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STATE OF L-ARGININE/ARGINASE SYSTEM AND DIHYDROGEN SULFIDE OF ORAL FLUID IN CHILDREN WITH PRECLINICAL IMBALANCE OF IRON AND THYROID HOMEOSTASIS

***Abstract**. The parameters of L-arginine/arginase system and dihydrogen sulfide of oral fluid in children with mild deficiency of iodine, latent iron deficiency, combined deficiency of iodine and iron and their effect on oral cavity were analyzed in the study. The increase in the content of NO2, NO2+NO3, peroxynitrite on the background of the enzymatic activity of arginase decreasing, especially in children with combined iodine and iron deficiency, were established. More marked changes were observed in boys.*

***Key words:** L-arginine, arginase, dihydrogen sulfide, oral fluid, iodine deficiency, latent iron deficiency.*

Introduction. Western Ukraine belongs to the endemic region with iodine deficiency in the biosphere, characterized by a high level of thyroid gland diseases. In recent years, residents of this region have had a tendency to worsen the dental status of the children's population. This dynamics is associated with a number of objective factors, such as: a decrease in the socio-economic status of the population, unbalanced nutrition, changes in environmental processes that have a negative impact on the balance of micro and macro elements, primarily essential ones (iodine, iron, selenium) [1]. Therefore, an in-depth study of the origin of dysfunction of thyroid gland and comorbid pathology remains relevant both for the physiology of the endocrine system and dentistry. The main cause of thyroid homeostasis disorders is an inadequate supply of iodine to the body, iodide organification disorders and the deficiency of other essential elements involved in the secretion of thyroid hormones, including iron, which is a cofactor of enzymes of thyreogenesis. These factors can affect the condition of the oral cavity, in particular, the permeability of the vascular wall of the mucous membrane. At the same time, it is known that nitric oxide (NO) plays an important role in regulating physiological and biochemical processes in the body as a whole and in the dentofacial area. NO detects, simultaneously activates and inhibits various metabolic processes in the human body, though it is active only for a few seconds. Under its influence, the regulation of signaling pathways, which trigger a series of adaptive-compensatory reactions of the organism, is carried out. Changes in the NO metabolism system can lead to hypoxic necrobiosis in the cells.

A biomarker for the study of dental status in the human body is an oral fluid, the characteristics of which are specific for prediction and detection of periodontal diseases, as well as qualitative changes in the oral cavity [2]. In recent years, salivary glands (SG) have been studied as a special organ for the control of the formation of NO entering the oral cavity with saliva, and contoured by the mechanism of autoregulation. At the same time, NO affects hemodynamics and proliferation of SG cells, neurotransmission and saliva secretion. L-arginine participates in such physiological processes as regulation of the immune response, maintenance of nitrogen balance, antiproliferative actions, and is a substrate for the formation of NO. In the human body there is a nitrite reductase system that provides the formation of nitrate (NO_3) and nitrite (NO_2), which do not exhibit vasoconstrictor action [3], L-arginine → NO→ NO_2→NO_3 – the cycle of NO metabolism [4]. Nitrite and nitrate reductase system is the largest exporter, providing the intake of NO [5]. Nitrite reductase reactions of oral bacterial microflora have a significant influence on NO production [6, 7]. It is known that almost 25% of plasma nitrates are excreted with saliva into the oral cavity, leading to an increase in nitrate concentration in the oral fluid ten times more, concerning their content in plasma [8]. NO excess leads to the formation of peroxynitrite, which has a signal effect and pathogenic influence on a cell, causing nitroso-active and oxidative stress of cells.

The purpose of the study was to study the parameters of the nitric oxide system in the oral fluid of children of the endemic region with a mild hypothyroid dysfunction, latent iron deficiency and combined microelementosis (iodine and iron deficiency).

Materials and methods of research. 78 children aged 6-11 years old who were randomized for age-sexual characteristics and clinical diagnosis were examined. All schoolchildren were divided into four groups: with adequate iodine and iron supplementation (gr. I, n=24), with mild iodine deficiency (gr. II, n=18), with latent iron deficiency (gr. III, n=17) and with combined deficiency of bioelements (gr. IV, n=19). Differentiation of diagnoses was carried out on the basis of complaints, anamnesis, clinical and laboratory methods of research. Functional state of thyroid gland was determined by the content of thyroid hormones in serum: thyroid stimulating hormone (TSH), free T_4 and T_3 hormones. Median ioduria was determined for establishing a level of iodine supplementation in children. To characterize the iron base, the content of serum iron and ferritin, the total iron binding capacity of the blood serum and the coefficient of transferrin saturation by iron were determined. The study of the NO system in the oral fluid was carried out by the content of NO^-, NO_2^-, $NO_2^-+NO_3^-$, H_2S, arginase and L-arginine activity [8]. Statistical analysis was performed using computer programs (Statistic Soft 7.0). Comparison of the samples was carried out according to the Student t-criterion. The error $p<0.05$ was considered statistically significant.

