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COMPARISON OF OXIDATIVE STRESS LEVELS IN HEALTHY CHILDREN AND CHILDREN WITH ALLERGIC RHINITIS

Ivana Arnaudova Danevska[1], Tatjana Jakjovska[1], Dragica Zendelovska[2], Emilija Atanasovska[2], Pavlina Dzekova-Vidimliski[3], Marija Petrushevska[2], Katerina Boshkovska[1], Gorica Popova[4], Elena Gjinovska Tasevska[4], Trajan Balkanov[2]

[1] University of Ss Cyril and Methodius, Faculty of Medicine, Institute for Respiratory Diseases in Children, Skopje, RN Macedonia

[2] University of Ss Cyril and Methodius, Faculty of Medicine, Institute of Preclinical and Clinical Pharmacology and Toxicology, Skopje, RN Macedonia

[3] University of Ss Cyril and Methodius, Faculty of Medicine, University Hospital of Nephrology, Skopje, RN Macedonia

[4] Institute for Respiratory Diseases in Children, Skopje, RN Macedonia

Corresponding author: Ivana Arnaudova Danevska, Institute for Respiratory Diseases in Children, Skopje, RN Macedonia, email: aivana77@yahoo.com

ABSTRACT

Background/aim: Allergic rhinitis (AR) is characterized by chronic inflammation of the nasal mucosa. Under the influence of exogenous factors - allergens, reactive oxygen species (ROS) are released during cellular metabolism. They induce a series of pathological changes in the mucosa. Oxidative stress is a result of an imbalance between the production of ROS and the ability to neutralize them. The aim of this study is to compare the levels of oxidative stress between healthy children and children with allergic rhinitis.

Material and methods: A total number of 60 children were included (30 healthy children and 30 children with AR). The oxidative stress index was determined by using the FRAS 5 (Free Radical Analytical System) Bravo system. Demographic characteristics, medical history, children's living conditions and eating habits were obtained from the questionnaire. Anthropometric measurements and the absolute number of eosinophils in the peripheral smear were performed on each child.

Results: This study showed high oxidative stress index and a significantly higher value of the absolute number of eosinophils in the peripheral smear in children with AR in comparison to healthy children ($p<0.05$). The group of children with AR had more atopic characteristics and was more exposed to passive smoking than healthy children.

Conclusion: Compared to healthy children, children with AR have a high index of oxidative stress, despite of the very high mean value of the concentration of water-soluble antioxidants in serum (PAT test) in the group of children with AR.

Keywords: allergic rhinitis, children, oxidative stress, d-ROMs, PAT, OSI

INTRODUCTION

Allergic rhinitis (AR) is an inflammatory disorder of the nasal mucosa that results from an allergic immune response to inhaled allergens in sensitized individuals. Allergic immune cascade in

the nasal mucosa can cause symptoms of varying severity and duration. These include nasal congestion/obstruction, rhinorrhea, itchy nose and/or sneezing, as well as conjunctival symptoms (itchy eyes, watery eyes, conjunctival congestion, haemosis, and periorbital edema). In addition to local symptoms, allergic rhinitis also presents with general symptoms such as fatigue, impaired concentration, and decreased productivity [1]. The Allergic Rhinitis and its Impact on Asthma (ARIA) recommendations classify AR according to the duration of symptoms (intermittent and persistent) and severity of symptoms such as sleep disturbance, disruption of daily activities, free time and/or sports, negative impact on school and work obligations, irritability etc (mild and moderate-severe). AR, traditionally, is divided into seasonal, perennial and occupational rhinitis [2].

