

CATION COMPOSITION OF ORAL SECRETION IN PRIMARY SCHOOL-AGED CHILDREN

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SUMMARY. Recurrent respiratory infections (RRIs) currently have the greatest medical and social burden among infectious diseases worldwide, with the highest prevalence and incidence rates of acute respiratory infections in children. Recent studies indicate the role of inadequate immune response and the development of recurrent acute respiratory infections (ARI) in children due to deficiencies in essential trace elements in the body, which are crucial for the proper functioning of physical barriers and the immune system. The hypothesis of our study assumed that, considering the recurrence of at least 6 episodes of ARI per year, children with RRIs may have changes in salivary ion levels, which could serve as a basis for local immune suppression of the upper respiratory mucosa.

The aim – to investigate the levels of metals in the saliva of children of primary school age with recurrent respiratory infections in comparison with episodically ill peers and establish the relationship of these changes with the state of local immunity.

Material and Methods. In the actual study, 40 children participated, with 30 of them (the main group) being children with recurrent respiratory infections and 10 other children who had episodic illnesses were assigned to the control group. The levels of metal cations in the saliva were examined using inductively coupled plasma atomic emission spectrometry, including essential metals such as copper, manganese, zinc, calcium, cobalt, potassium, magnesium, sodium, phosphorus, selenium, iron, chromium, and sulfur. Additionally, levels of conditionally essential metals like lithium, nickel, and boron were measured. The levels of secretory IgA and lysozyme in saliva were determined using the enzyme-linked immunosorbent assay method.

Results. The main group was not homogeneous; therefore, we divided it into two clusters. Respondents in one cluster had significantly lower relative levels of essential ion content in saliva. Specifically, calcium was lower in 64 % ($p=0.003$), copper in 76.5 % ($p=0.001$), manganese in 41.1 % ($p=0.008$), magnesium in 64 % ($p=0.02$), and zinc in 58.8 % ($p=0.027$). This fact can be explained by their increased losses due to frequent inflammatory processes in the upper respiratory tract, resulting in higher daily replenishment needs compared to their peers. Correlation analysis revealed a connection between illness incidence and the levels of copper ($p=0.008$), sodium ($p=0.00005$), and phosphorus ($p=0.028$), indicating potential associations between saliva metal profiles and the frequency of ARI in children with RRIs. The presence of dental caries correlated with lithium ($p=0.02$), copper ($p=0.032$), and nickel levels ($p=0.037$). Integral indicators of local immunity, such as lysozyme and secretory IgA, exhibited correlations with metal levels in saliva (Li, Cu, Ni, Na, Co, P, Zn), confirming the hypothesis of their role in regulating immune reactions in the oral cavity.

Conclusions. Cluster analysis of the metal profiles of the group of children with RRI shows its heterogeneity, 43 % of representatives of this cohort had significantly lower levels of calcium ($p=0.003$), copper ($p=0.001$), manganese ($p=0.008$), magnesium ($p=0.02$) and zinc ($p=0.027$) than the control group. Which may indicate the presence of deficient conditions in some children with PRI as a result of the increased need for their recovery. The presence of caries, the level of SIgA, lysozyme (clinical indicators of local immunity of the oral cavity) have certain relationships with the levels of essential and conditionally essential levels of salivary metals (Li, Cu, Ni, Na, Co, P, Zn), which indicates the benefit of the connection between the metal profile of saliva and the state of local immunity.

KEY WORDS: children; RRIs; trace elements; saliva metal profile; ionome.

Introduction. Recurrent respiratory infections (RRIs) currently impose the greatest medical and social burden among clusters of infectious diseases worldwide, with the highest prevalence and incidence rates of acute respiratory infections in children [1, 2]. RRIs in children typically result in frequent visits to healthcare professionals, worsen quality of life and socio-economic conditions of families, and contribute to polypharmacy and irrational use of antibiotics. On the other hand, RRIs affect the health status and physical development of children, shape transient immune response characteristics in these patients, reduce their adaptive abilities and socialization, impact academic performance, and worsen intra-family relationships [3, 4].

The balance of macro- and microelements in various biological fluids of the body is one compo-

nent that determines human health status. Recent studies indicate the role of deficiencies in essential microelements in the body in disrupting adequate immune response and the development of recurrent acute respiratory infections (ARIs) in children, which are crucial for the proper functioning of physical barriers and the immune system [5]. For instance, according to Baima G. et al. (2022) [6], an imbalance in salivary ion levels may be associated with local inflammation and oxidative tissue damage in the oral cavity, which aligns with findings from other authors (Kalaivani Natarajan 2016, Sumit Gaur 2017, Marín Martínez 2018, Sejdini M. 2018, Elif Inonu 2019, Polletto A.C 2020, Federica Romano 2020) regarding the significance of changes in salivary ion balance and the risks of developing oral cavity diseases [7–13].

