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Exhaled Nitric Oxide and Aeroallergen Sensitization in Asthmatic Children

Azot monoksid u izdahnutom vazduhu i alergijska senzibilizacija kod dece sa astmom

Snežana Živanović[1,2], Ljiljana Šaranac[1,2], Bojko Bjelaković[1,2], Slobodanka Petrović[3] and Zorica Živković[4,5]

[1]Clinic of Paediatrics, Clinical Center Nis, Serbia
[2]Medical Faculty, University of Nis, Serbia
[3]Institute for Child and Youth Health Care of Vojvodina, Paediatric Clinic, Department of Pulmonology, Novi Sad, Serbia
[4]Children's Hospital for Lung Diseases and Tuberculosis, Medical Center "Dr Dragiša Mišović", Belgrade, Serbia
[5]Faculty of Pharmacy Novi Sad, Serbia

Summary

Introduction: The examination of nitric oxide in exhaled air concentration in children suffering from asthma and to establish the relation with the degree of sensitization to aeroallergens.

Material and Methods: The examination included fifty-two children (aged 12.40 ± 2.35 years), twenty-eight male (53.85%) and twenty-four female (46.15%), with the average length of suffering from asthma 8.33 ± 3.93 years. The degree of sensitization to aeroallergens was determined by skin prick testing and assessed using the atopic index (AI).

Results: The average value of FeNO in exhaled air of children suffering from stable allergic asthma was 43.92 ± 35.63 ppb, and after a four week anti-inflammatory treatment it decreased to 34.92 ± 32.04 ppb ($p<0.05$). In relation to AI, the level of FeNO in exaled air was 41.00 vs. 40.69 vs 50.88 ppb, in the given order without statistically significant difference. The highest values of FeNO in exhaled air were present in children suffering from a mixed type of sensitisation, 56.85 ppb (Me 48.50) in comparison to sensitisation to seasonal allergens 15.29 ppb (Me 12) and indoor allergens 32.22 ppb (Me 26). Allergic rhinitis, the duration of asthma and the gender were not significantly related to the values of FeNO in exhaled breath, while significant was the negative correlation between the body mass index and FeNO, $r = -0,43$ ($p<0.01$).

Conclusion: Children suffering from allergic asthma possess increased values of nitric oxide in exhaled air, which is a useful indicator of daily dosage adjustment in patients treated with anti-inflammatory drugs

Key words: asthma, children, nitric oxide, senzitisation

Sažetak

Uvod: Cilj našeg ispitivanja je bio merenje azot monokisida u izdahnutom vazduhu (FeNO) kod dece sa astmom i korelacija sa stepenom senzibilizacije na aeroalergene.

Metodologija: U ispitivanje je uključeno 52 dece (uzrast od 12.40 ± 2.35 godina), 28 dečaka (53.85%) i 24 devojčica (46.15%). Prosečna dužina trajanja astme je bila 8.33 ± 3.93 godina.Stepen senzibilizacije na aeroalergene određivan je kožnim testom i procenjivan atopijskim indeksom (AI).

Rezultati: Prosečna vrednost FeNO kod dece sa stabilnom alergijskom astmom je bio 43.92 ± 35.63 ppb, a nakon 4 nedelje anitinflamatorne terapije, vrednost FeNO se smanjila na 34.92 ± 32.04 ppb ($p<0.05$). U odnosu na AI, vrednosti FeNO su bile 41.00 vs. 40.69 vs 50.88 ppb, bez statisičke značajnosti. Najviša vrednost FeNO izmerena je kod dece sa polisenzibilizacijom, 56.85 ppb (Me 48.50), dok su kod dece senzibilisane samo na sezonske alergene vrednosti FeNO bile 15.29 ppb (Me 12) i kod dece senzibilisane na alergene unutrašnje sredine FeNO vrednosti su bile 32.22 ppb (Me 26). Alergijski rinitis, dužina trajanja astme i pol ispitanika nisu bili značajno povezani sa izmerenim vrednostima FeNO. Značajna negativna korelacija je utvrđena između indeksa telesne mase (BMI) i FeNO, $r = -0,43$ ($p<0.01$).