According to the third phase of the International Study of Asthma and Allergies in Childhood (ISAAC), having taken place from 1999-2004, the prevalence of allergic rhinoconjunctivitis was 8.5% in 6-7 year old children and 14.6% (1.0-45%) in 13-14 year old adolescents worldwide [3]. In 2006 Vlashki et al. established and compared the prevalence rates of asthma, hay fever, eczema and the risk factors associated with these diseases by the self-reported data obtained through the ISAAC phase 3 questionnaires by 5507 respondents from 5 regions in the Republic of North Macedonia. In Macedonia in general, in Skopje, Veles, Tetovo, Ohrid and Eastern Macedonia the following prevalence rates were established: 9.4%, 9.5%, 11.6%, 9.5%, 7.6%, 8.9%, respectively by regions for current allergic rhinoconjunctivitis symptoms and 6.3%, 4.4%, 4.9%, 7.4%, 8.2%, 6.4% respectively by regions for ever diagnosed hay fever. When compared to the prevalence in the world, Macedonia has a moderately low prevalence of hay fever with the possibility of underdiagnosis of this disease [4]. The trend of the prevalence of hay fever in the Republic of North Macedonia was investigated also by Vlashki et al. through 3 cross-sectional studies (2002, 2006 and 2016), in which 4804 adolescents aged 12-15 years were included. Namely, the prevalence of sneezing or nasal discharge or obstruction other than cold or flu for 2002, 2006 and 2016 was 23.1%, 19.9%, 39.3%, respectively by years ($p<0.001$); and the prevalence of ever having been diagnosed with hay fever by a physician was 6.7%, 4.4%, 15.4%, respectively per year ($p<0.001$). Research has shown a significant increase in the prevalence of hay fever among adolescents over a 14-year period.

Under the influence of exogenous factors, such as allergens, free radicals are released during cellular metabolism, i.e., reactive oxygen species (ROS), such as hydroxyl radicals, superoxides and peroxides [5].

Direct exposure to environmental air is an additional source of ROS exposure that is unique to the airways. Exogenous sources include: cigarette smoke [6, 7], passive smoking [8], gas cooking [9], external pollution, i.e., passing trucks on the street of residence [10] and polluted air, containing ozone [11].

The primary defense against ROS are endogenous antioxidants, which can be divided into enzymatic and non-enzymatic categories. Enzymatic antioxidants include the superoxide dismutase (SOD) family, catalase, glutathione peroxidase, glutathione C-transferase and thioredoxin [12]. The non-enzymatic category of antioxidants includes low molecular weight compounds such as glutathione, ascorbic acid, urate, α-tocopherol, bilirubin and lipoic acid [13].

Several authors have suggested the role of the oxidative stress in the etiopathogenesis of AR [14-18]. They found that patients with AR have higher oxidative stress in comparison to healthy individuals and the introduction of the antioxidant therapy could be helpful in preventing allergic rhinitis.

Our study was focused on assessment of oxidative stress levels in healthy children and children with allergic rhinitis and its comparison between these groups of children. Oxidative stress occurs as a result of an imbalance between free radicals and antioxidants. Our hypothesis is that children with allergic rhinitis have higher levels of oxidative stress than healthy children. For this purpose, we have analyzed the serum from healthy children and children with AR by well-established photometric fast analytical method. .

MATERIAL AND METHODS

Study design (Patients)

The study was conducted from March 2021 until March 2022 at the Institute for Respiratory Diseases in Children in Skopje and at the Institute for Preclinical and Clinical Pharmacology

with Toxicology at the Medical Faculty in Skopje. The study was approved by the Ethics Committee of the Medical Faculty, University Ss Cyril and Methodius, Skopje, Republic of North Macedonia. The study included children with AR and healthy children (as a control group) of both sexes, at the age between 4 and 14 years. Children with AR had positive skin prick test (SPT) for inhaled allergens, presence of at least 2 symptoms of allergic rhinitis (local: nasal congestion/obstruction, rhinorrhea, sneezing, itchy nose and itchy eyes, or general symptoms: disturbed sleep and negative effects on physical activity).

Clinical characteristics and laboratory data

Demographic characteristics (place of residence, ethnicity, etc), medical history, children's living conditions (heating, gas cooking, passive smoking, animals in the home, frequency of passing trucks on the street of living during working days) and eating habits were obtained from the questionnaire that was specially designed for this study.

Based on anthropometric measurements (body height, body weight), Body mass index (BMI) was calculated using the standard formula and expressed as weight (kg)/height (m^2). International BMI cutoff points established by Cole et al. [19] were used for overweight and obesity by gender, for ages between 2 and 18 years, defined BMI over 25 kg/m^2 for overweight and 30 kg/m^2 for obesity aged 18 years.

The scoring of the symptoms of allergic rhinitis was done by scoring local and general symptoms. A 3-point response scale was used for scoring of all individual items (never, rare, often). According to the scoring of the symptoms, the patients with AR were divided into 3 categories:

- mild AR (2-4 points)

- moderately severe AR (5-7 points, of which at least 1 is a general symptom)

- severe AR (> 7 points, of which at least 1 is a general symptom).

