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# MARKERS OF INFLAMMATION AND ANTIOXIDANT SYSTEM IN THE ORAL FLUID OF 12-YEAR-OLD CHILDREN, DEPENDING ON THE BODY MASS INDEX

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The results of biochemical studies of the oral fluid in 12 years old children with different body mass index indicate a significant increase in urease activity in children both from the side of hypo- and hypertrophy. This fact indicates a high level of contamination with opportunistic bacteria in the oral cavity in children of the observed groups at the initial stage of the study. The activity of the secretory enzyme lysozyme is reduced in Chernomorsk children with hypotrophy; however, an increase in the activity of lysozyme in the oral fluid, as a rule, indicates the initial stage of the disease, or a period of remission. The high activity of lysozyme can be considered as an adaptive response to the development of pathology. The imbalance of these indicators causes the development of dysbiosis, which leads to pathological changes in the oral cavity. The obtained results indicate the increase of the degree of dysbiosis in the oral cavity in children both in Odesa (1.61 and 1.30) and in the Chernomorsk (3.08 and 4.02). In our opinion, dysbiotic and inflammatory reactions in the oral cavity are protective in relation to the parodont tissues.

**Key words:** children, inflammation, body mass index, oral fluid, dental diseases.

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# МАРКЕРИ ЗАПАЛЕННЯ ТА АНТИОКСИДАНТНОЇ СИСТЕМИ В РОТОВІЙ РІДИНІ ДІТЕЙ 12 РОКІВ В ЗАЛЕЖНОСТІ ВІД ІНДЕКСУ МАСИ ТІЛА

Результати біохімічних досліджень ротової рідини у дітей 12 років з різним індексом маси тіла вказують на достовірне збільшення активності уреази у дітей як з гіпо- так і з гіпертрофією. Цей факт свідчить про високий рівень контамінації умовно-патогенними бактеріями в порожнині рота у дітей груп спостереження на початковому терміні дослідження. Активність секреторного ферменту лізоциму знижена у дітей м. Чорноморська з гіпотрофією, однак підвищення активності лізоциму в ротовій рідині свідчить про початкову стадію захворювання, або про період ремісії. Високу активність лізоциму можна розглядати як адаптаційну відповідь на розвиток патології. Дисбаланс цих показників викликає розвиток дисбіозу, що призводить до патологічних змін в порожнині рота. Отримані результати свідчать про зростання ступеня дисбіозу в порожнині рота у дітей як в Одесі (1,61 и 1,30), так і в Чорноморську (3,08 и 4,02). На наш погляд, дисбіотична та запальна реакції в порожнині рота є захисними по відношенню до пародонта.

**Ключові слова:** діти, запалення, індекс маси тіла, ротова рідина, стоматологічні захворювання.

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According to the latest WHO data, a child's health is 60 % dependent on a balanced diet. A large number of the population of Ukraine has a deficiency in the diet of animal products (milk, meat, fish, eggs), a deficiency of fresh plant foods (fruit, vegetables and other plants), and excessive consumption of animal fats; bakery and flour products. Of particular concern is low physical activity among children and adolescents, as well as increased consumption of carbohydrates in the form of simple sugars (confectionery, candy, juices, carbonated drinks) [5]. When ingesting easily digestible carbohydrates, bacteria receive sugar, break it down to form acid, triggering demineralization processes. An intensive learning process, high rates of physical and mental development of school-age children, a significant neuropsychic load at school and at home, needs all the necessary nutrients to enter the child's body with food. Compliance with a high-quality diet of school-age children in combination with optimal physical activity is aimed at the comprehensive prevention of non-communicable diseases in children [6].

According to the Cabinet of Ministers of Ukraine (CMU) Resolution dated November 22, 2004, No. 1591 "On the approval of nutritional standards in educational and children's institutions of health improvement and recreation" (as amended on March 23, 2016 No. 211), the nutritional standards in general educational institutions for one-time meals for students (the rate per student, g) are: proteins – 31.8 (including those of animal origin – 15); fats – 30.7 (including vegetable – 10); carbohydrates – 143. Energy value – 975 kcal.

Peculiarities of human nutrition play a significant role in the predisposition to certain diseases [6]. This especially applies to diseases of a nutritional nature, which can also include dental caries [13]. The development of dental caries is mainly determined by the intake of sugar into the oral cavity. Excessive

consumption of easily digestible carbohydrates affects not only the condition of the teeth, but also the degree of obesity [11], the development of metabolic syndrome [4] and type 2 diabetes mellitus [7].

Studies by some authors indicate that overweight and obesity can be risk factors for the development of parodontal disease [14]. Other authors emphasize that there is no statistically significant relationship between obesity and parodontal status. Increased body mass index, however, may be a predictor of bleeding gums [12].

