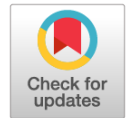


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# Associations between caries experience and oral fluid mineral content among patients in Arctic Russia: a study in the Nenets Autonomous Area

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## ABSTRACT

The relationship between the intensity of caries and mineral composition of oral fluid in adolescents in the Arctic zone of Russia was investigated using the example of the Nenets Autonomous Okrug. This study included 171 healthy boys and girls aged 15–17 years. The intensity of caries was assessed using the index caries + fillings + extracted permanent teeth and its components. Unstimulated oral fluid was collected into sterile tubes. Sodium, potassium, magnesium, phosphorus, and total and ionized calcium content and pH were examined in the oral fluid. Considering the pronounced right-sided asymmetry of the components of the index caries + fillings + extracted permanent teeth, calculations were performed using Poisson regression models. Results were presented as relative risks with 95% confidence intervals and as tests for trend. The prevalence of caries in the sample population did not differ by sex and was 87.8% for boys and 93.3% for girls ( $p = 0.221$ ). On average, 1.8 carious teeth per person were detected in boys and 1.4 in girls ( $p = 0.021$ ). No differences were found in the number of filled ( $p = 0.167$ ) and extracted ( $p = 0.981$ ) teeth. Additionally, the total sodium content in oral fluid was directly proportional to the index of caries + fillings + extracted permanent teeth ( $p = 0.040$ ) and number of carious teeth ( $p < 0.001$ ). The total oral fluid calcium was significantly associated with both the caries + fillings + extracted permanent teeth index ( $p = 0.019$ ) and number of filled teeth ( $p = 0.001$ ). Inverse relationships were found between the number of filled teeth and magnesium ( $p = 0.028$ ) and phosphorus ( $p = 0.037$ ) content. The study showed the presence of statistically significant relationships between the mineral composition of oral fluid and index of caries + fillings + extracted permanent teeth and its components. Thus, unfavorable living conditions in the Arctic zone provide additional risk factors for the occurrence of dental diseases. The results of the study, if confirmed in other populations, can be used to develop models for predicting the development and progression of caries in adolescents of the Far North. Further studies of the mineral composition of oral fluid are required to obtain more complete clinical and laboratory data, considering confounding factors such as nutrition and type of filling material.

**Keywords:** Arctic; Far North; dental diseases; caries; mineral composition of oral fluid; index caries + fillings + extracted permanent teeth; Nenets Autonomous Okrug; ionomics.

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# Минеральный состав ротовой жидкости и интенсивность кариеса у подростков Арктической зоны России на примере Ненецкого автономного округа

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## АННОТАЦИЯ

Оценивается связь между интенсивностью кариеса и минеральным составом ротовой жидкости у подростков в Арктической зоне Российской Федерации на примере Ненецкого автономного округа. Обследованы 171 относительно здоровые юноши и девушки в возрасте 15–17 лет. Интенсивность кариеса оценивали с помощью индекса кариес + пломбы + удаленные постоянные зубы и его компонентов. Сбор нестимулированной ротовой жидкости проводили в стерильные пробирки. В ротовой жидкости исследовали содержание натрия, калия, магния, фосфора, общего и ионизированного кальция, а также pH. Учитывая выраженную правостороннюю асимметрию компонентов индекса кариес + пломбы + удаленные постоянные зубы расчеты проводили с использованием регрессионных моделей Пуассона. Результаты представляли в виде относительных рисков с 95 % доверительными интервалами, а также в виде тестов для тренда. Выявлено, что распространенность кариеса в выборочной совокупности не различалась по полу и составила 87,8 % у юношей и 93,3 % у девушек ( $p = 0,221$ ). У юношей в среднем на человека было выявлено 1,8 кариозных зуба против 1,4 у девушек ( $p = 0,021$ ). Различий в количестве запломбированных ( $p = 0,167$ ) и удаленных ( $p = 0,981$ ) зубов не обнаружено. Установлено, что содержание общего натрия в ротовой жидкости было прямо пропорционально связано с индексом кариес + пломбы + удаленные постоянные зубы ( $p = 0,040$ ) и числом кариозных зубов ( $p < 0,001$ ). Общий кальций ротовой жидкости был значимо связан как с индексом кариес + пломбы + удаленные постоянные зубы ( $p = 0,019$ ), так и с количеством запломбированных зубов ( $p = 0,001$ ). Обратные связи были выявлены между количеством запломбированных зубов и содержанием магния ( $p = 0,028$ ) и фосфора ( $p = 0,037$ ). В целом проведенное исследование показало наличие статистически значимых связей между минеральным составом ротовой жидкости и индексом кариес + пломбы + удаленные постоянные зубы и его компонентами. Таким образом, неблагоприятные условия проживания в Арктической зоне обуславливают дополнительные факторы риска возникновения стоматологических заболеваний. Результаты исследования при их подтверждении в других совокупностях могут использоваться для разработки моделей прогнозирования развития и прогрессирования кариеса у подростков Крайнего Севера. Для получения более полных клинико-лабораторных данных требуется проведение более углубленных исследований минерального состава ротовой жидкости, с учетом смешивающих факторов, таких как питание и вид пломбировочного материала.