The percentage of eosinophils in nasal secretions was determined by the following procedure: A soft sterile swab was taken from both nostrils, applied on a glass slide, a thin smear was made and the product was dried at room temperature for about 30 minutes. Once dried, it was placed in a dye tub. First, the May-Grunwald fixative was applied for 2-3 minutes, then the product was rinsed with distilled water, put back in the dye tub and we applied diluted Giemsa dye. The product was stained for 15 minutes, then rinsed with distilled water, shaken to remove excess water and allowed to dry at room temperature for about 30 minutes. After that, the sample was microscoped with 100 x magnification (immersion) with a drop of cedar oil previously placed on the product. The eosinophils were found in the microscopic field, if any, and the percentage ratio of eosinophils to neutrophils was determined up to one hundred counted cells in the microscopic fields.

In all study participants, blood count was taken in order to determine the absolute number of eosinophils in peripheral smear.

Method for determination of d-ROMs, PAT and Oxidative stress index

In healthy children and children with AR, a serum sample was collected and analysed after collection. The FRAS 5 Bravo system (H&D srl - Parma, Italy), was used to determine the oxidative stress index. The d-ROMs test is a photometric test that allows to assess the pro-oxidant status in a biological sample by measuring the concentration of hydroperoxides (ROOH). It is expressed in U. Carr (1 U. Carr = 0.08 mg H_2O_2/dl), a unit of free radical scales used only by international scientific community. The normal d-ROMs reference values are 250–300 U. Carr. PAT is a test which can be used to determine the antioxidant power (both scavenger and antioxidant haematic concentration), expressed in U. Carr (1 U. Carr = 1.4 μmol/L Vitamin C). The reference values of the PAT test are 2200–2800 U. Carr. The system summarizes the results obtained from the d-ROMs and PAT test in one value – oxidative stress index (OSI), with normal reference values less than 40.

Statistical analysis

Statistical analyses were performed using Statistica for Windows, Version 17.0 (SPSS Inc., Chicago, IL, USA). Investigated parameters were presented as mean value ± standard deviation. The comparison of parametric variables between children with AR and the control group of healthy children was done with Student's t-test. Pearson's correlation coefficient was used for the determination of the correlation between two parametric variables. The Chi-squared test ($\chi 2$ test) was used in order to compare non-parametric variables between children with AR and the control group of

healthy children. A value of $p<0.05$ was considered statistically significant.

RESULTS

A total of 60 children with a mean age 8.1 ± 3.1 years (range: from 4 to 14 years) were included, 30 healthy (mean age 7.9 ± 3.4 years) and 30 children with AR (mean age 8.3 ± 2.9 years). There was no presence of co-existing medical conditions (cardiac, endocrinological, gastrointestinal, haematological, hepatic or renal), no medication was used and nobody participated in other clinical studies over the last 2 months in both groups.

The comparison of anthropometric characteristics: body weight, body height and BMI between study groups, as well as the absolute number of eosinophils in the peripheral smear are shown in Table 1. The group of children with AR had a significantly higher value of the absolute number of eosinophils in the peripheral smear (446.3±331.4/mm3) compared to the group of healthy children (144.2 ± 98.7/mm3).

According to the severity of the symptoms in children with AR, the mean symptom score was 7.9±2.5, which is classified as severe AR.

The mean value of the percentage of eosinophils in nasal secretions in children with AR was 8 ± 15%.

There was a significant positive correlation between the absolute number of eosinophils in the peripheral smear in all study participants with the values of reactive oxygen metabolites ($r = 0.375$, $p = 0.004$) and oxidative stress index ($r = 0.365$, $p = 0.005$). The correlation between the absolute number of eosinophils in peripheral smear with the values of water-soluble antioxidants was negative and statisticaly non signifficant ($r = - 0.080$, $p=0.552$).

There was a positive but non significant correlation between the absolute number of eosinophils in the peripheral smear from the children with AR with reactive oxygen metabolites ($r =0.041$, $p=0.828$) and the oxidative stress index ($r = 0.017$, $p=0.930$). The correlation between the absolute number of eosinophils in the peripheral smear from the children with AR with the values of water-soluble antioxidants was negative and statisticaly non significant ($r = - 0.249$, $p=0.185$).