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The hypothesis of the current study is that, considering the recurrence of at least 6 episodes of ARI per year, children with RRIs may have changes in salivary ion levels, which could serve as a basis for local immune suppression of the upper respiratory mucosa.

The aim – to investigate the levels of metals in the saliva of children of primary school age with recurrent respiratory infections in comparison with episodically ill peers and establish the relationship of these changes with the state of local immunity.

Material and Methods. The study involved 40 children, with 30 of them (main group) being children with recurrent respiratory infections that met the criteria of the Inter-society Consensus on Recurrent Respiratory Infections in 2021 [2]. The remaining 10 children who had episodic illnesses were assigned to the control group. Written consent for the examination of their children was obtained from the parents. The study was conducted in accordance with the principles of bioethics outlined in the Helsinki Declaration "Ethical Principles for Medical Research Involving Human Subjects" and the "Universal Declaration on Bioethics and Human Rights" (UNESCO).

Inclusion criteria for the main group were children aged 5–7 years with recurrent respiratory infections in a state of somatic well-being at the time of the study, according to the consensus. The control group consisted of children aged 5–7 years who had respiratory illnesses less than 5 times per year.

Exclusion criteria: children with signs of acute infectious processes or exacerbation of chronic infectious foci at the time of the study, presence of dental metal structures, severe hereditary diseases (cystic fibrosis, immunodeficiencies, etc.), and refusal of the child or parents to participate in the study.

Saliva (oral cavity secretions) was collected between 8 and 10 a.m. Donors were asked to rinse their mouths with 100 ml of distilled water before sample collection. Then, 5 ml of unstimulated saliva were collected in plastic tubes placed on ice and stored at a temperature of -20 degrees Celsius until further analysis in the laboratory.

The levels of metal cations in the oral cavity secretions were examined, including:

1. Essential metals: copper, manganese, zinc, calcium, cobalt, potassium, magnesium, sodium, phosphorus, selenium, iron, chromium, sulfur.

2. Conditionally essential metals: lithium, nickel, boron.

Measurement of metal levels in saliva was performed at the laboratory of NVTК "Center," State Institution "DMA Ministry of Health of Ukraine," using the inductively coupled plasma atomic emission spectrometry method on iCAP 7000 Duo (iCAP 7200 Duo modification) by Thermo Fisher Scientific. Multi-element standard for ICP VIII Certipur® (diluted in

nitric acid) was used as the standard solutions.

Measurement of secretory IgA and lysozyme levels was carried out at the "Pharmacies of Medical Academy" LLC using a photometer for microplates HiPo MPP-96, with the assistance of DKO078IgA Saliva ELISA DiaMetra Italy and Human LZM (Lysozyme) ELISA Kit Elabscience test systems.

The statistical analysis of the data was performed using traditional methods of descriptive statistics, utilizing licensed software programs such as Microsoft Excel and SPSS trial v.29. Since over 90 % of the data deviated significantly from a normal distribution, nonparametric statistical methods were preferred. The obtained results were considered statistically significant at a significance level (p-value) less than 0.05.

Results and Discussion. Respondents from the main group (n=30) and the control group (n=10) did not differ significantly in terms of age and gender. Based on the data obtained in the actual study, the mean levels of essential and conditionally essential metal cations in saliva did not show statistically significant differences when comparing children from the main and control groups. This led to the necessity of conducting hierarchical cluster analysis using the Ward's method. The dendrogram plot of the indicators of essential and conditionally essential micronutrient cation content in children from the main group is presented in Fig. 1.