**The purpose** of the study was to establish a link between metabolic disorders in the body of children, differing in BMI, with the development of dental pathology.

**Materials and methods.** An examination was carried out on 72 pupils of school No. 6 of Chernomorsk and 79 pupils of gymnasium No. 1 of Odesa. The research was carried out in groups of children separately from Odesa and the city of Chernomorsk, assuming that children live in cities of the same climatic zone, but study in different educational institutions. We also took into account the different social level of families (living conditions, the nature of food, parental employment, the ability of families to engage children in different sections, etc.). Moreover, there is no developed network of sports clubs in Chernomorsk. Only a part of families have the opportunity to pay for children's sports in Odesa. After assessing their physical development, all children were divided into three groups depending on body mass index (BMI): normotrophy (BMI=20-25), hypertrophy (BMI>25), hypotrophy (BMI <20). First, we calculated the BMI by measuring the height (cm) of each child and determining the weight (kg). The results are presented in table 1.

Table 1

**Indicators of correspondence of height, weight and BMI in 12-year-old children**

| Indices height/weight | Chernomorsk (n=72) | | Odesa (n=79) | |
|---|---|---|---|---|
| | boys (n=41) | girls (n=31) | boys (n=43) | girls (n=36) |
| height (cm) | 150.19±0.41 | 149.0±0.47 | 151.04±1.01 | 154.17±1.22 |
| norm* | 144-155 | 146-155 | 144-155 | 146-155 |
| weight (kg) | 41.92±0.56 | 37.38±0.61 | 44.57±1.91 | 43.58±2.14 |
| norm* | 34.4-45.1 | 36-45.4 | 34.4-45.1 | 36-45.4 |
| BMI | 17.99±0.18 | 16.94±0.11 | 19.20±0.75 | 18.11±0.69 |
| norm* | 15.9-18.6 | 15.6-18.8 | 15.9-18.6 | 15.6-18.8 |

Note: * – norm indicators correspond to the data of centile tables.

There was a certain dependence of the BMI indicator on the place of residence. The 72 examined children of Chernomorsk had normotrophy in 45.83 %, malnutrition in 19.44 %, and hypertrophy in 34.72 %. Of the 79 children 12 years old of Odesa, 25.32 % had the same height and weight, 36.71 % had hypotrophy, and 37.97 % had hypertrophy.

Biochemical analysis was performed in the liquid portion of the mixed oral fluid of children. The collection of saliva was carried out in the morning, before meals, in centrifuge tubes for 10 minutes in accordance with the recommendations [10].

The determination of the activity of urease (a marker of microbial contamination) in the oral fluid was carried out by a method based on the ability of urease to split urea to ammonia. The intensity of the color of the sample is directly proportional to the activity of urease, which was expressed in μcatal/l of ammonia, formed in 1 second in 1 liter of oral fluid [10].

Determination of the level of lysozyme (an indicator of nonspecific immunity) in the oral fluid of children was carried out by the bacteriolytic method. When lysozyme interacts with the substrate of bacteria *Micrococcus lysodeikticus*, a clarification of the substrate solution is observed, which is recorded spectrophotometrically. The degree of clarification is proportional to the activity of lysozyme, which was expressed in u/l of oral fluid [8].

The degree of dysbiosis was calculated from the ratio of the relative activities of urease and lysozyme according to Levitsky [9]. Normally, in healthy individuals, this indicator equals 1. In the presence of dysbiotic phenomena>1. The more pronounced the dysbiosis, the greater the indicator of the degree of dysbiosis.

In saliva, the level of inflammation markers was determined: the content of malondialdehyde (MDA) and elastase activity [1]. MDA content – by reaction with 2-thiobarbituric acid. Normally, the concentration of MDA in the oral fluid is 0.3±0.07 mmol/l.

Biochemical studies of the activity of the leukocyte enzyme elastase, reflecting the degree of inflammatory processes in the oral cavity, were carried out in the oral fluid of children. The elastase activity

was assessed by the degree of hydrolysis of the synthetic substrate N-t-BOC-L-alanine-p-nitrophenyl ester (BOC) ("Sigma", USA) by the Visser method. Elastase activity is expressed in microcatals per 1 liter of oral fluid. Normally, elastase activity in the oral fluid is 8.0±1.0 μkat/l.

The results were processed by variational and statistical methods of analysis on an IBM PC in SPSS SigmaStat 3.0 and StatSoft Statistica 6.0 software [3].

**Results of the study and their discussion.** Our studies to determine the level of a marker of microbial contamination of the oral cavity - urease activity in the oral fluid - indicate its significant increase in children with deviations from the norm, both towards hypertrophy and towards hypotrophy. This pattern is seen in both children of Odesa, and children of Chernomorsk. As for the index of nonspecific immunity of the oral cavity (lysozyme activity in oral fluid), its level is significantly increased in children of Odesa with BMI deviations from the norm. At the same time, in the oral fluid of children from Chernomorsk with BMI<20, the activity of lysozyme is slightly reduced, which indicates an insufficient level of antimicrobial protection in the oral cavity of these children (table 2).