There was a positive but non significant correlation between the absolute number of eosinophils in the peripheral smear from the healthy children with reactive oxygen metabolites ($r =0.349$, $p=0.074$), with oxidative stress index ($r = 0.307$, $p=0.119$), and with water-soluble antioxidants ($r = 0.280$, $p=0.157$).

The mean serum concentration of reactive oxygen metabolites expressed by the d-ROMs test in children with AR was 403.4 ± 91.3 U. Carr, which is a high level of oxidative stress. In the group of healthy children, the mean value of the serum concentration of reactive oxygen metabolites was 280.0 ± 49.9 U. Carr, and this is within the normal range of values (Fig.1). The mean value of the PAT test in children with AR was 2935.2 ± 939.1 U. Carr, a very high value. The mean value of the PAT test in healthy children was 2657.6 ± 310.1 U. Carr, and according to the stated reference values, this is within the

Table 1. *Anthropometric characteristics, absolute number of eosinophils in peripheral smear in children with AR and healthy children*

	Children with AR **X±SD**	**Healthy children** **X±SD**	**p**
Weight (kg)	33.9±15.2	30.4±15.5	0.389
Height (cm)	133.7±17.9	130.3±22.3	0.520
BMI (kg/m^2)	18.2±4.0	16.8±3.3	0.192
Absolute number of eosinophils in peripheral smear (number/mm^3)	446.3±331.4	144.2 ± 98.7	<0.001

normal range (Fig. 2). There was a statistically significant difference in the mean values of oxidative stress index in children with allergic rhinitis and healthy children. In children with allergic rhinitis, it was 95.1 ± 45.9, which is a high value of the index of oxidative stress. In healthy children, however, it was 32.6 ± 13.3 and this is within the normal reference values (Fig. 3).

Data obtained from the questionnaire on the demographic characteristics, living conditions of the children, atopic characteristics, and eating habits are presented in Table 2. In the group of children with allergic rhinitis, the number of male children was significantly higher compared to the group of healthy children (20 vs. 9, p=0.009). In the obtained data about living conditions, there was significant difference in exposure to passive smoking and combustion products of domestic gas cooking. Namely, the group of children with AR was more exposed to passive smoking than the healthy children. On the other hand, the group of healthy children was more exposed to the combustion products of domestic gas cooking than children with AR. Regarding the atopic characteristics of the subjects, there was a significant difference between children with AR and the healthy control group in relation to family history of atopy, history of atopic dermatitis, symptoms of allergic rhinitis, and positive skin tests to inhalant allergens, but not in the history of asthma, food, and drug allergy. A significant difference was detected in some of the eating habits (red meat, cereals and pasta intake) between the two groups (Table 2).

DISCUSSION

Allergic rhinitis is an inflammatory disorder of the nasal mucosa, and ROS contribute to the pathogenesis of allergic disorders [20,21]. The presence of chronic inflammation in the upper airway epithelium, as in allergic rhinitis, may contribute to the development of significant and persistent oxidative stress. Recent studies have investigated molecular pathways of oxidative stress and antioxidants in AR and the potential therapeutic options with dietary antioxidants for AR. It was found that transcription factors, such as Nrf2 (nuclear factor erythroid 2-related factor 2) and NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells), in murine models of AR as well as nasal mucosa epithelial cells of patients with AR regulate the antioxidants' response. Several possible therapeutic antioxidants that are abundant in natural dietary resources have been studied and have promising results by inhibiting several oxidative stress pathway markers [14].

Direct exposure to enviromental air is an additional source of ROS which is unique for the airways. For example, inhaling cigarette smoke results in increased exposure to both superoxide and hydrogen peroxide. Cigarette smoke leads to lung damage as a result of exposure to nitric oxide and nitrites [6,7]. The study by Mitchell et al. [8] (Phase 3 analysis of the ISAAC program) suggests a significant association of smoking in both parents and symptoms of rhinoconjunctivitis, but no current asthma symptoms in a group of adolescents aged 13-14 years. In our study, 19 out of 30 children with allergic rhinitis were exposed to passive smoking. In 2005 Willers et al. [9] investigated the effect of gas cooking, kitchen ventilation, products of indoor combustion, and respiratory and allergic effects in children. The results indicated that gas cooking was, in itself, associated with the onset of nasal symptoms in young children, rather than with the other respiratory symptoms examined. Regarding the exposure to external pollution (determined by the frequency of passing trucks on the street) Vlashki et al. [10] found a positive association between the frequency of passing trucks on the street and the occurrence of wheezing, dry night cough, symptoms of allergic rhinitis, and rash followed by itching in young adolescents. In our study, in 19 of all 30 children with allergic rhinitis, trucks sometimes passed on the street during the working days.

