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SALIVARY TOXIC METALS AND LOCAL IMMUNITY IN YOUNGER SCHOOL-AGE CHILDREN WITH RECURRENT RESPIRATORY INFECTIONS

Abstract. Recently, there has been growing interest among scientists and clinicians in non-invasive diagnostic methods utilizing saliva as a diagnostic medium. Changes in the levels of specific salivary microelements play a crucial role in dental caries, periodontal disease exacerbations, local immune disorders. Since the status of the immune system at the entry gate is associated with the frequency of acute respiratory infections, our research aimed to explore the relationships between understudied toxic microelement influences in saliva and indicators of local immunity in children with recurrent respiratory infections. Purpose: to investigate the levels of toxic and potentially toxic metals in the saliva of primary school-age children and analyze their correlation with oral cavity local immune markers. The study involved 30 children aged 5-7 years with recurrent respiratory infections (main group) and 10 practically healthy children who experienced episodic acute respiratory infections (control group). The number of acute respiratory infection episodes in the main group was 7.64 (1.02) (M (SD)), while in the control group, it was 1 (0.63). The study was conducted in accordance with the Helsinki Declaration of the World Medical Association regarding the ethical principles for medical research involving human subjects. Saliva samples were measured for levels of toxic metals, including aluminum (Al), lead (Pb), barium (Ba), thallium (Tl), cadmium (Ca), strontium (Sr), bismuth (Bi), and potentially toxic metals, such as silver (Ag), gallium (Ga), and indium (In). Measurements were performed using inductively coupled plasma atomic emission spectroscopy. The levels of secretory IgA and lysozyme, as indicators of local immunity, were measured using a photometer for microplates (HiPo MPP-96) with the help of test systems. Comparative analysis of data on the content of toxic and potentially toxic metals in children from the main

and control groups allowed us to establish significantly higher levels of Al, Sr, Ag, and In in the saliva of children with recurrent respiratory infections. The high frequency of acute respiratory infections in children with recurrent episodes is associated with the levels of lead, cadmium, and indium in saliva. Moreover, elevated levels of lead and cadmium in saliva exert a suppressive influence on secretory IgA, an integral indicator of oral cavity local immunity. On the other hand, the presence of dental caries in children with recurrent respiratory infections is associated with a high content of indium, which, in turn, also shapes the phenomenon of recurrent respiratory infections. We discovered that children aged 5 to 7 years with recurrent respiratory infections, during the period of clinical well-being, have higher average levels of toxic (Al, Sr) and potentially toxic (Ag, In) metal ions in saliva compared to their episodically ill peers. The study confirms the impact of increased levels of cadmium and lead in saliva on secretory IgA and the influence of high levels of Cd, Pb, and In ions on the development of recurrent respiratory infections.

Keywords: toxic metals, salivary ions, school-age children, recurrent respiratory infections.

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ТОКСИЧНІ МЕТАЛИ СЛИНИ ТА ЛОКАЛЬНИЙ ІМУНІТЕТ У ДІТЕЙ МОЛОДШОГО ШКІЛЬНОГО ВІКУ З РЕКУРЕНТНИМИ РЕСПІРАТОРНИМИ ІНФЕКЦІЯМИ

Анотація. Останнім часом зростає інтерес науковців та клініцистів щодо неінвазивних методів діагностики з використанням слини, як діагностичного середовища. Зміна вмісту певних мікроелементів слини є визначальною при карієсі, загостреннях хвороб пародонту, порушеннях місцевого імунітету тощо. Оскільки стан імунітету вхідних воріт асоційований з частотою захворюваності на гострі респіраторні інфекції, напрямком нашого дослідження було обрано пошук взаємозв'язків між недостатньо вивченим впливом токсичних мікроелементів слини та показниками місцевого імунітету у дітей з рекурентними респіраторними інфекціями. Мета: дослідити рівні

