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Effect of Age and Eosinophil Number on Fractional Exhaled Nitric Oxide Level in Non-Asthmatic Children in Shanghai

Wei Liu[1#], Jizhi Chu[2#], Li Sun[3#], Zhiqin Shen[1], Yan Liu[1], Qing Peng[4], and Xiwen Gao[4]

[1]Medicine,Minhang Gumei Community Health Center, Shanghai 201102, China
[2] Gumei Community Health Center, Minhang, Shanghai 201102, China
[3] Laboratory of the second people's Hospital of Jiaxing 314000, Zhejiang Province, China
[4] Department of Respiratory Diseases, Minhang District Central Hospital, Shanghai 201199, China

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ABSTRACT

This study aimed to identify the relationship between fractional exhaled nitric oxide (FeNO) level and potential factors in non-asthmatic children from Shanghai, China.

From March to April 2012, the school-aged children fulfilling the inclusion criteria were recruited. The FeNO levels of non-asthmatic children were detected by the Nano Coulomb nitric oxide analyzer. Questionnaires were recorded, including personal data, family illness history and daily habits. In addition, not only the number of leukocytes and eosinophils but also the level of hemoglobin in peripheral blood, were measured via the automated blood cell analyzer. All data were statistically analyzed with SPSS version 17.0 software and the correlation of these potential factors with FeNO level was calculated via Kendall's rank correlation.

A total of 132 healthy children (aging 6-13 years) were enrolled in Minhang District, Shanghai, China. The mean value of FeNO level was 15.05 ppb. The correlation analyses revealed that age (R=0.190, $p=0.029$) and eosinophil number (R=0.575, p=0.000) were significantly and positively correlated with FeNO levels. The FeNO levels of individuals aged 10-13 years was significantly higher than those of the individuals aged 6-9 years (22.65± 18.82 ppb vs. 15.28 ± 9.78 ppb, p<0.05). However, other potential factors were not significantly correlated with FeNO level.

The FeNO levels in healthy school-aged children may reflect airway eosinophilic inflammation levels, and was affected by eosinophil count and age significantly.

Keywords: Asthma; Airway inflammation; Eosinophils; Fractional exhaled nitric oxide

Corresponding Author: Xiwen Gao, PhD;
Department of Respiratory Diseases, Minhang District Central Hospital, NO.170 Xinsong Road, Xinzhuang Town, Minhang District, Shanghai 201199, China. Tel: (+86 21) 6492 3400, Fax: (+86 21) 6492 3400, E-mail: xiwengao001@hotmail.com
#equal contribution according to corresponding author´s request

INTRODUCTION

Asthma, an acute or chronic inflammatory disease of the airways, was characterized by variable and recurring symptoms, reversible airflow obstruction, and

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bronchospasm.[1,2] Asthma was caused by a combination of genetic and environmental factors and its incidence was increased year by year since the 1970s.[3] Fractional exhaled nitric oxide (FeNO) was proposed as a biomarker for airway inflammation in asthma as well as other respiratory diseases.[4,5] The availability of nitric oxide (NO) analyzers and the publication of official guidelines made the measurement of FeNO a useful tool in the diagnosis and monitoring treatment of various pediatric airway disorders.[6,7]

Currently, FeNO level could be measured either online or offline via non-invasive technique. Several methods were used to measure FeNO in children and the choice was dependent on their age and cooperation.[8,9] Online measurement allowed FeNO testing with a real-time display of NO breath profiles, which is available in stationary individuals only.[10] The single breath online measurement was the gold standard in school-aged children, because it was easy to perform, reproducible and acceptable by children.[11] The children were asked to inhale to near total lung capacity and to exhale at a constant flow of 50 mL/s until NO level was identified.[9] For the non-asthmatic children in certain regions, the normal FeNO level was needed to detect, which was essential for the application of FeNO measurement on everyday clinical diagnoses.[7] Many relevant reference values have been reported in the past decades.[8,9]

Currently, FeNO values was detected in healthy children aged 4-18 years from the Italy,[12] Canada,[13] America,[4] Hong Kong,[14] Taiwan[15] and China (Shenyang).[5] Most of the studies revealed comparable results. In different references, FeNO level was varied between countries. The human species was one of the most important variables[14] and the FeNO level of children from western countries could not be directly applied to Asian individuals.[5,8,14] For example, some studies showed that gender and age affected the normal FeNO level while this was not observed in other studies.[13,16] However, some studies have shown different FeNO levels among Asians, which might be related to regional difference and sample characteristic. There were FeNO reference values for the northern areas of China, but no reports about other areas.[5] To perfect the reference values of FeNO level in Chinese individuals, it was necessary for us to supplement the FeNO reference values in Shanghai, China.

