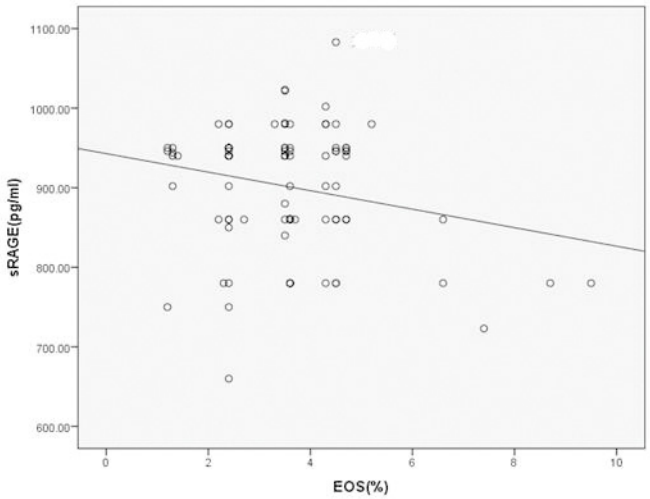


Figure 2. Spearman's rank correlation test showed that there was no correlation between sRAGE and EOS in children with recurrent wheezing.

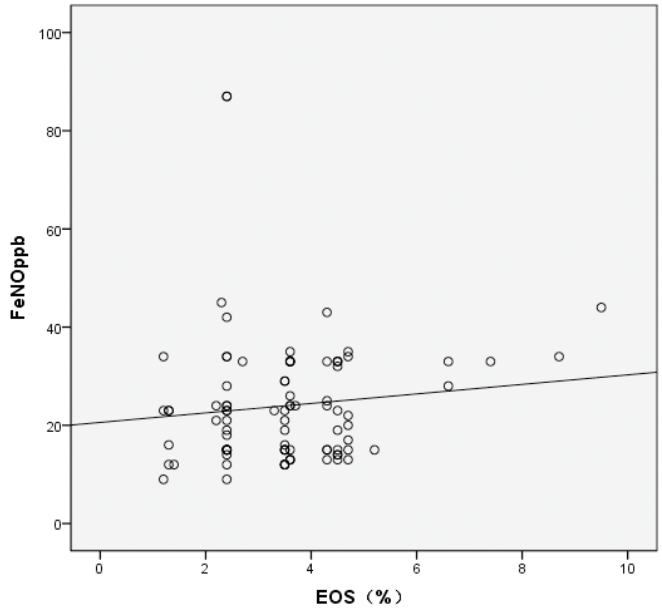
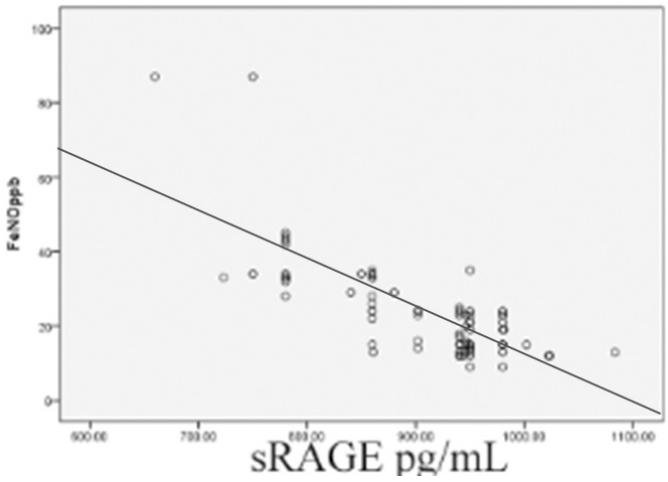


Figure 3. Spearman's rank correlation test showed that there was correlation between FeNO and sRAGE in children with recurrent wheezing.



anti-inflammatory drugs has been shown to reduce the FeNO level in children with wheeze.[14] In this study, we found that the FeNO level in these children decreased significantly after inhaled Pulmicort therapy, confirming that the FeNO level is closely related to airway inflammation. In addition to applications in diagnosis and guiding treatment for asthma in children, FeNO measurements can help with the differential diagnosis of other diseases such as cystic fibrosis (CF), primary ciliary dyskinesia (PCD) and chronic lung disease (CLD).[15-17]

FeNO levels are influenced by many factors, including the detection method, environmental factors such as smoking, and other comorbidities.[18] In addition, there are individual differences. Therefore, the value of the detection of FeNO is limited and it should be complemented with other indices for an accurate diagnosis of childhood asthma.