токсичних та потенційно токсичних металів слини у дітей молодшого шкільного віку та проаналізувати їх зв'язок з показниками місцевого імунітету ротової порожнини. До дійсного дослідження були залучені 30 дітей 5-7 років, які мали рекурентні респіраторні інфекції (основна група) та 10 практично здорових дітей, які хворіли на гострі респіраторні інфекції (ГРІ) епізодично (контрольна група). Кількість епізодів ГРІ в основній групі становила 7,64 (1,02) (M (SD)), та 1 (0,63) в контрольній групі. Дослідження було проведено відповідно до Гельсінської декларації Всесвітньої медичної асоціації про етичні принципи проведення наукових медичних досліджень за участю людей. В слині вимірювали рівні токсичних металів – алюміній (Al), свинець (Pb), барій (Ba), талій (Tl), кадмій (Cd), стронцій (Sr), вісмут (Bi) та потенційно токсичних – срібло (Ag), галій (Ga), індій (In). Вимірювання проведено методом атомно-емісійної спектроскопії з індуктивно-зв'язаною плазмою. Вимірювання рівнів секреторного IgA та лізоциму, як показників місцевого імунітету, проводилось фотометром для мікропланшетів HiPo MPP-96 з допомогою тест-систем. Порівняння даних щодо вмісту токсичних та потенційно токсичних металів у дітей основної та контрольної груп дозволило нам констатувати достовірно вищі рівні Al, Sr, Ag, In слини у дітей з РРІ. Високу кратність ГРІ у дітей з рекурентним перебігом структурують рівні свинцю, кадмію та індію слини. При цьому підвищений рівень свинцю та кадмію слини чинить пригнічувальний вплив на рівень секреторного IgA як інтегрального показника місцевого імунітету ротової порожнини. З іншого боку, наявність карієсу у дітей з РРІ обумовлює високий рівень вмісту індію, який, в свою чергу, також структурує феномен РРІ. Нами було виявлено, що діти віком від 5 до 7 років з РРІ в періоді клінічного благополуччя мають вищі за епізодично хворіючих одноліток середні рівні іонів токсичних (Al, Sr) та умовно-токсичних (Ag, In) металів слини. Доведено вплив підвищеного вмісту кадмію і свинцю слини на рівень секреторного IgA та вплив високого рівня іонів Cd, Pb, In на формування рекурентного перебігу ГРІ.

Ключові слова: токсичні метали, іоном слини, діти шкільного віку, рекурентні респіраторні інфекції

Introduction. In contemporary medical practice, the spectrum of non-invasive diagnostic investigations utilizing saliva, among others, is expanding, with particular significance in assessing children's health. Saliva serves as a diagnostic medium as it contains serum blood components, making it suitable for diagnosing various pathological conditions [1, 2]. Salivary secretions have already been successfully used for monitoring kidney diseases, preventing cardiometabolic risks, detecting drug use, as well as etiological diagnosis of bacterial and viral nucleic acids in acute respiratory cluster diseases in humans [3].

Research on salivary ionomics as one of the direct biomarkers of health status is currently being explored as an essential component of assessing local immunity

in the oral cavity. Numerous recommendations from scientists position it as an optimal diagnostic approach. It has been proven that changes in salivary microelement content play a crucial role in conditions such as dental caries, periodontal exacerbations and more [4]. Clinicians are particularly interested in toxic elements present in saliva, as the oral cavity becomes a target zone for their toxicity.

Since the state of immunity at the entry gate is associated with the frequency of acute respiratory infections, the focus of this study was to investigate the interrelationships between poorly studied toxic microelements in saliva and indicators of local immunity in children with recurrent respiratory infections (RRI).

The aim of this study was to examine the levels of toxic and potentially toxic metals in the saliva of younger school-age children and analyze their association with indicators of local immunity in the oral cavity.

Materials and Methods. For the present study, 30 children aged 5-7 years with recurrent respiratory infections (main group) and 10 practically healthy children who experienced episodic acute respiratory infections (ARI) (control group) were recruited. The main group had an average of 7.64 (1.02) (M (SD)) episodes of ARI, while the control group had 1 (0.63) episode.

Inclusion criteria for the main group were: children aged 5-7 years with recurrent respiratory infections according to the Intersocial Consensus 2021 [5]. Inclusion criteria for the control group were: children aged 5-7 years who experienced episodic ARI (practically healthy children).

Exclusion criteria included: children with active ARI or exacerbation of chronic conditions, presence of dental metal restorations, and parental or guardian refusal to participate in the study.

The present study was conducted in accordance with the Helsinki Declaration of the World Medical Association on ethical principles for medical research involving human subjects. Informed consent was obtained from all parents or guardians of the participating children.

