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## MEASUREMENT OF NITRIC OXIDE IN EXHALED AIR IN PRIMARY SCHOOL CHILDREN IN RUŽOMBEROK

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**Ключові слова:** оксид азоту, FeNO, забруднення повітря, еозинофільне запалення, діти, астма

**Ключевые слова:** оксид азота, FeNO, загрязнение воздуха, эозинофильное воспаление, дети, астма

**Abstract.** Measurement of nitric oxide in exhaled air in primary school children in Ružomberok. Czubaj-Kowal M., Friediger T., Hudáková Z., Sokolowski M., Walczak-Kapolka E., Aštaryová I., Lesňáková A. Nowadays, there are a number of respiratory illnesses that directly affect children. Exhaled nitric oxide measurement (FeNO) is one of the modern methods of investigation used for diagnosing and controlling allergic eosinophilic airway inflammation. It is a simple, painless and time-saving method for the patient. Because of its non-invasiveness and simplicity, it is a valuable diagnostic method, especially in younger children. Measurement of FeNO is a recognized biomarker and an accurate quantitative indicator in the detection, assessment and monitoring of airway inflammation, including asthma. However, only a few studies assess the relationship between FeNO and level of air pollution. The aim of our study is to determine the relationship between FeNO in third-grade students of primary schools and the level of air pollution in Ružomberok. 146 children aged 8-10 years were included in the research. This is the first stage of a wider research project involving FeNO measurements in children at different times of the year. The goal of the research is to determine the concentration of FeNO in the studied group of children in the autumn when air pollution is lower than in second stage which is observed in winter. The study showed elevated values of FeNO > 20 ppb in 11% of the tested children and correct values < 20 ppb in 89% of them. Level of average air pollution in survey days for PM10 ranged from 23 to 40  $\mu\text{g}/\text{m}^3$  and for NO<sub>2</sub> – from 28 to 44  $\mu\text{g}/\text{m}^3$ . The study shows that the significant majority of children have normal levels of nitric oxide in the exhaled air when air pollution is low. Analysis of the second phase of FeNO measurements in winter, when air pollution was higher, will allow to compare the results and assess the correlation between the FeNO value and air pollution.

**Реферат.** Вимірювання оксиду азоту в повітрі, що видихається, у дітей початкової школи в Ружомбероці. Чубай-Коваль М., Фрідігер Т., Худакова З., Соколовський М., Вальчак-Каполька Е., Астарьова І., Лещакова А. У наш час існує низка респіраторних захворювань, які безпосередньо уражають дітей. Вимірювання оксиду азоту (FeNO) - один із сучасних методів дослідження, який застосовується для діагностики та контролю алергічного запалення еозинофільних дихальних шляхів. Це простий, безболісний і економічний для часу пацієнта метод. Через свою неінвазивність та простоту він є цінним методом діагностики, особливо в дітей молодшого віку. Вимірювання FeNO є визнаним біомаркером і точним кількісним показником при виявленні, оцінці та моніторингу запалення дихальних шляхів, включаючи астму. Однак лише деякі дослідження оцінюють взаємозв'язок між FeNO та рівнем забруднення повітря. Метою нашого дослідження є визначення взаємозв'язку між FeNO у дітей третього класу початкових шкіл та рівнем забруднення повітря в

*Ружомбероку. У дослідженні взяли участь 146 дітей віком 8-10 років. Це перший етап більш широкого дослідницького проекту, що передбачає вимірювання FeNO у дітей у різний час року. Метою дослідження є визначення концентрації FeNO у досліджуваній групі дітей восени, коли забруднення повітря нижче, ніж на другій стадії, що забезпечується взимку. Дослідження показало підвищені значення FeNO > 20 ppb в 11% тестованих дітей та правильні значення < 20 ppb у 89% з них. Рівень середнього забруднення повітря за спостережувані дні для PM10 коливався від 23 до 40 мкг/м<sup>3</sup>, а для NO<sub>2</sub> – від 28 до 44 мкг/м<sup>3</sup>. Дослідження показує, що значна частина дітей має нормальний рівень оксиду азоту в повітрі, що видихається, коли забруднення повітря низьке. Аналіз другої фази вимірювань FeNO взимку, коли забруднення повітря було вищим, дозволить порівняти результати та оцінити співвідношення між значенням FeNO та забрудненням повітря.*