In the present study, healthy school-aged children in Minhang District, Shanghai, were recruited and their normal FeNO levels were measured. The correlation between FeNO level and potential factors were analyzed and calculated, providing the basis for the diagnosis and monitoring of airway disorders.

MATERIALS AND METHODS

Study Subjects

From March to April 2012, the schoolchildren, 6-13 years old, were recruited in Minhang District, Shanghai, China. The inclusion criteria were listed as follows: no reported history of asthma; no oral or inhaled corticosteroid therapy in the last month; no acute infection or fever one week prior to study; no eating or strenuous exercise one hour before testing.

Our study was approved by the local Education Bureau and by the department of Institutional Human/Animal Care and Use Committee of Minhang District Central Hospital, Shanghai, China. Written informed consent was provided by parents for the consent of the underage children.

Measurement of FeNO

Single-breath online measurements of FeNO were taken by the YZB/Su 0034-2010 Nano Coulomb Nitric Oxide Analyzer (ShangWo Biotech Co. Ltd., Wuxi, China) based on its guidelines, which were consistent with the American Thoracic Society (ATS)/European Respiratory Society (ERS) Recommendations for Standardized Procedures for the Online and Offline Measurement of Exhaled Lower Respiratory Nitric Oxide and Nasal Nitric Oxide, 2005.[6]

All children did not eat, drink or exercise heavily in the morning before testing. Children were relaxed and standing quietly during test. Every child breathed according to the instructions of technical personnel. If the data were not available after testing six times, the measurement was rejected. For every child, the measurements were repeated at least 3 times and the data of FeNO values were averaged. Each measurement consisted 10 min testing and 2 min rest.

Measurement of General Information

All children completed a questionnaire, including age, gender, height, weight, body mass index (BMI), medical history (such as asthma, allergic rhinitis and fever in the last two weeks), family history of asthma, smoking behavior of parents and drug use in the last month.

Table 1. General information of enrolled non-asthmatic children

Parameter	Min	Max	Statistics (x ± s)
Number			132
Gender (male/female)			70/62
Age (years)	6	13	9.92 ± 2.67
Weight (kg)	15.7	74.1	36.84 ± 13.05
Height (cm)	98.4	171.4	140.50 ± 19.62
BMI (kg/m^2)	13.54	29.07	18.02 ± 3.05
Parental smoking history (yes/no)			22/110
Family history of asthma (yes/no)			16/116
Leukocytes (×10^9/L)	4.1	11.4	7.37 ± 1.78
Eosinophils (×10^9/L)	0.02	0.91	0.35 ± 0.26
Hemoglobin (g/L)	109	142	122.83 ± 7.77

BMI: body mass index. x ± s: mean ± standard deviation.

Measurement of Blood Cells

The blood was drawn from each child by using anticoagulant tubes and was analyzed via a Sysmex XE-2100 automated hematology analyzer (Sysmex, Kobe, Japan). Not only the number of leukocytes and eosinophils, but also the level of hemoglobin in peripheral blood, were measured.

Statistical Analysis

All data were statistically analyzed with SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Information of numeric variables were presented as mean ± standard deviation (x ± s). The t-test was used for comparison between groups. In order to identify the correlation between FeNO and factors, variables were analyzed using Pearson correlation analysis and R values were calculated using Kendall's rank correlation. $P<0.05$ was accepted as a statistically significant difference.

RESULTS

A total of 132 non-asthmatic children fulfilling the inclusion criteria, with an average age of 9.92 ± 2.67 (6-13) years were enrolled. The general information of these enrolled children are shown in Table 1. For 70 boys/62 girls, the mean value of FeNO was found to be these enrolled children are shown in Table 1. For 70 boys/62 girls, the mean value of FeNO was found to be 15.05 ppb (95% confidence interval (CI): 13.1-17.2 ppb).