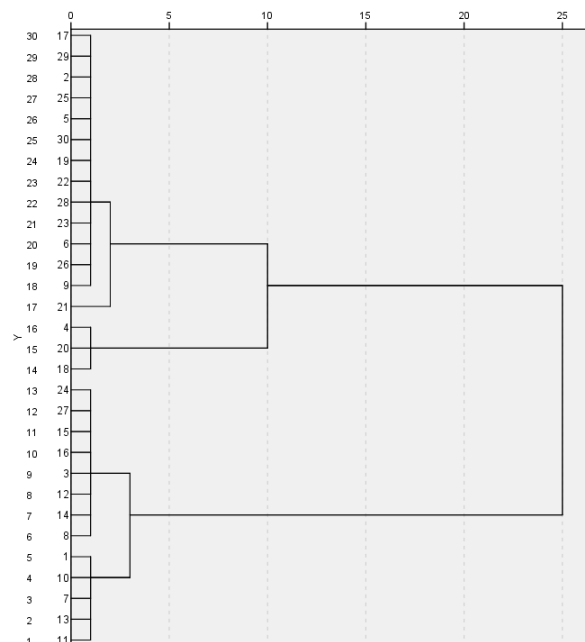


Fig. 1. Dendrogram of cluster analysis of essential and conditionally essential micronutrient cation content in children with RRIs obtained using the Ward's method.

According to the dendrogram, we can conclude that the main group of children with RRI was not ho-

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mogeneous, which allowed us to divide the main group into 2 major clusters: subgroup Main 1 (n=13) and subgroup Main 2 (n=17). Further comparative analysis of the characteristics of saliva ion components in children with RRI was conducted within these two subgroups (Cluster 1 and Cluster 2), comparing them with the results from the control group (practically healthy peers).

Taking into account the heterogeneity of data from different authors regarding the normal levels of metals in saliva in the selected age group (5–7 years) and considering the industrial region affiliation of these patients, we decided to consider the indicators of the control group as normal (within the range of Q1-Q3).

With the aim of identifying possible a priori statistically significant differences in the indicators of salivary metal cation levels among the participants of these groups, we used the calculation of the Kruskal-Wallis test (Table 1).

To determine the posterior difference between the cluster subgroups and the control group, we conducted an analysis with pairwise comparisons among all components (subgroup 1, subgroup 2, control group) using the Mann-Whitney test. We found that only children from cluster 2 of the main group and the control group had a statistically significant difference in the levels of certain salivary metals (Fig. 2).

According to our data, children from the second subgroup with RRIs had significantly lower relative levels of only essential salivary ions, specifically calcium by 64 % (p=0.003), copper by 76.5 % (p=0.001), manganese by 41.1 % (p=0.008), magnesium by 64 % (p=0.02), and zinc by 58.8 % (p=0.027). It is possible

Table 1. Results of determining the a priori statistically significant differences in the indicators of salivary metal profiles among the participants of the study groups.

	Kruskal-Wallis test (p)
Chrome	0.024
Lithium	0.006*
Copper	0.0001*
Manganese	0.0001*
Zinc	0.002*
Selenium	0.129
Calcium	0.0001*
Cobalt	0.001*
Iron	0.021*
Potassium	0.0001*
Magnesium	0.001*
Sodium	0.007*
Nickel	0.005*
Phosphorus	0.001*
Sulfur	0.004*
Boron	0.395

that the decreased levels of these essential salivary metals in children from cluster 2 of the main group can be explained by their increased losses due to frequent inflammatory processes in the upper respiratory tract and, as a result, higher daily requirements for replenishment compared to their peers [14].

To confirm or refute the hypothesis of the actual study, we conducted a correlation analysis of the relationships between the levels of essential and conditional essential salivary metals and the incidence of RRI over the past year in cluster 2 of the main group (n=17) and in children from the control group.

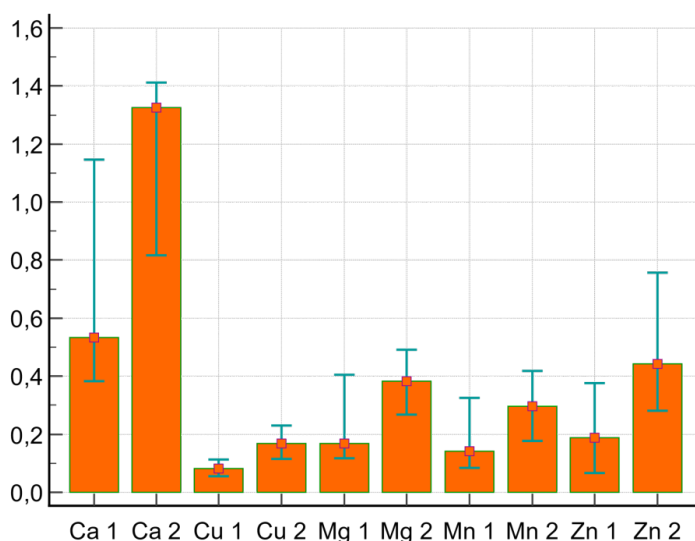


Fig. 2. Comparison of mean levels of essential salivary metals in children from cluster 2 of the main group and the control group.