Unstimulated mixed saliva samples were collected in the morning on an empty stomach from 30 children aged 5-7 years with recurrent respiratory infections (main group) and 10 practically healthy children who experienced episodic acute respiratory infections (control group). The saliva samples were collected in sterile plastic Eppendorf tubes (5 mL) placed on ice. Patients were instructed not to eat or brush their teeth two hours before saliva collection. Prior to sample collection, the oral cavity was rinsed with distilled water. The saliva samples were then stored frozen at -20 degrees Celsius until laboratory analysis.

Levels of toxic metals, including aluminum (Al), lead (Pb), barium (Ba), thallium (Tl), cadmium (Ca), strontium (Sr), and bismuth (Bi), as well as potentially toxic metals, including silver (Ag), gallium (Ga), and indium (In), were measured in the saliva samples. Measurement was performed using inductively coupled plasma atomic emission spectrometry on an iCAP 7000 Duo instrument (modification iCAP 7200 Duo) by Thermo Fisher Scientific. The minimum detection limit (LOD) for

most metals in saliva ranged from 0.1 to 0.5 parts per billion (ppb). Multi-element standard for ICP VIII Certipur® (Merck) was used as the standard solution.

Measurement of secretory IgA and lysozyme levels was conducted using a photometer for microplates (HiPo MPP-96) with DKO078IgA Saliva ELISA DiaMetra Italy and Human LZM (Lysozyme) ELISA Kit Elabscience test systems. Analyses were performed according to the manufacturer's instructions using provided controls.

Statistical analysis was carried out using Microsoft Excel and SPSS trial v.26 software. Normality of data distribution in the groups was assessed using the Shapiro-Wilk test. Since most parameters exhibited a normal distribution, parametric statistical tests were applied. Results were considered statistically significant at $p < 0.05$.

Comparison between the main and control groups did not reveal a statistically significant difference in age and gender distribution ($p > 0.05$), indicating comparability between these groups in other parameters.

Results and Discussion. According to our data, the highly dangerous toxic metals, thallium, and gallium, were not detected in the saliva samples of both the main and control groups. However, levels of the following metals in saliva – Al, Pb, Ba, Ca, Sr, Bi, Ag, and In – were determined during the actual investigation.

When comparing the data regarding the content of toxic and potentially toxic metals in the saliva of children from the main and control groups using the Mann-Whitney test, we obtained results that allowed us to identify distribution differences of these compounds in saliva depending on the presence or absence of recurrent respiratory infections in children aged 5-7 years.

Table 1

Comparison of the content of toxic and potentially toxic metals in the saliva of children from the main and control groups

Metals toxicity	Metal	The level of statistical significance of the difference (p) in metal content between the main and control groups
Toxic metals	Aluminum	0,009*
	Barium	0,052
	Cadmium	0,264
	Lead	0,059
	Strontium	0,02*
	Bismuth	0,414
Potentially toxic metals	Argentum	0,027*
	Indium	0,004*

*statistically significant differences

The comparison of the mean levels of metal ions in the saliva of children, which showed statistically significant differences (Figure 1), led us to conclude that children with RRI had higher levels of toxic elements in their saliva, specifically strontium and aluminum, compared to their relatively healthy counterparts. Additionally, they exhibited higher levels of potentially toxic elements, such as silver and indium, indicating changes in the salivary ionome of children with RRI even in a clinically asymptomatic state. This suggests the release of potentially hazardous metal ions into saliva, which, according to other researchers, can impact the immune response [6].