**Ключевые слова:** Арктика; Крайний Север; стоматологические заболевания; кариес; минеральный состав ротовой жидкости; индекс кариес + пломбы + удаленные постоянные зубы; Ненецкий автономный округ; иономика.

## Как цитировать

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## 以涅涅茨自治区为例，俄罗斯北极地区青少年口腔液的矿物质成分和龋齿强度

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### 摘要

以涅涅茨自治区为例，评估了俄罗斯联邦北极地区青少年龋齿强度与口腔液矿物质成分之间的关系。研究对象为171名15-17岁相对健康的男孩和女孩。使用龋齿+补牙+填充物+拔除恒牙指数及其组成部分评估龋齿强度。使用无菌试管收集未经刺激的口腔液。检测口腔液中钠、钾、镁、磷、总钙和离子钙的含量以及 pH 值。考虑到龋齿+补牙+拔除恒牙指数的各组成部分明显右向不对称，因此采用泊松回归模型进行计算。结果以95%置信区间的相对风险以及趋势检验的形式呈现。结果发现，样本中的龋齿患病率没有性别差异，男性为87.8%，女性为93.3% ( $p = 0.221$ )。男性平均每人发现1.8 颗龋齿，而女性为1.4 颗 ( $p = 0.021$ )。补牙 ( $p = 0.167$ ) 和拔牙 ( $p = 0.981$ ) 的数量没有差异。研究发现，口腔液中的总钠含量与龋齿+补牙+拔除恒牙指数 ( $p = 0.040$ ) 和龋齿数量 ( $p < 0.001$ ) 成正比。口腔液总钙与龋齿+补牙+拔除恒牙指数 ( $p = 0.019$ ) 和补牙数量 ( $p = 0.001$ ) 均有明显相关性。补牙数量与镁含量 ( $p = 0.028$ ) 和磷含量 ( $p = 0.037$ ) 呈反向关系。总之，研究显示口腔液中的矿物质成分与龋齿+补牙+拔除恒牙指数及其组成部分之间存在统计学意义上的显著关系。因此，北极地区不利的生活条件是导致牙科疾病的额外风险因素。这项研究的结果如果在其他人群中得到证实，可用于建立模型，预测极北地区青少年龋齿的发生和发展。为了获得更完整的临床和实验室数据，有必要对口腔液的矿物质成分进行更深入的研究，同时考虑到营养和填充材料类型等干扰因素。

**关键词：** 北极；极北；牙科疾病；龋齿；口腔液矿物质成分；龋齿+补牙+拔除恒牙指数；涅涅茨自治区；离子学。

### 引用本文

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## BACKGROUND

Dental caries is a common health problem among adolescents worldwide. It is more significant in the special conditions of the Arctic zone of the Russian Federation (AZRF), which are characterized by a harsh climate, underpopulated areas, a significant proportion of small and indigenous peoples in the population structure, the absence of a federal transport system, high costs of living, and shortage of medical personnel, which leads to difficulties in providing dental care [1].

In adolescence, pubertal growth spurt occurs, accompanied by hormonal changes and long-term mineralization of bone tissue and teeth [2]. Owing to the characteristics of traditional nutrition in remote areas and body adaptive mechanisms, adolescents may have lower calcium, phosphorus, potassium, and magnesium levels [3], which also affects dental health.