Table 2

**Activity of urease and lysozyme in the oral fluid of children of Odesa and Chernomorsk depending on BMI (M±m)**

| No | Groups | Urease activity, мkat/l | Lysozyme activity, u/l |
|---|---|---|---|
| Odesa | | | |
| 1 | BMI=20-25 | 0.071±0.069 | 78±10 |
| 2 | BMI>25 | 0.214±0.010; p<0.001 | 147±18; p<0.05 |
| 3 | BMI<20 | 0.177±0.014; p<0.01; $p_1$<0.05 | 149±20; p<0.05; $p_1$>0.8 |
| Chernomorsk | | | |
| 1 | BMI=20-25 | 0.046±0.0608 | 83±13 |
| 2 | BMI>25 | 0.130±0.011; p<0.01 | 76±12; p>0.5 |
| 3 | BMI<20 | 0.120±0.010; p<0.01; $p_1$>0.3 | 53±8; p<0.05; $p_1$>0.05 |

Note: p – an indicator of the significance of differences compared with group 1; $p_1$ – compared with group 2.

Table 3 shows the results of determining the degree of oral dysbiosis by the method of Levitsky A. P. The obtained results indicate the increase of the degree of dysbiosis in the oral cavity in children both in Odesa (1.61 and 1.30) and in Chernomorsk (3.08 and 4.02). The low degree of oral dysbiosis in children of Odesa on the background of a high level of parodontal indices can be explained by the increased (almost twice as compared with the norm) activity of lysozyme in their oral fluid. An increase in the activity of the antimicrobial enzyme lysozyme in the oral fluid, as a rule, is observed at the initial stages of dental diseases or during the period of remission, when high activity of lysozyme can be considered as an adaptive response to the development of pathology. The most significant increase in the degree of dysbiosis was found in the oral cavity of children of Chernomorsk.

Table 3

**Degree of oral dysbiosis, salivation rate and pH of the oral fluid in children from Odesa and Chernomorsk depending on BMI (M±m)**

| No | Groups | Degree of dysbiosis | |
|---|---|---|---|
| | | Odesa | Chernomorsk |
| 1 | BMI=20-25 | 1.00±0.15 | 1.00±0.15 |
| 2 | BMI>25 | 1.61±0.22; p<0.05 | 3.08±0.35; p<0.01 |
| 3 | BMI<20 | 1.30±0.18; p>0.2; $p_1$>0.2 | 4.02±0.59; p<0.001; $p_1$<0.05 |
| Odesa | | | |
| | | Salivation rate, ml/min | pH, unit |
| 1 | BMI=20-25 | 0.71±0.10; | 7.78±0.16 |
| 2 | BMI>25 | 1.08±0.15; p<0.05 | 6.34±0.18; p<0.01 |
| 3 | BMI <20 | 0.80±0.10; p>0.3; $p_1$>0.05 | 6.22±0.19; p<0.01; $p_1$>0.5 |
| Chernomorsk | | | |
| 1 | BMI=20-25 | 0.74±0.10 | 6.71±0.24 |
| 2 | BMI>25 | 0.72±0.13; p>0.5 | 7.10±0.25; p>0.2 |
| 3 | BMI<20 | 0.77±0.16; p>0.5; $p_1$>0.5 | 7.33±0.42; p>0.05; $p_1$>0.3 |

Note: p – an indicator of the significance of differences compared with group 1; $p_1$ – compared with group 2.

Table 3 also presents the results of determining the rate of salivation and the pH of the oral fluid of Odesa and Chernomorsk children.

From the presented data it can be seen that in children of Odesa, the salivation rate significantly increases only in persons with increased body weight, and the reaction of the oral fluid in persons becomes weakly acidic. Increased acidity in the mouth increases the risk of caries (this is a favorable environment for the reproduction of pathogenic microorganisms), as well as a provocateur of parodontal disease and other dental problems. Low pH in saliva is a sign of chronic acidification, a chronic process that negatively affects bone tissue. An acidic environment interferes with the normal assimilation of calcium, and the body draws its deficiency from the reserve. In Chernomorsk children, the salivation rate does not depend on the BMI index and, in contrast to Odesa children, the slightly acidic oral fluid becomes slightly alkaline. The shift in pH is due, in our opinion, to an alimentary factor.

Table 4 shows the results of determining the level of biochemical markers of inflammation in the oral fluid. In children of Odesa with BMI>25 or BMI<20, a decrease in the level of inflammation markers is observed, while in children of Chernomorsk they change slightly, with the exception of a slightly reduced level of MDA in children with hypertrophy.