Zakljucak: Deca koja boluju od alergijske astme imaju povišene vrednosti FeNO, što je koristan indikator odgovora na antiinflamatornu terapiju

Ključne reči: astma, deca, azot monoksid, senzibilizacija

Introduction

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation (1).Many phenotypes have been identified: allergic asthma, non allergic asthma, late-onset asthma, asthma with fixed airflow limitation and asthma with obesity (1). The level of exhaled nitric oxide fraction (FeNO) is elevated in patients with asthma and FeNO may be involved in airway inflammation. Because NO is generated from L-arginine by various cells in the airway including airway and alveolar epithelial cells, vascular endothelial cells, smoth muscle cells, and alveolar macrophages in consequence of

the inflammatory process, the concentration of exhaled NO is proposed to be a non-invasive and facile test or marker to assess eosinophilic airway inflammation in asthma, even in children. Exposure to allergen in sensitized individuals may contribute to airway inflammation. FeNO measurements may provide information on pathological processes, and response to treatment, within the distal lung (2). Studies on children have suggested that levels of FeNO are higher in patients with atopic asthma compared with levels in patients with non-atopic asthma and atopic patients without asthma.The aim of this study was to examin nitric oxide in exhaled air in children suffering from asthma and to establish the relation with the degree of sensitization to aeroallergens.

Material and Methods:

The examination included fifty-two children (aged 12.40 ± 2.35 years), twenty-eight male (53.85%) and twenty-four female (46.15%), with the average duration of asthma 8.33 ± 3.93 years. Inclusion criteria were:children and adolescents with asthma from 7 to 18 years , without signs and symptoms of acute infection one month before the test and the stable phase of the disease. Exclusion criteria were:age less than 7 and more than 18 years, acute exacerbations of asthma, acute viral infection and other chronic diseases: cystic fibrosis, bronchopulmonary dysplasia, primary ciliar dyskinesia.

Pulmonary Function testing

Lung function was measured by baseline spirometry with spirometer Spirovit SP1 (Schiller).

FeNO Measurements

FeNO levels were measured according to the ATS / ERS guidelines by using the NIOX NO monitoring system (Niox mino, Aerocrine AB, Solna, Sweeden) before spirometry tests, so that parameters were within the limits specified by the ATS guidelines. FeNO was measured online with an expiratory flow of 50 ml/s and subjects exhaled against resistance to prevent upper airway contamination.

Allergy Test procedure

Allergic sensitization was determined in all subjects by skin prick tests (SPTs) on common aeroallergens: grass pollen mix, tree pollen mix, wed pollen mix, dust mite mix, house dust mite (*Dermatophagoides pteronyssinus*), cat and dog epithelia, mold mix (Institute for Virusology, Vaccine and Serum, Torlak, Serbia). Histamine and physiological saline were positive and negative controls respectively. A wheal diameter of 3 mm or greater than the negative saline control was considered as a positive result, and sensitization was confirmed. SPT response were converted into an atopic index (0: negative to all aeroallergens, 1: positive to 1-2 aeroallergens, 2: positive to 3-4 aeroallergens, 3: positive to more than 5 aeroallergens).

Statistical Analysis

Data were analyzed using the statistical package for social sciences version 10.0 for Windows (SPSS, Inc., Chicago, IL). Categorical variables are expressed as number of items and percentage. Contiunuous variables are expressed as the mean ± standard deviation (and median). Data were tested for normality (Shapiro Wilk test). Comparison within groups was done using paired t test and Wilcoxon Signed Ranks Test. Comparison between two groups was done using the unpaired t test or Mann-Whitney U test. A p value of <0.05 was considered statistically significant.

Results

Clinical Characteristic of Patients

The total study population consisted of 52 children (28 male, mean age 12,06±2,40 years, and 24 female, mean age 12,79±2,27 years) with intermittent and mild-to-moderate persistent asthma. Among them, 34 (69.38%) also had a diagnose of allergic rhinitis. Mean duration of the diseasewas 8,33 ±3,93years.There were no differences between regarding age, sex, duration of the disease, atopic status and lung function at any time, except for FeNO before and after treatment (Table 1).