In the control group, no statistically significant correlations were found. In cluster 2 of the main group, we found a correlation between the incidence of RRI and the levels of copper ($p=0.008$), sodium ($p=0.00005$), and phosphorus ($p=0.028$). This suggests the presence of certain associations between salivary metal profiles and the frequency of RRI in the group of children with RRI. According to the literature, the level of phosphorus and sodium in saliva is closely related to oral health and serves as a prognostic indicator of periodontal diseases. The antimicrobial action of electrolytes, such as Na, present in saliva, increases in response to infection in the oral cavity [15,16]. Copper is an essential component of antioxidant enzymes and prevents tissue damage caused by reactive oxygen species. There is evidence that Cu can dissolve in mixed saliva during demineralization caused by tooth decay [17]. These facts prompted our next step in searching for possible correlations between data on existing dental caries (disruption of local immunity of the oral cavity) in children from the main group ($n=30$) and salivary metal levels (Table 2).

Table 2. Data of correlation analysis between the presence of dental caries in the oral cavity of children with RRI and salivary metal levels

Name of saliva metal	The presence of caries (r;p)
Lithium	-0.378; 0.02
Copper	0.315; 0.032
Nickel	0.331; 0.037

The obtained data are consistent with similar international studies. It has been proven that lithium reduces the incidence of dental caries in humans [18, 19], unlike copper [20].

Most studies on nickel are associated with its release from metal dental constructions and emphasize that nickel forms complexes with arsenides and sulfides, which are known carcinogens, allergens, and mutagens [21]. Therefore, the observed correlation between salivary metal levels and dental caries in children with RRI in our study requires further investigation.

The final stage of our research was to search for possible correlations between salivary metal profiles and indicators of local immune response in the oral cavity of children from Cluster 2 of the main group. The results are presented below (Fig. 3).

Taking into account the fact that lysozyme is an enzyme that breaks down bacterial cell wall peptidoglycan, leading to their lysis and is more active against gram-positive bacteria (providing antibacterial defense), and secretory IgA acts by blocking the adhesion of viruses to epithelial cells and suppressing intracellular viral replication, with indirect antibacterial effects mediated through the activation of phagocytes and lymphocytes (predominantly antiviral effect) [22], we can confirm the hypothesis regarding the role of micronutrients in the regulation of local immunity processes.

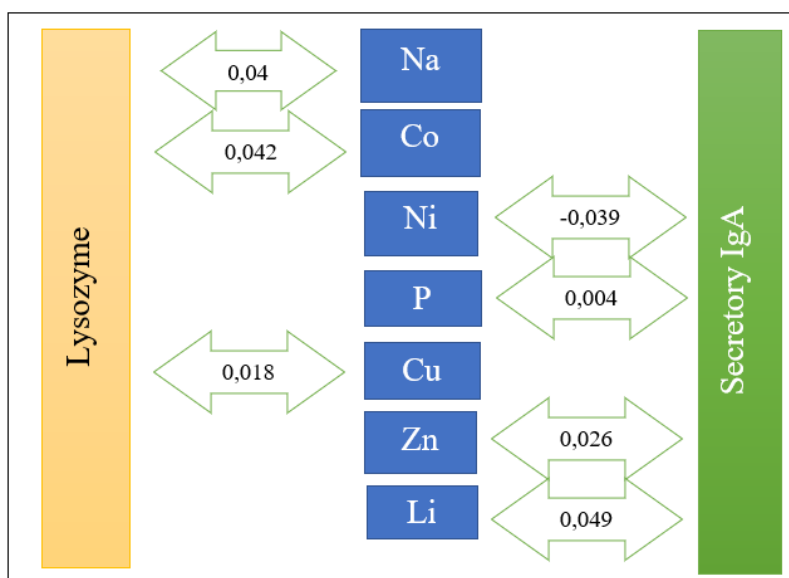


Fig. 3. Correlation analysis between metal levels, lysozyme, and secretory IgA in saliva of children from Cluster 2 of the main group (p levels).

Conclusions. Cluster analysis of the metal profiles in the group of children with RRI indicates its heterogeneity, as 43 % of the participants in this cohort had significantly lower levels of calcium

($p=0.003$), copper ($p=0.001$), manganese ($p=0.008$), magnesium ($p=0.02$), and zinc ($p=0.027$) compared to the control group. This may suggest the presence of deficiency states in some children with RRI

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The presence of dental caries, levels of SigA and lysozyme (clinical indicators of local immunity in the

oral cavity) exhibit certain correlations with levels of essential and conditionally essential metals in saliva (Li, Cu, Ni, Na, Co, P, Zn) indicating a link between salivary metal profile and the state of local immunity.