Table 4

**The level of biochemical markers of inflammation of the oral fluid of children in Odesa and Chernomorsk depending on BMI (M±m)**

| No | Groups | MDA content, mmol/l | Elastase activity, мkat/l |
|---|---|---|---|
| Odesa | | | |
| 1 | BMI=20-25 | 0.202±0.018 | 0.197±0.020 |
| 2 | BMI>25 | 0.167±0.020; p>0.05 | 0.076±0.009; p<0.01 |
| 3 | BMI<20 | 0.130±0.018; p<0.05; $p_1$>0.05 | 0.072±0.010; p<0.001; $p_1$>0.5 |
| Chernomorsk | | | |
| 1 | BMI=20-25 | 0.149±0.013 | 0.150±0.018 |
| 2 | BMI>25 | 0.114±0.015; p<0.005 | 0.176±0.020; p>0.3 |
| 3 | BMI<20 | 0.152±0.012; p>0.5; $p_1$<0.01 | 0.176±0.018; p>0.3; $p_1$=1.0 |

Note: p – an indicator of the significance of differences compared with group 1; $p_1$ – compared with group 2.

It is necessary to pay attention to the low activity of elastase in the oral fluid of children of Odesa. The greatest amount of elastase is found in neutrophils. Since this enzyme has a neutrophilic origin, we can assume the presence of delayed immunodeficiency in children with deviations in the body weight of adolescents in Odesa. Neutrophil elastase reduces the inflammatory response to invasion by microorganisms. The results of biochemical studies of the oral fluid in 12 years old children with different BMI indicate a significant increase in urease activity in children with BMI deviations from the norm in examined settlements, both from the side of hypo- and hypertrophy. This fact indicates a high level of contamination with opportunistic bacteria in the oral cavity in children of the observed groups [2]. The activity of the secretory enzyme lysozyme is reduced in Chernomorsk children with BMI<20, however, an increase in the activity of lysozyme in the oral fluid, as a rule, indicates the initial stage of the disease, or a period of remission. The high activity of lysozyme can be considered as an adaptive response to the development of pathology. An imbalance of these indicators causes the development of oral dysbiosis, on the background of which degenerative changes in the oral cavity develop, which to a large extent contributes to a decrease in the level of the protective systems of the oral cavity. The obtained results indicate the increase of the degree of dysbiosis in the oral cavity in children both in Odesa (1.61 and 1.30) and in the Chernomorsk (3.08 and 4.02). Thus, the degree of dysbiosis in the oral cavity of children, especially of Chernomorsk, clearly reflects the relationship between antimicrobial protection in the oral cavity and the degree of contamination of opportunistic and pathogenic microflora. The microflora of the oral cavity is a highly sensitive indicator system that reacts with qualitative and quantitative changes to alterations in the state of various organs and systems of the body, especially in childhood. It is important to note that the development of dysbiosis can also occur in the event of malnutrition by persons who abuse sweets [11, 15].

The development and course of parodontal disease and caries in children on the background of hypo- and, especially, hypertrophy has its own characteristics. These comorbid conditions are factors that provoke diseases of parodontal tissues and hard tissues of teeth and worsen their course. It should be noted that the treatment of children with such comorbid diseases has certain difficulties, which is associated with

an additional aggravating pathogenesis and a large number of drugs that are not always sufficiently effective and safe for this category of patients [8].

In our studies, we have shown the dependence of the acidity of saliva on the rate of salivation. In our opinion, the shift in pH is due to the alimentary factor.

In adolescents of Odesa, whose BMI index deviates from the norm, we observe a decrease in the level of inflammation markers, while in children of Chernomorsk they change insignificantly. On the background of deviations in body weight both in the direction of hypo- and in the direction of hypertrophy in the oral cavity, there are microbiocenosis disorders (urease activity), more pronounced in children of Chernomorsk, and a decrease of defense reactions (a decrease of elastase activity and MDA level) in children of Odesa. To address the issue of causality and effect in the case of overweight and underweight with caries and parodontal disease, further research is needed to determine whether hypo- and hypertrophy are true risk factors for the development of dental diseases, especially among children [6, 12, 13, 14, 15]. We hypothesized that an insufficient level of inflammatory response may be the cause of the formation of chronic parodontitis.

## Conclusion

The obtained data indicate the beneficial effects of hypotrophy and hypertrophy for the development of dental diseases in children. In our opinion, dysbiotic and inflammatory reactions in the oral cavity are protective against the parodontium. Thus, prospects for further research consist in the development of an effective method for the prevention of major dental diseases in school-age children, depending on the body mass index, with the use of an appropriate therapeutic and prophylactic complex and adjusting the diet and oral hygiene.

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