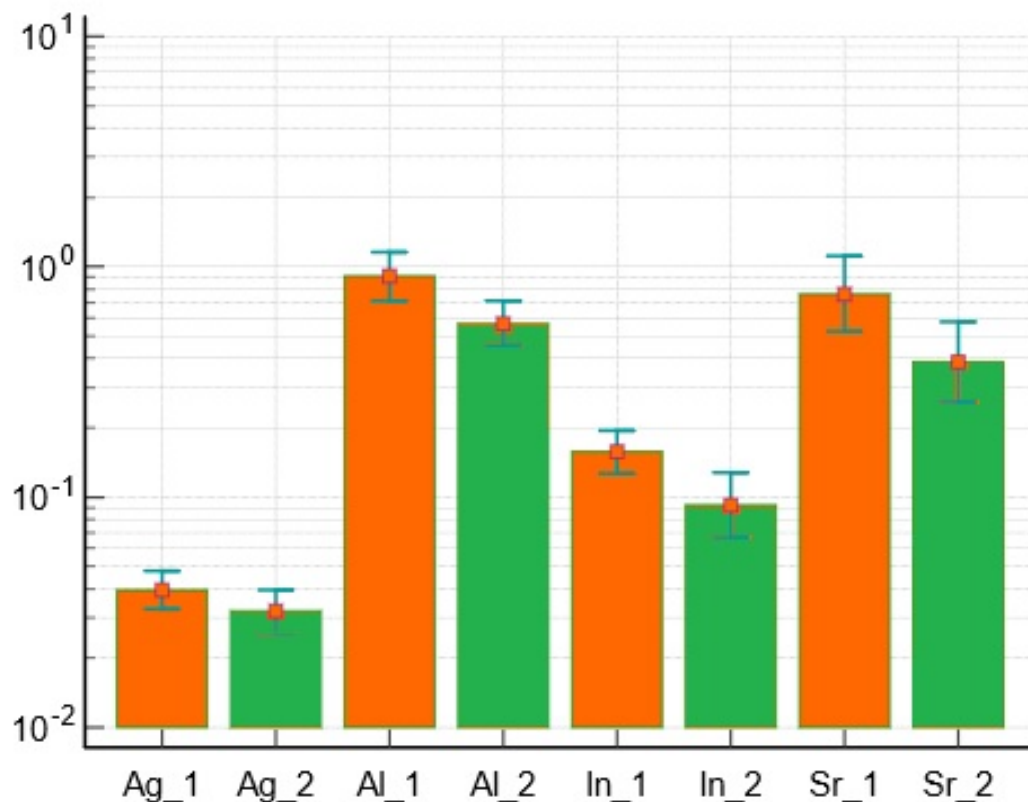


Fig.1. Comparison of mean metal levels in the saliva of children from the main group (1) and the control group (2) (mean with 95% confidence interval and logarithmic transformation).

Taking into account the purpose of the current study, we conducted a correlation analysis of the examination results of children in the main group (children with RRI) regarding the levels of toxic and potentially toxic metals in saliva, medical history data, and the results of immunological assessment (s-IgA, lysozyme) (Table 2).

Table 2

Correlations between the levels of metal ions in saliva, indicators of local immunity, and the phenomenon of RRI

Metals toxicity	Metal	Indicator	Bond strength and direction (r)	Level of statistical significance (p)
Toxic metals	Cadmium	Number of episodes of ARI	0,680	0,001344
	Lead		0,512	0,017851
	Lead	s-IgA level	-0,539	0,012707
	Cadmium	s-IgA level	-0,508	0,018680
Potentially toxic metals	Indium	Number of episodes of ARI	0,526	0,015017
	Indium	Caries	0,535	0,013377

According to our data, the following factors influenced the parameter of the number of ARI episodes, which defines and structures the RRI phenomenon: saliva cadmium, lead, and indium levels (Figure 2). In other words, the high frequency of ARIs in children with recurrent RRI is structured by the levels of lead, cadmium, and indium in saliva. Additionally, the elevated levels of lead and cadmium in saliva exert a reverse effect on the secretory IgA level, which serves as an integral indicator of local immunity in the oral cavity. On the other hand, the presence of dental caries in children with RRI predisposes to or determines a high level of indium content, which, in turn, also structures the RRI phenomenon.