Results of the research and their discussion. As a result of the study, there were significant differences between the investigated parameters depending on the changes of the micronutrient panel. Thus, in the case of a mild iodine deficiency in boys, an increase in NO_2 content (by 15 times, $p_{1-2}<0.05$), NO_2+NO_3 (almost by four times, $p_{1-2}<0.05$) in the oral fluid was observed regarding the control values. In girls of the same group, the increase of peroxynitrite (by two times, $p_{1-2}<0.01$) relative to healthy peers, was found. Boys with latent iron deficiency had increased NO_2 (by 3.6 times, $p_{1-3}<0.01$) in the oral fluid, as compared to control. More significant parameters changes were observed in girls of this group, in particular, reduction of NO_2 (by 45%, $p_{2-3}<0.05$) against the background of peroxynitrite content increase in oral fluid (almost by three times, $p_{1-3}<0.05$). Changes in the balance in the L-arginine/arginase system were the most significant in combined iodine and iron deficiency. Boys of group IV had an increase in NO metabolism products in oral fluid: NO_2 – by 8.3 times, $p_{1-4}<0.001$), NO_2+NO_3 (by 3.3 times, $p_{1-4}<0.05$), peroxynitrite (by 2.8 times, $p_{1-4}<0.05$) against the background of arginase activity decreasing (by 33%, $p_{1-4}<0.05$) as compared to the data of control group of children. It should be mentioned that girls of the same age had only a significant increase in the content of peroxynitrite in oral fluid by 2.5 times ($p_{1-4}<0.01$) relative to the source data. Attention is drawn to the significant discrepancies between the indicators of children in study groups III and IV. Thus, girls with combined iodine and iron deficiency had an increase in the content of NO_2 in the oral fluid (by 3 times, $p_{3-4}<0.05$), NO_2+NO_3 (by 47%, $p_{3-4}<0.05$) in relation to data in group III. Accumulation of nitrates and nitrites in oral fluid with decreasing activity of arginase is a dangerous sign of inflammatory processes in the mucous membrane of the oral cavity and gums. It has been established that NO affects the aetiology and pathogenesis of periodontal diseases as a result of reduction of antimicrobial activity of oral fluid [9, 10]. There is a metabolic acidosis in the tissues, which contributes to the development of inflammatory and dystrophic processes, accompanied by gum hyperemia [11]. This effect is due to increased vascular wall permeability, an inhibitory effect on platelet aggregation and osteoclast activation.

Conclusion. Changes in the NO system in the oral fluid of school-age children indicate a disturbance in the balance of the L-arginine/arginase system in preclinical stages of thyroid homeostasis and the development of iron deficiency anemia. Particular attention is paid to the change in the rates in children with combined microelementosis and the gender difference in the indicators in the studied groups. Such differences can be the result of poor hygiene in boys against the background of microelement

Table 1

State of L-arginine/arginase system and dihydrogen sulfide of oral fluid in children with iodine deficiency (ID), latent iron deficiency (LID), combined deficiency of bioelements (ID+LID), adequate iodine and iron supplementation children aged 6-11 years old (M±m)

Indexes	Group I (control)		Group II (ID)		Group III (LID)		Group IV (ID+LID)	
	Boys (n=8)	Girls (n=8)	Boys (n=9)	Girls (n=9)	Boys (n=8)	Girls (n=8)	Boys (n=10)	Girls (n=8)
H2S, (μmol / l)	67.22±11.33	68.18±8.23	70.18±3.93	78.25±3.74	72.91±6.89	63.3±0.85	71.74±5.89	75.5±11.53
NO2, (μmol / l)	0.27±0.09	0.77±0.62	4.17±1.18 $p_{1-2}<0.05$	1.09±0.24	0.98± 0.18 $p_{1-3}<0.01$	0.42±0.05 $p_{2-3}<0.05$	2.23±0.31 $p_{1-4}<0.001$	1.22±0.15 $P_{3-4}<0.001$
NO2+NO3, (μmol / l)	1.44±0.32	2.97±1.54	5.39±1.70 $p_{1-2}<0.05$	2.60±1.25	2.79± 1.03	2.20±0.14	4.81±1.37 $p_{1-4}<0.05$	3.24±0.36 $P_{3-4}<0.05$
Peroxynitrite, (μmol / l)	5.30±0.91	3.69±1.08	12.23±3.98	11.98±1.65 $p_{1-2}<0.01$	10.96±3.15	10.57±2.72 $p_{1-3}<0.05$	14.63±3.58 $p_{1-4}<0.01$	9.09±0.79 $P_{1-4}<0.01$
L-arginine, (μg / ml)	37.03±5.06	28.25±7.67	42.28±6.13	42.12±7.88	37.67±9.01	40.13±0.42	38.52±9.73	37.3±3.11
Arginase, (μmol / min • mg)	0.27±0.04	0.23±0.09	0.28±0.06	0.24±0.06	0.205±0.02	0.25±0.03	0.18±0.01 $p_{1-4}<0.05$	0.33±0.07

Note. P with Arabic numerals is a significant difference between the indicators in the respective research groups

imbalance, which potentially negatively affects the stomatological status of children. The revealed changes can affect the oral cavity, mineralizing and anti-inflammatory functions of the oral fluid, with subsequent effects on hard tissue of the teeth, periodontal and mucous membranes.

Prospects for further research. Investigation of dental status in children – residents of endemic regions and schoolchildren with iron deficiency anaemia. Implementation of modern preventive measures for pupils with microelement imbalance, who form the risk group of endocrine, hematologic and dental pathology.

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