The RAGE/AGE system is involved in the pathogenesis of a variety of diseases, such as coronary heart disease, atherosclerosis, hypertension, chronic obstructive pulmonary disease (COPD), heart failure, and hypercholesterolemia.[19] In children, serum levels of sRAGE have shown a negative correlation with obesity and complications related to obesity.[20] In particular, the sRAGE level is crucial in the diagnosis and treatment of respiratory diseases, and sRAGE can be regarded as a marker in COPD patients.[21] Elevated serum levels of sRAGE in children with bronchiolitis can be used as an inflammatory biomarker of bronchiolitis and/or lung injury.[22] sRAGE is closely related to the severity of bronchial asthma and may be a target for intervention.[23] Our previous study showed that sRAGE in alveolar lavage fluid is positively correlated with alveolar damage in newborn animals with oxygen-induced lung injury.[24]

In this study, we performed FeNO measurements in children under five years of age with recurrent wheezing. At the same time, we detected serum levels of sRAGE and EOS counts in these children. We found that FeNO levels were significantly increased, while serum levels of sRAGE significantly reduced in children with persistent wheezing. Moreover, we found a significant negative correlation between FeNO and serum sRAGE levels. Notably, FeNO decreased while serum sRAGE levels increased after inhaling corticosteroids, again exhibiting a negative correlation.

As discussed above, FeNO levels are influenced by many factors, and the potential benefit of FeNO monitoring is equivocal.[25] To our knowledge, this is the first study to reveal a negative correlation between the FeNO level and sRAGE serum level in children with recurrent wheezing. These data indicate that the combination of FeNO and the sRAGE serum level may provide a better diagnosis of asthma in children. Compared to conventional diagnostic methods for asthma, the combination of FeNO and sRAGE as biomarkers for asthma avoids invasive procedures, and could accurately evaluate the progression of asthma because the levels of FeNO and sRAGE are correlated with the development of asthma.

Taken together, our results suggest that FeNO and serum sRAGE levels are negatively correlated in children with recurrent wheezing. However, this was a single center study, the sample size was relatively small, and the stratification of the children into high-risk and low-risk groups was not validated. Therefore, it is necessary to carry out large-scale clinical multi-center studies to test the use of FeNO and sRAGE as biomarkers for the prediction of asthma in children.

Conflict of interest

None declared.