Furthermore, the correlation analyses revealed that age had significantly positive correlation with FeNO levels (R = 0.190, p=0.029, Table 2). However, it was also interesting to find that 9 years old was a turning point of FeNO levels. For 45 individuals aged 6-9 years, the FeNO level was 15.28 ± 9.78 ppb, while for the rest 87 individuals aged 10-13 years, the FeNO level was 22.65 ± 18.82 ppb. There was a significant difference in FeNO levels between these two groups (p=0.032). There was also a significant positive correlation between the number of eosinophils and FeNO level (R = 0.575, p=0.000, Table 2).

Table 2. Correlation analysis of FeNO and potential factors

Correlation	R-value	P-value
Gender	-0.096	0.179
Age	0.190$^{\#}$	0.029
Weight	0.131	0.134
Height	0.166	0.057
BMI	0.026	0.769
Leukocytes	0.170	0.052
Eosinophils	0.575$^{\#\#}$	0.000
Hemoglobin	0.077	0.379
Smoking of parents	0.022	0.760
Family history of asthma	0.119	0.098

$^{\#}$: $p<0.05$. $^{\#\#}$: $p<0.01$. BMI: body mass index.

However, there was no significant correlation between FeNO levels and other potential factors (Table 2), including gender (R=-0.096, $p>0.05$), weight (R=0.131, $p>0.05$), height (R=0.166, $p > 0.05$), BMI (R=0.026, $p>0.05$), leukocytes number (R=0.170, $p>0.05$), hemoglobin level (R=0.077, $p>0.05$), smoking of parents (R=0.022, $p>0.05$) and family history of asthma (R = 0.119, $p>0.05$).

DISCUSSION

Since Alving, et al. reported firstly that the FeNO level in asthma patients was significantly higher than that in normal subjects,[17] measurement and clinical application of FeNO in airway inflammation have become a research hotspot.[6] Recently, FeNO level has been used as a useful biomarker of airway inflammation.[18,19] Therefore, it is important to analyze all potential and actual factors that might affect FeNO level.

In the present study, the mean value of FeNO level of non-asthmatic children in Minhang District was 15.05 ppb (95 % CI: 13.1-17.2 ppb) and FeNO level was increased along with age, which was consistent with many researches of healthy school children confirming that age is positively correlated with FeNO level.[12,15,20] Taylor et al.[21] have proposed that the range of normal FeNO level was 5-9 ppb, the slightly high level was 20-34 ppb and the severe high level was over 35 ppb. For children of the yellow race, Wong et al.[14] have recommended that the mean FeNO level of males was 17.0 ppb (range, 10.7-36.6 ppb) and that of females was 9.1 ppb (range, 7.8-17.6 ppb). Meanwhile, we found that the FeNO levels of individuals aged 10-13 years was significantly higher than those of the individuals aged 6-9 years. Similar results have been reported in the study of Linn et al. where FeNO was found to be significantly higher in subjects aged over 9 years than younger subjects.[22] The correlation between FeNO level and gender is still controversial. Several studies have verified that FeNO level of males is significantly higher than that of females.[23,24] Meanwhile, no correlation between FeNO level and gender has been identified in other reports as well as our study.[12,25-27] These differences might be due to the genetic, regional difference and the sample size. To establish the normal reference value of FeNO level for Chinese children, a larger sample size is needed for future study.

In addition, the number of eosinophils in peripheral blood was significantly and positively associated with FeNO level in healthy school-aged children. Berry et al. have reported that a significant positive relationship was found between the FeNO level and sputum eosinophil count.[28] Furthermore, FeNO levels in atopic–eosinophilic subjects were about two-fold higher than those in atopic subjects with low blood eosinophils as well as that in non-atopic subjects with high or low blood eosinophils.[29] Therefore, the result of our study is consistent with the above previous researches to some extent. Other factors, such as individual information (gender, weight, height and BMI), leukocyte counts, hemoglobin level and parents behavior (smoking and asthma), could not statistically affect the FeNO level.

In conclusion, we found that age and the number of eosinophils in peripheral blood were significantly and positively correlated with FeNO level of healthy school-aged children in Minhang District, Shanghai. It will provide the basis for diagnosis and monitoring of airway disorders in the future based on the studies of FeNO level with a larger sample size from different countries and areas.

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