Exhaled nitric oxide measurement (FeNO) is one of the modern, simple and noninvasive method of investigation used for diagnosing and controlling eosinophilic inflammation. Measurement of bronchial FeNO is a recognized biomarker and an accurate quantitative indicator in the detection, assessment and monitoring of airway inflammation, including bronchial asthma [9, 10, 11, 12, 13]. In connection with asthma, FeNO is currently the most sensitive method for the determination of eosinophilic airway inflammation [1]. Nitric oxide is a molecule with high biological activity and plays an important role in physiology and human pathology as well. Its effect on the body depends on its concentration. Low concentration regulates homeostasis of the circulatory, respiratory, immune and nervous systems. High concentration is pro-inflammatory and cytotoxic directly or via active metabolites. The first presence of NO in the exhaled air was confirmed by Gustafsson in 1991 and the first guidelines principles for the measurement of nitric oxide in exhaled air were published by the American Thoracic Society in 1999. FeNO is able to determine the presence and amount of nitric oxide in an exhaled air stream, which the lungs of a healthy person excrete only in a minimal amount [4, 5]. In recent years, the measurement of FeNO has been increasingly used in the diagnosis of respiratory diseases in children. A lot of scientific publications show a good correlation between FeNO and the results of other diagnostic tests. In children, FeNO is a specific indicator of asthma exacerbation and treatment response. It can be repeated for each cooperating child. In patients with eosinophilic inflammation, the amount of FeNO increases, which is caused by activated airway epithelial cells in inflammation of the airways. It is used for evaluation of the activity of the airway inflammatory process. FeNO values respond quickly to the treatment or worsening of the disease. The result is immediately visible and treatment is adjusted accordingly [2]. FeNO measurement can help us with diagnosis of bronchial asthma, along with other diagnostic methods such as spirometry, bronchoprovocation tests, and monitoring the effectiveness of the treatment. FeNO allows to determine the lowest effective

dose of inhaled corticosteroids that sufficiently controls inflammation in the airways. This method is possible since the age of 4 years [3]. Other functional diagnostic tests also help diagnose bronchial asthma early, in addition to a thorough medical history and physical examination. FeNO is preferred to other methods such as bronchial mucosal biopsy or bronchial lavage that are invasive, time-consuming, and uncomfortable [6]. Many studies confirm the usefulness of this method in the diagnosis of respiratory diseases in children. However, only a few studies assess the relationship between FeNO and level of air pollution, which is the aim of our study.

The aim of the study is to determine the relationship between the concentration of nitric oxide in exhaled air in children of the third grade of primary school and the level of air pollution in Ružomberok. The whole project includes two stages: Stage 1 September 19<sup>th</sup>, October 8<sup>th</sup> and Stage 2 February 4<sup>th</sup> and 5<sup>th</sup>. The study presents the results of the first stage of the measurement performed on September 19<sup>th</sup>, 2018 and October 8<sup>th</sup>, 2018. There is a large company producing paper and cellulose pulp in Ružomberok, but at the same time, there are variables that have a large impact on the level of air pollution in Ružomberok, which are not related to the paper and pulp production. In addition, there is long-distance international transport near the city, which also causes air pollution. According to modelling and analysis results, it contributes to average annual PM10 and NO<sub>2</sub> concentrations in ambient air [7].

#### MATERIALS AND METHODS OF RESEARCH

Our study examined all third-grade children who were in school on the days of the research. The aim of the research is to determine the concentration of nitric oxide in the exhaled air in the studied group of children in autumn, when the air pollution is lower than in winter. This is the first stage of a wider research project involving FeNO measurements in children at different times of the year. The research of the first phase of the project included children of the third grade of elementary schools at the age of 8-9 years, several children were at the age of 10 years, whose parents gave their written consent prior to this measurement. Including the invitation to the

research parents received information regarding the type, purpose and methodology of the test. Our study examined all third-grade primary school children who were at school on the days when the measurements were taken on September 19th and October 8th, 2018.

**Pre-Examination Preparation:** Children should be rested 15 minutes prior to examination and should not eat, drink or exercise for 2 hours prior to examination.

**Measurement method:** The study was carried out by measuring the concentration of nitric oxide in the exhaled air using Nitric oxide analyser MediSoft Belgium. The examination consisted of slow inhalation and exhalation through a mouthpiece with an antibacterial filter. The safety of the examination was ensured by non-invasiveness, simplicity of the test and absence of side effects. The examination was painless and lasted 1-2 minutes. The child holds the device with his/her hands and exhales as much as possible outside the device. Through a special mouthpiece with filter, the child breathes outside the device as much as possible, holds its breath briefly and exhales into the device for 6 seconds with moderate effort. The correct exhalation intensity is seen on the computer screen or is audibly controlled. The result of the examination is available within 2 minutes. The measurement of nitric oxide in exhaled air was performed twice in each child. Before the test, children were instructed by the doctor who performed the test and the way in which the test was performed was shown.

According to the generally accepted methodology for this type of research, the determination of

nitric oxide concentration in exhaled air is given in dimensionless quantities ppb (parts per billion,  $10^{-9}$ ). The limit value of FeNO measurement result in children was 20 ppb, as assumed in most studies.

In our study we divided the increased FeNO values into three groups:

Group I – NO concentration range 21-50 ppb

Group II – NO concentration range 51-99 ppb

Group III – NO concentration range > 99 ppb

#### RESULTS AND DISCUSSION

146 children aged 8-10 years from elementary schools in Ružomberok participated in the first phase of the research. The measurement of nitric oxide concentration in the exhaled air was performed in each child according to the planned research methodology. The range of FeNO values in the whole studied population ranged from 2 to 55 ppb. The increased values ranged from 22 to 55 ppb (Table). Measurement values of exhaled nitric oxide concentration in 130 examined children (89.0%) were normal FeNO < 20 ppb and in 16 examined children (11.0%) FeNO were increased > 20 ppb (Fig. 1). Among 16 children (11%) with increased FeNO > 20 ppb, 15 of them were in group I (FeNO 21-50 ppb) and 1 child was in group II (FeNO 51-99 ppb), which was 6.25% of the group of children with increased results. There was no child in Group III (Fig. 2). Average values of air pollution (smog) in Ružomberok during the FeNO measure in children for PM10 ranged from 23 to 40  $\mu\text{g}/\text{m}^3$  and for NO<sub>2</sub> from 28 to 44  $\mu\text{g}/\text{m}^3$  and were below the WHO and EU standard 50  $\mu\text{g}/\text{m}^3$ .