Our study shows that the mean d-ROMs test in healthy children was within normal range, while in children with AR it indicated high levels of oxidative stress. In the group of children with AR, the mean value of the PAT test was very high, contrary to healthy children, where it was within normal limits (albeit still close to the upper limit). The normal to high PAT values in both groups of children we obtained may be due to their eating habits, i.e., the almost daily consumption of fresh fruits and vegetables. By contrast, Agarwal et al. [22] found that patients with allergic rhinitis had low levels of total plasma antioxidant activity as a result of inflammation leading to oxidant overload from enhanced lipid peroxidation. Therefore, fortifying the diet with antioxidants along with anti-inflammatory drugs can be an effective ap-

Table 2. *Demographic characteristics, living conditions, atopic characteristics and eating habits of the subjects*

Questionnaire	AR (n=30)		Healthy children (n=30)		p
Gender (Male/Female)	20	10	9	21	0.009
Place of residence (city/village)	25	5	25	5	1.000
Ethnicity (Macedonian/Albanian)	23	7	27	3	0.299
Mother's education - university degee (yes/no)	15	15	15	15	1.000
Father's education - university degee (yes/no)	15	15	8	22	0.110
Type of heating in the home (central or electricity / air conditioning)	17	13	19	11	0.792
Gas cooking in the home (yes/no)	4	26	13	17	0.020
Passive smoking in the home (yes/no)	19	11	8	22	0.009
Frequency of passing trucks on residential street during weekdays (never/sometimes)	11	19	11	19	1.000
Animals in home (yes/no)	12	18	9	21	0.588
Family history for allergy (yes/no)	24	6	4	26	<0.001
Atopic dermatitis (yes/no)	13	17	1	29	<0.001
Allergic rhinitis (yes/no)	30	0	0	30	<0.001
Asthma (yes/no)	4	26	0	30	0.112
Drug allergy (yes/no)	2	28	0	30	0.491
Food allergy (yes/no)	5	25	0	30	0.052
Skin prick test (yes/no)	29	1	0	30	<0.001
Clinically significant disease (yes/no)	0	30	0	30	1.000
Use of medicines (yes/no)	4	26	0	30	0.112
Use of nasal topic corticosteroids in the last 2 months (yes/no)	5	25	0	30	0.052
Type of food preparation (baked or boiled/fried)	20	10	20	10	1.000
Spicy food (yes/no)	14	16	21	9	0.115
Fast food (yes/no)	10	20	7	23	0.567
Bread (white/integral)	24	6	27	3	0.471
Juices (carbonated/natural)	5	25	6	24	1.000
Fruit intake (1-2 times a week/ ≥ 3 weeks)	10	20	10	20	1.000
Vegetable intake (1-2 times a week/ ≥ 3 weeks)	9	21	9	21	1.000
Fish intake (1-2 times a week/ ≥ 3 weeks)	29	1	28	2	1.000
Red meat intake (1-2 times a week/ ≥ 3 weeks)	6	24	16	14	0.015
Cereals intake (1-2 times a week/ ≥ 3 weeks)	26	4	16	14	0.010
Pasta intake (1-2 times a week/ ≥ 3 weeks)	26	4	17	13	0,020
Participation in other clinical studies in the last 2 months (yes/no)	0	30	0	30	1.000