Parameters	Total
Number of patients (N)	52
Age (years)	12.40±2.35
Duration of the disease (years)	8.33±3,93years
Sex: Male / Female	28 (53.85%) / 24 (46.15%)
Body mass index-percentile	55,31±32.19
Allergic rhinitis	34 (69.38%)
FeNO 1 total (ppb) before treatment	43,92±35,63
FeNO 2 total (ppb) after treatment*	34,92±32,04*
Sex	
Male/	44,43±40,68 vs. 36,25±37,57
Female (FeNO 1 and FeNO 2)	43,33±29,52 vs. 33,38±24,82
Treatment and FeNO (ppb)	
FeNO 1 :Without therapy/ICS	43,70±29,09 ; 95CI 32,84-54,56 ***vs***. 44,23±43,74; 95CI 24,83-63,62
FeNO 2: LTRA/ LTRA+ ICS	33,70±24,58; 95CI 24,52-42,88 ***vs***. 36,59±40,68; 95CI 18,56-54,63
Atopic index N° (%)	
1 / 2 / 3	26 (50.00%) / 16(30,77%) / 10(19.23%)
FEV1 % predict	91.10±13.93

**p<0,05*

Table 1.Clinical characteristics of patients

Average values of FeNO levels were increased in all subjects. FeNO levels were significantly reduced after the antiinflammatory therapy (p<0,05), while no significant difference was obtained in relation to gender. Although the value of FeNO levels decreased after three months of treatment, the difference was not statistically significant.

Table 2.FeNO (ppb) in relation to atopic index

Atopic index -AI	N	%	FeNO1				FeNO2
			X	±SD	Me	X	±SD
Negative - **AI 0**	1	1,85%					
Positive up to 2 allergens- **AI 1**	27	50,00%	41,00	±29,86	32,00	34,07	±23,78
Positive up to 4 allergens- **AI 2**	16	29,63%	40,69	±38,79	30,50	36,88	±46,29
Positive up to/or more then 5 allergenes- **AI 3**	10	18,52%	50,88	±42,35	38,00	37,25	±25,87

** - p<0.01; FeNO1 (before the treatment), FeNO2 (after the treatment)

The levels of FeNO were higher in children with the higher atopic index (polysensitisation).

Table 3.FeNO and indoor allergens (dust mite mix, house dust mite, cat and dog epithelia, mold)

SPTs	N	NO 1			NO 2		
		X	± SD	Me	X	± SD	Me
Indoor allergens +	44	a*46,77	± 34,92	38,00	a*b*38,09	± 33,63	30,50
Indor allergens -	8	28,25	± 37,74	14	17,50	± 11,08	14,00

*a - positive vs negative b ; FeNO2 vs NO1, *- p<0,05*

The levels of FeNO were higher in children with indoor aeroallergens senzitisation compared with children without senzitisation (p <0.05, Mann-Whitney test).

The level of FeNO were reduced significantly after the antiinflammatory therapy in children sensitized to indoor aeroallergens (p <0.05; Wilcoxon Signed Rank test).

Table 4.FeNO (ppb) and sensitisation on aeroallergens

SPTs	N	FeNO1			FeNO2		
		X	± SD	Me	X	± SD	Me
Negative test	1						
Seasonal allergens +	7	15,29	± 9,66	12,00	18,86	± 11,23	14,00
Perennial allergens +	18	32,22	± 23,30	26,00	23,44	± 13,56	23,00
Mixed type of sensitisation	26	56,85	± 38,35	48,50	48,23	± 39,48	43,00

The value of FeNO were increased in children with the mixed type of sensitization. There was no significant difference in relation to the type of sensitization and first and second FeNO measuring.

Body mass index and FeNO

Median value of BMI-P was 55.31±32.19. The negative correlation between FeNO and BMI-P was statistically significant (r = -0,43; *p<0.01*).

The negative correlation between FeNO and BMI-P after the treatment still remains significant (r= - 0.28; *p<0.05*).