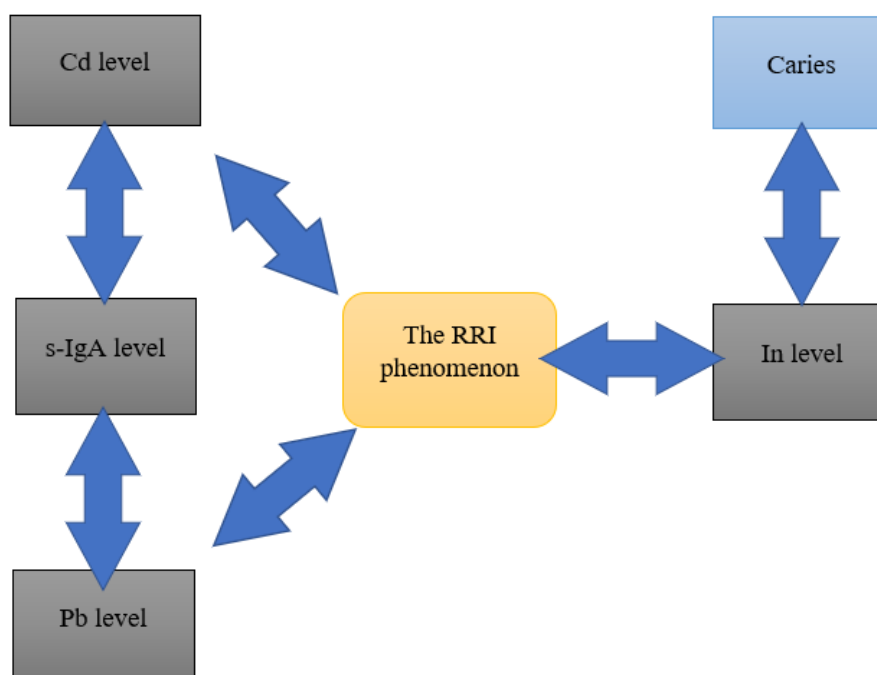


Fig. 2. Relationship between the RRI phenomenon and saliva metal level, secretory IgA and dental caries.

The generalization of our obtained data allows us to conclude the presence of a multilevel construct of the influence of toxic and potentially toxic metals on the formation of recurrent respiratory infections in children aged 5 to 7 years. This is supported by the confirmed associations between the levels of toxic and potentially toxic metals in saliva and the state of local immunity, as well as the presence of chronic infectious foci (dental caries).

Regarding the discussion of our novel findings on the relationship between the state of saliva ions and their impact on local immunity and their association with chronic infection foci (dental caries) in children, it is noteworthy that in the group of children with dental caries, the levels of strontium (Sr) and silver (Ag) were significantly higher compared to the group of examined children with healthy teeth. Additionally, it has been demonstrated that the level of strontium is significantly elevated due to dental caries, regardless of whether it was treated [7]. Aluminum, on the other hand, has a toxic effect on salivary glands even at low doses [8], which may indirectly contribute to the development of dental caries. Furthermore, according to the literature, cadmium and lead ions in saliva are considered caries-stimulating metals [9,10], therefore patients with periodontal diseases have higher levels of saliva cadmium, which can lead to exacerbations [14,15]. The International Agency for Research on Cancer (IARC) classifies cadmium and its compounds as a Group 1 carcinogen, associated with oral cavity cancer [11,12,13].

Considering the existing studies on the impact of toxic metals in saliva on immune response mechanisms, there are publications regarding the influence of the toxic metal cadmium on cell proliferation, differentiation, apoptosis, cell signaling, and other mechanisms of immune control [12,13]. The role of cadmium in local immunity of the oral cavity has been confirmed, as it stimulates cells to produce prostaglandins, cytokines (interleukins, tumor necrosis factor- α), and matrix metalloproteinases (MMP). According to the literature, the high content of indium ions in saliva can induce cytotoxicity [17], and we believe that further research is needed to address its long-term effects on the human body [18], especially considering the increasing environmental levels of indium due to its widespread use in industry.

In summary, the obtained findings emphasize the complex relationship between toxic and potentially toxic metals in saliva, local immune status, and the presence of dental caries in children with recurrent respiratory tract infections. Further research is warranted to deepen our understanding of these associations and their implications for health.

Thus, we can conclude that the impact of increased levels of cadmium and lead on the reduction of secretory IgA in children aged 5 to 7 years with recurrent respiratory infections in a state of clinical well-being, as observed in the actual study, may serve as a basis for the recurrent course of respiratory infections in these patients. By summarizing our findings and reviewing the literature on this specific issue, we can assert the coherence between the results of our own research and the existing literature regarding the influence of toxic metals on immune status.

Conclusions. Children aged 5 to 7 years with RRI in a state of clinical well-being exhibit higher average levels of toxic metals – aluminium ($p=0.009$), strontium ($p=0.02$) and potentially toxic metals – silver ($p=0.027$), indium ($p=0.004$) in saliva compared to episodically ill peers.

The influence of elevated levels of cadmium and lead in saliva on the level of secretory IgA has been demonstrated in children aged 5 to 7 years with RRI in a state of clinical well-being.

It has been established that high levels of cadmium ($p=0.001$), lead ($p=0.017$) and indium ($p=0.015$) ions have an impact on the development of recurrent episodes of respiratory infections in children aged 5 to 7 years with RRI in a state of clinical well-being.

Conflict of interest: None.

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