#### Elevated FeNO values

Elevated FeNO values		
21 – 50 ppb	51 – 99 ppb	>99 ppb
22, 22, 22, 24, 24, 26, 27, 27, 28, 29, 29, 31, 36, 46, 48	55	no results

The 1-hour and 3-hour maximum values for NO<sub>2</sub> levels in Ružomberok during the FeNO measure in children were:

September 19<sup>th</sup>, 2018

m1h – maximum 1-hour average  
of NO<sub>2</sub>: 44 [ $\mu\text{g}/\text{m}^3$ ]

m3h – maximum 3-hour average  
of NO<sub>2</sub>: 39 [ $\mu\text{g}/\text{m}^3$ ]

October 8<sup>th</sup>, 2018

m1h – maximum 1-hour average  
of NO<sub>2</sub>: 29 [ $\mu\text{g}/\text{m}^3$ ]

m3h – maximum 3-hour average  
of NO<sub>2</sub>: 28 [ $\mu\text{g}/\text{m}^3$ ].

NO<sub>2</sub> in 2018 did not exceed the hourly limit value at any monitoring station. Exceeding the limit value for the protection of human health for hourly concentrations did not occur at any monitoring station. In 2018, no case of exceeding the alert threshold was discovered. The critical level for vegetation protection (30  $\mu\text{g}/\text{m}^3$  per calendar year expressed as NO<sub>2</sub>) was not exceeded at any of the EMEOP stations in 2018. The values were way below the lower limit for ASHM vegetation protection [8].

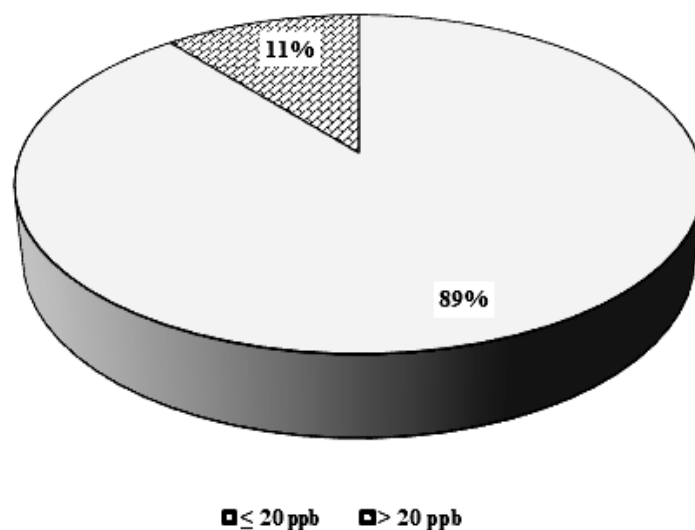


Fig. 1. Range of FeNO values in all examined children

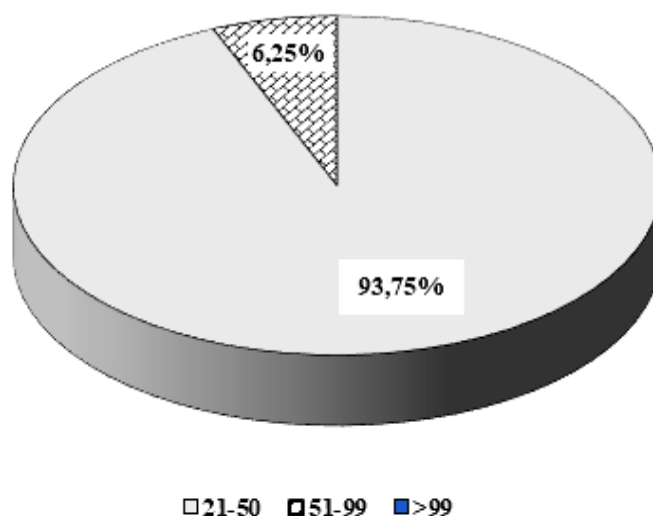


Fig. 2. Range of FeNO in children with increased values

**CONCLUSIONS**

1. The first stage of FeNO measurements in children in the autumn of 2018, when air pollution was low, showed that most children (89%) had normal FeNO values.

2. Analysis of the second phase of FeNO measurements in the winter of 2019, when air pollution was higher, will allow to compare the results and assess the correlation between the FeNO value and air pollution.

3. Children with elevated FeNO values are recommended for control tests and diagnostics for respiratory diseases, including asthma.

4. The second phase of the measurements and their comparative analysis will be presented in the next study.

Conflict of interests. The authors declare no conflict of interest.

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