Figure 1. *d-ROMs test in children with AR and healthy children*

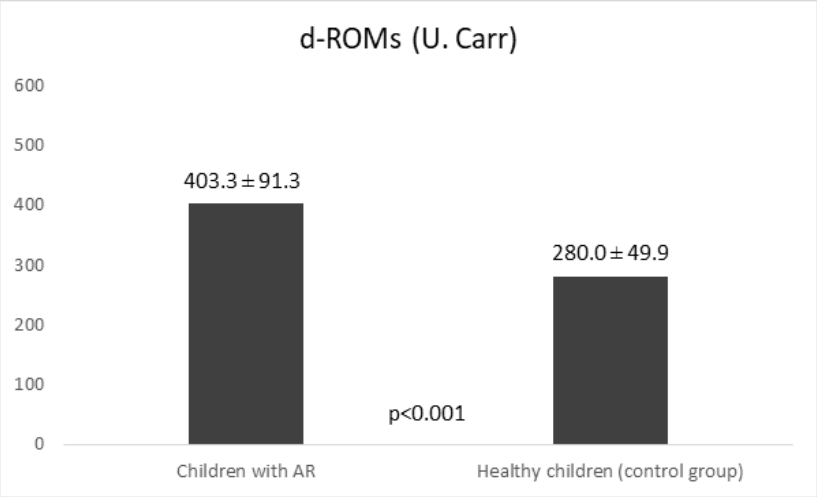


Figure 2. *PAT in children with AR and healthy children*

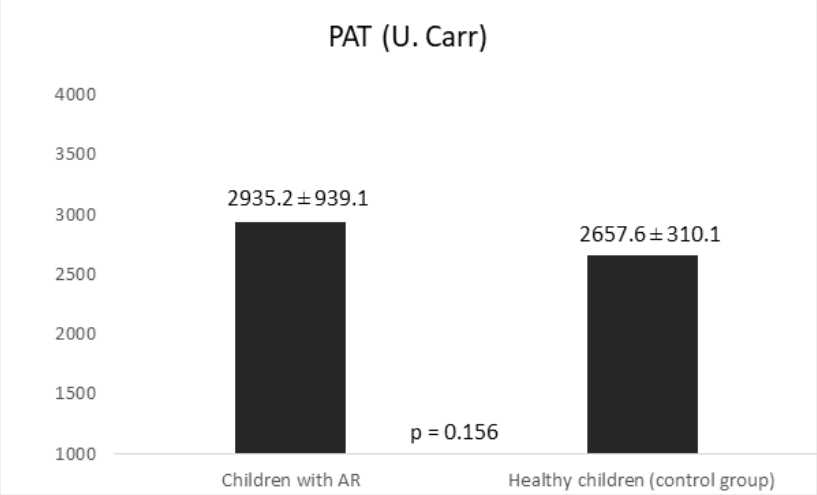
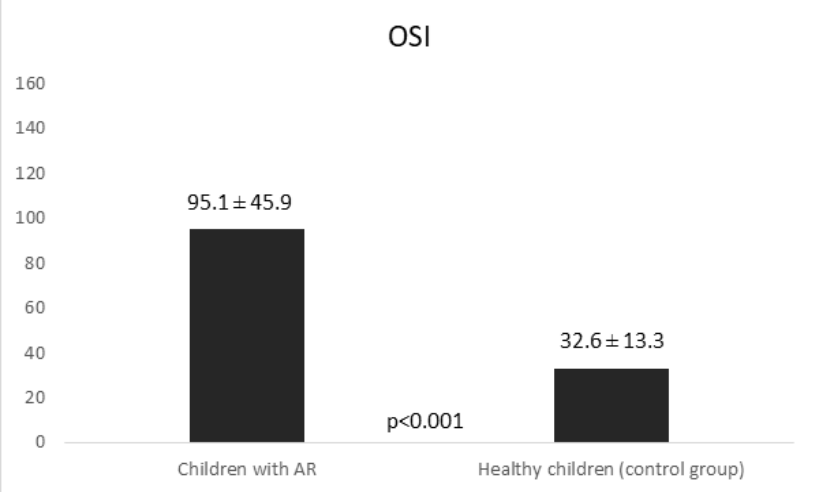


Figure 3. *OSI in children with AR and healthy children*



proach in preventing the progression of allergic rhinitis.

Oxidative stress occurs as a result of an imbalance between free radicals and antioxidants. This was established by Akbay et al. [15] by comparing myeloperoxidase (MPO) activity and vitamins A and E as enzymatic and non-enzymatic antioxidants, respectively, and the oxidative stress determined by malondialdehyde (MDA) levels in a group of patients with AR and healthy individuals. The average level of MDA was significantly higher in patients with AR, and the average level of MPO, vitamin A and E were significantly lower in the group of patients with AR. Meanwhile, Sim et al. [16] concluded that patients with AR had systemically elevated oxidative stress and systemically elevated levels of total antioxidant capacity compared with a group of healthy individuals. We have obtained similar results, but with a different method.

Sagdic et al. [17] concluded that, despite their clinically stable conditions, oxidative stress exists in allergic rhinitis patients and particularly in asthma patients, suggesting its role in the pathogenesis of these disorders.