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References

1. Brand PL, Baraldi E, Bisgaard H, Boner AL, Castro-Rodriguez JA, Custovic A, et al. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. Eur Respir J. 2008;32:1096-110.
2. Boonsawat W, Boonsawat W, Thinkhamrop B. Evaluation of asthma control by inhaled corticosteroids in general practice in Thailand. Asian Pac J Allergy Immunol. 2015;33:21-5.
3. Boonsawat W, Thompson PJ, Zaeoui U, Samosorn C, Acar G, Faruqi R, Poonnoi P. Survey of asthma management in Thailand - the asthma insight and management study. Asian Pac J Allergy Immunol. 2015;33:14-20.
4. Papi A, Nicolini G, Boner AL, Baraldi E, Cutrera R, Fabbri LM, et al. Short term efficacy of nebulized beclomethasone in mild-to-moderate wheezing episodes in preschool children. Ital J Pediatr. 2011; 37:39
5. Cheng S, Chen H, Wang A, Xie M, Xie J, Osanai K, Zhao J, Xu Y, Xiong W, Zhou M. Lentiviral vector-mediated delivery of lysophosphatidylcholine acyltransferase 1 attenuates airway inflammation in ovalbumin-induced allergic asthmatic mice. Asian Pac J Allergy Immunol. 2015;33:320-9.
6. Oh MA, Shim JY, Jung YH, Seo JH, Young Kim H, Kwon JW, et al. Fraction of exhaled nitric oxide and wheezing phenotypes in preschool children. Pediatr Pulmonol. 2013;48:563-70.
7. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS: clinical practice guideline interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med. 2011;184:602-15.
8. Zhang L, Postina R, Wang Y. Ectodomain shedding of the receptor for advanced glycation end products: a novel therapeutic target for Alzheimer's disease. Cell Mol Life Sci. 2009;66:3923-35.
9. Guo WA, Knight PR, Raghavendran K. The receptor for advanced glycation end products and acute lung injury/acute respiratory distress syndrome. Intensive Care Med. 2012;38:1588-98
10. Zhang H, Shu L, Cai X, Wang Z, Jiao X, Liu F et al. Gender and age affect the levels of exhaled nitric oxide in healthy children. Exp Ther Med. 2013;5:1174-1178.
11. Amado MC, Portnoy JM.Diagnosing asthma in young children. Curr Opin Allergy Clin Immunol. 2006;6:101-5.
12. Nakajima N, Mochizuki H, Muramatsu R, Hagiwara S, Mizuno T, Arakawa H. Relationship between exhaled nitric oxide and small airway lung function in normal and asthmatic children. Allergol Int. 2011;60:53-9
13. Bastain TM, Islam T,Berhane KT, McConnell RS,Rappaport EB, Salam MT,et al. Exhaled nitric oxide,susceptibility and new-onset asthma in the Children's Health Study. Eur Respir J. 2011;37:523-31
14. Moeller A, Franklin P, Hall GL, Turner S, Straub D, Wildhaber JH,et al. Inhaled fluticasone dipropionate decreases levels of nitric oxide in recurrently wheezy infants. Pediatr Pulmonol 2004; 38: 250–255
15. Keen C, Olin AC, Edentoft A, Gronowitz E, Strandvik B. Airway nitric oxide in patients with cystic fibrosis is associated with pancreatic function, Pseudomonas infection, and polyunsaturated fatty acids. Chest. 2007;131:1857-64.
16. Zihlif N, Paraskakis E, Lex C, Van de Pohl LA, Bush A.Correlation between cough frequency and airway inflammation in children with primary ciliary dyskinesia. Pediatr Pulmonol. 2005;39:551-7.
17. Williams O, Dimitriou G, Hannam S, Rafferty GF, Greenough A. Lung function and exhaled nitric oxide levels in infants developing chronic lung disease. Pediatr Pulmonol. 2007;42:107-13.

18. Ferrante G, Malizia V, Antona R, Corsello G, La Grutta S. The value of FeNO measurement in childhood asthma: uncertainties and perspectives. Multidiscip Respir Med 2013;8:50.
19. Prasad K. Low levels of serum soluble receptors for advanced glycation end products, biomarkers for disease state: myth or reality. Int J Angiol. 2014;23:11-6.
20. Accacha S, Rosenfeld W, Jacobson A, Michel L, Schnurr FJ, Shelov S, et al. Plasma advanced glycation end products (AGEs), receptors for AGEs and their correlation with inflammatory markers in middle school-age children. Horm Res Paediatr. 2013;80:318-27.
21. Gopal P, Rutten EP, Dentener MA, Wouters EF, Reynaert NL. Decreased plasma sRAGE levels in COPD: influence of oxygen therapy. Eur J Clin Invest. 2012;42:807-14.
22. García-Salido A,Oñoro G,Melen GJ, Gómez-Piña V, Serrano-González A, Ramírez-Orellana M, et al. Serum sRAGE as a Potential Biomarker for Pediatric Bronchiolitis: A Pilot Study. Lung. 2015;193:19-23.
23. El-Seify MY, Fouda EM, Nabih ES. Serum level of soluble receptor for advanced glycation end products in asthmatic children and its correlation to severity and pulmonary functions. Clin Lab. 2014;60:957-62.
24. Tian Z, Li Y, Ji P, Zhao S, Cheng H. Mesenchymal stem cells protects hyperoxia-induced lung injury in newborn rats via inhibiting receptor for advanced glycation end-products/nuclear factor κB signaling. Exp Biol Med. 2013;238:242-7.
25. Gomersal T, Harnan S, Essat M, Tappenden P, Wong R, Lawson R, Pavord I, Everard ML. A systematic review of fractional exhaled nitric oxide in the routine management of childhood asthma. Pediatr Pulmonol. 2016;51:316-28.