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КАТІОННИЙ СКЛАД СЕКРЕТУ РОТОВОЇ ПОРОЖНИНИ У ДІТЕЙ МОЛОДШОГО ШКІЛЬНОГО ВІКУ

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РЕЗЮМЕ. Рекурентні респіраторні інфекції (PPI) наразі мають найбільший медико-соціальний тягар у кластері інфекційних хвороб у світі з найвищими показниками розповсюдженості та захворюваності на гострі респіраторні інфекції у дитячому віці. Дослідження останніх років вказують на роль у порушенні адекватної імунної відповіді та розвитку рекурентного перебігу гострих респіраторних інфекцій (ГРІ) у дітей, есенціальних мікроелементів в організмі, які забезпечують належне функціонування фізичних бар'єрів та імунної системи. Гіпотезою нашого дослідження було припущення, що з огляду на повторюваність щонайменше 6 разів на рік епізодів ГРІ, діти з PPI можуть мати зміни іоному слини, що буде виступати підґрунтям зниження місцевого імунітету слизових верхніх дихальних шляхів.

Мета – дослідити рівні металів у слині дітей молодшого шкільного віку з рекурентними ГРІ у порівнянні з однолітками, які хворіють епізодично, та встановити зв'язок цих змін зі станом місцевого імунітету.

Матеріал і методи. У нашому дослідженні взяли участь 40 дітей, 30 з них (основна група) були з рекурентним перебігом респіраторних хвороб; 10 інших дітей, які хворіли епізодично, були віднесені до групи контролю. У секреті ротової порожнини методом атомно-емісійної спектроскопії з індуктивно-зв'язаною плазмою перевіряли рівні катіонів металів: есенціальних – мідь, марганець, цинк, кальцій, кобальт, калій, магній, натрій, фосфор, селен, залізо, хром, сірка; умовно есенціальних: літій, нікель, бор. Методом ферментного імуносорбентного аналізу визначали рівні секреторного IgA та лізоциму слини.

Результати. Основна група не була однорідною, тому нами було проведено її поділ на два кластери, респонденти одного із яких мали вірогідно нижчі відносні показники вмісту есенціальних іонів слини, а саме кальцію на 64 % ($p=0,003$), міді на 76,5 % ($p=0,001$), марганцю на 41,1 % ($p=0,008$), магнію на 64 % ($p=0,02$) та цинку на 58,8 % ($p=0,027$). Цей факт можна пояснити їх підвищеними втратами на фоні частих запальних процесів верхніх дихальних шляхів, і, як наслідок, вищими, ніж в одноліток, щоденними потребами відновлення. При кореляційному аналізі було знайдено зв'язок між захворюваністю та рівнями міді ($p=0,008$), натрію ($p=0,00005$), фосфору ($p=0,028$), що може свідчити про наявність певних взаємозв'язків між металопрофілем слини та частотою захворюваності на ГРІ у групі дітей з PPI. Наявність карієсу корелювала з рівнями літію ($p=0,02$), міді ($p=0,032$) та нікелю ($p=0,037$). Інтегральні показники місцевого імунітету – лізоцим та секреторний IgA – мали кореляційні зв'язки з рівнями металів слини (Li, Cu, Ni, Na, Co, P, Zn), що підтверджувало гіпотезу про їх роль у регуляції імунних реакцій у ротовій порожнині.

Висновки. Кластерний аналіз металопрофілів у групі дітей з PPI свідчить про її неоднорідність, 43 % представників цієї когорти мали вірогідно менші, в порівнянні з групою контролю, рівні кальцію ($p=0,003$), міді ($p=0,001$), марганцю ($p=0,008$), магнію ($p=0,02$) та цинку ($p=0,027$). Це може вказувати на наявність дефіцитарних станів у частини дітей з PPI в результаті підвищеної потреби їх відновлення. Наявність карієсу, рівень SIgA, лізоциму (клінічні показники місцевого імунітету ротової порожнини) мають певні взаємозв'язки з рівнями есенціальних та умовно есенціальних рівнів металів слини (Li, Cu, Ni, Na, Co, P, Zn), що свідчить на користь зв'язку між металопрофілем слини та станом місцевого імунітету.

КЛЮЧОВІ СЛОВА: діти; PPI; мікроелементи в слині; металопрофіль слини; іоном.

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