A study by Sequeira et al. [18], published in 2012, examined the changes in several parameters of oxidative stress and antioxidant status in patients with allergic rhinitis. It suggests that a strategy of well-balanced antioxidant therapy, based on reducing the production of endogenous ROS and increasing the overall antioxidant capacity of human cells, could be helpful in preventing allergic rhinitis.

In summary, the mean value of the oxidative stress index of the healthy children group was low, contrasting to the group of children with allergic rhinitis, where the high mean value of the oxidative stress index was obtained.

Reviewing the available literature, it can be concluded that there are several studies dealing with oxidative stress in AR, especially in children. The authors would like to emphasize that this is the first study that investigates oxidative stress in children with AR that was conducted in RN Macedonia.

The authors suggest that the study was limited as it was performed on a small number of subjects at one single center. Seeing that the determination of markers of oxidative stress had to be performed at the Institute of Preclinical and Clinical Pharmacology, it was technically difficult to send the serum samples immediately. Therefore, serum samples were collected and frozen at -80 C before their processing.

CONCLUSION

From the data available so far and their analysis in this paper, it could be concluded that there is an evident difference between oxidative stress in healthy children and children with allergic rhinitis. Namely, children with allergic rhinitis have significantly higher oxidative stress levels compared to healthy children. These results provide information about the potential role of oxidative stress in allergic rhinitis.

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Резиме

КОМПАРАЦИЈА НА НИВОТО НА ОКСИДАТИВЕН СТРЕС КАЈ ЗДРАВИ ДЕЦА И ДЕЦА СО АЛЕРГИСКИ РИНИТИС

Ивана Арнаудова Даневска[1], Татјана Јаќовска[1], Драгица Зенделовска[2], Емилија Атанасовска[2], Павлина Џекова-Видимлиски[3], Марија Петрушевска[2], Катерина Бошковска[1], Горица Попова[4], Елена Ѓиновска Тасевска[4], Трајан Балканов[2]

[1] Универзитет „Св. Кирил и Методиј“, Медицински факултет, Институт за белодробни заболувања кај децата, Скопје, РС Македонија

[2] Универзитет „Св. Кирил и Методиј“, Медицински факултет, Институт за претклиничка и клиничка фармакологија и токсикологија, Скопје, РС Македонија

[3] Универзитет Св. Кирил и Методиј, Медицински факултет, Универзитетска клиника за нефрологија, Скопје, РС Македонија

[4] Институт за белодробни заболувања кај децата, Скопје, РС Македонија

Вовед/цел: Алергискиот ринитис (АР) се карактеризира со хронично воспаление на назалната мукоза. Под влијание на егзогени фактори, во овој случај алергените, доаѓа до ослободување на слободни радикали во текот на клеточниот метаболизам, т.н. реактивни кислородни специи – ROS (reactive oxygen species). Тие предизвикуваат низа патолошки промени во мукозата. Оксидативниот стрес е резултат на нерамнотежа меѓу продукцијата на ROS и способноста нив да ги неутрализира. Истражувањето има цел споредување на нивото на оксидативен стрес меѓу здрави деца и деца со алергиски ринитис.

Материјал и методи: Беа вклучени вкупно 60 деца, од кои 30 здрави и 30 деца со АР, на кои им беше одреден индексот на оксидативен стрес со употреба на FRAS 5 (Free Radical Analytical System) Bravo систем. Демографските карактеристики, медицинската историја, условите во кои живеат и навиките во исхраната на децата, беа обезбедни преку прашалници. За секое дете беа направени антропометриски мерења и беше одреден апсолутен број еозинофили во периферна размаска.

Резултати: Оваа студија покажа висок индекс на оксидативен стрес и значително повисока вредност на апсолутниот број еозинофили во периферната размаска кај децата со АР во споредба со здравите деца ($p < 0,05$). Група деца со АР имаа повеќе атописки карактеристики и беа повеќе изложени на пасивно пушење во однос на здравите деца.

Заклучок: Во споредба со здравите деца, децата со АР имаат висок индекс на оксидативен стрес и покрај многу високата средна вредност на концентрацијата на хидросолубилни антиоксиданси во серум (PAT-тест) во групата деца со АР.

Клучни зборови: алергиски ринитис, деца, оксидативен стрес, d-ROMs, PAT, OSIa