Discussion

The aim of this study was to examin the values of nitric oxide in exhaled air in children suffering from asthma and to establish the relation with the degree of sensitization to aeroallergens. 52 children were tested, of which 34 had allergic rhinitis in addition to asthma. Only 1 child was non-atopic, while all the rest had positive skin prick tests on aeroallergens and the diagnosis of allergic asthma (intermittent and stable mild persistent asthma). All patients with allergic asthma have elevated levels of NO in exhaled air. No correlation was found between FeNO level and age, gender, weight or BMI. In our study, children senzitised on aeroallergens had elevated levels of FeNO, especially children senzitized on indoor allergens in comparison with the levels of FeNO in children who were not sensitized. The difference was statistically significant. This phenomenon is due to the expectation that children spend more time indoors during the year. Other authors found no difference in the FeNO levels between mono- and polysensitized allergic asthmatic, indicating that the number of allergens had no effect on NO exhalation (3,4). Concentration of RAST values or severity of reaction of the skin prick tests had not been investigated. The highest levels of FeNO were seen in subjects with both atopy and asthma. Scott M at all. found that the FeNO values were positively associated with increased atopic index as evidenced by increased FeNO together with increased skin prick testing positivity, as well as with increased severity of atopic asthma evidenced by the number of attacks of wheezing. FeNO and current inhaled corticosteroid use were not significantly associated. (5) In asthmatic patients, the atopic phenotype is caracterised by significant relationship seen between FeNO and frequency of wheeze. FeNO values in non-atopic asthmatic patients is not significantly related to wheezing frequency, which is an important finding since nearly half of the patients with asthma are non-atopic (6).In our study, only one patient had non-atopic asthma and we were not able to make a comparison of the level of FeNO and atopic or non-atopic asthmatic subjects. This is the biggest disadvantage of our study.

Indicator of asthma severity and the amount of medication the patients receive, did not correlate with the FeNO levels (5). We obtained similar results in our study. Several study have examined the response of FeNO to inhaled corticosteroids (ICS). Willson et al. demonstrated a rapid fall in FeNO after 4 weeks of ciclsonide therapy, followed by an increase following drug washout (7). The ability of FeNO to predict a response to corticosteroid treatment in asthma and other airways disease has been assessed. The most compelling study demonstrated that patients with a high FeNO (>47 ppb at 250 mL·s -1) responded best to ICS, in terms of lung function and improvement in airways hyperresponsiveness. The improvement occurred in patients with a high FeNO and was irrespective of the underlying airways diagnosis (8). Other studies have shown similar results in paediatric populations (9,10). A small study of 26 children examined the FeNO levels change with or without montelukast compared to a control group receiving placebo. FeNO levels decreased when treatment was started, and increased when treatment was discontinued (11). Fritscher et al. found that montelukast added to fluticasone gained a small decrease in alveolar NO, suggesting a change in small airway inflammation (12). Similar results have been described in preschool children (13). In our study, the addition of montelukast to inhaled corticosteroids did not affect significantly the FeNO level.

The main goal of asthma treatment is the prevention of asthma exacerbations, using the lowest dose of corticosteroid. This approach relies on predicting and targeting asthma exacerbations accurately. FeNO is a reasonably good marker of eosinophilic inflammation which

has been shown to predict preventable asthma exacerbations. FeNO is helpful in guiding ICS therapy in patients with asthma. However, in order to replace the peak flow measurements or symptom score management plans, FeNO has two important points: first, normal ranges of FeNO values affected by the age, height and sex need to be established and secondly, the affect of other confounding variables, including atopy, need to be clarified (14).

Conclusions

In conclusion, our results confirmed that children with allergic asthma have increased values of nitric oxide in exhaled air, which is a useful indicator of daily dosage of anti-inflammatory drugs. FeNO has an additional advantage for patient care detecting the eosinophilic airway inflammation, determining the likelihood of corticosteroid responsiveness, monitoring of airway inflammation and unmasking of otherwise unsuspected nonadherence to corticosteroid therapy (15,16).

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Correspondance to:
Snezana Zivanovic
Clinic of Paediatrics, Clinical Center Nis
Medical Faculty, University of Nis
Zorana Đinđica 81, 18000 Nis, Serbia
zsneza@yahoo.com