In AZRF, adolescents have a high level of caries prevalence, and the intensity varies widely from 2.5 in Norilsk to 8.4 in Salekhard [4, 5]. The course of dental caries is associated with several risk factors, including limited access to dental services [6], low oral hygiene awareness [7], a diet rich in carbohydrates and industrially processed foods [8], and drinking water composition [6, 9]. Currently, besides the pathogenesis of dental caries, close attention is paid to the ionomics of mixed saliva (oral fluid) [10], which can be due to various reasons. Homeostasis is maintained in the oral cavity by specific composition of the oral fluid, which includes a certain amount of trace elements, proteins, and immunoglobulins. This composition is crucial in maintaining local immunity and dental tissue mineralization [11]. Enamel, being on the borderline of interaction with the external environment, is exposed to various influences. Preservation of the properties and structure of enamel is possible only with a constant dynamic equilibrium of enamel with oral fluid, implemented through the physicochemical metabolism of sodium, potassium, calcium, phosphorus, magnesium, bicarbonate, and other components. Thus, a buffer equilibrium occurs and post-eruptive maturation of the enamel and remineralization of tooth structures become possible after their demineralization [12]. This is confirmed by experiments using radioactive isotopes [13].

Literature data show varied results on the relationship between the mineral composition of oral fluid and intensity of caries. For example, it is known that calcium and magnesium levels and an increase in the oral fluid pH significantly affect the protection of hard dental tissues. However, some studies have revealed weak relationships

between the ionic composition of the oral fluid and level of the caries + fillin g + extracted permanent teeth (CFE) index. Nevertheless, results should be obtained carefully owing to the peculiarities and differences in the methods of data collection and processing [14, 15]. The heterogeneity of published research results raises a serious need for high-quality research in ionomics in different territories and age groups. Such studies have not previously been conducted in AZRF.

**The study aimed** to analyze the relationship between the intensity of caries and mineral composition of oral fluid in adolescents in AZRF, using the example of the Nenets Autonomous District (NAD).

## MATERIALS AND METHODS

As part of the state assignment no. 056-00121-18-00, an observational, cross-sectional, one-stage examination of 171 adolescents (82 boys and 89 girls) aged 15–17 years, permanently residing in the NAD, was performed. A sample of participants was selected from secondary schools nos. 1 and 4 in Naryan-Mar (the administrative center of the NAD) and schools in the Iskateley, Krasnoe, and Telviska villages. Additionally, to include adolescents belonging to the category of small and indigenous peoples of the North, students at a boarding school in Naryan-Mar were included. These children were born in remote rural areas with a lack of schools and other conditions for learning; however, at the time of the examination, they lived and studied in a boarding school in the administrative center of the district. The examination was conducted according to the methodology of the European Bureau of the World Health Organization, 5<sup>th</sup> edition [16]. The sample population included pediatric patients without severe general somatic pathology and disability, studying at the selected school and present in classes during the study period. Voluntary informed consent was signed by all students included in the sample. CFE index and its components were used to assess the intensity of caries.

The participants were familiarized with instructions for collecting oral fluid in advance. Unstimulated oral fluid was collected in the dental office on an empty stomach or with a fasting pause of at least 2 hours, before the dental examination. Furthermore, drinking water and other beverages, smoking, chewing gum, and brushing the teeth were not allowed before the procedure. To collect the material, 10 ml sterile graduated glass tubes were used. The participant tilted his head down and collected the fluid independently, while pressing the test tube to his lower lip and pushing the accumulated oral fluid into it with his tongue. After obtaining a sufficient

amount of foam-free fluid, the material was distributed by the researchers into four Eppendorf tubes using sterile pipettes and immediately froze it at  $-20^{\circ}\text{C}$ . Then, the containers were transported in dry ice in cooler bags by air to Arkhangelsk without defrosting. The material was stored in refrigerators at  $-80^{\circ}\text{C}$ . Saliva samples were analyzed at the Central Research Laboratory of the Northern State Medical University.

Oral fluid samples, after complete thawing, were thoroughly mixed on a Micro-spin FV-2400 vortex (Biosan, Latvia) and centrifuged at 10.000 rpm for 7 minutes on a MiniSpin minicentrifuge (Eppendorf, Germany). Total calcium, magnesium, and phosphorus in the resulting supernatant were determined on a biochemical automatic analyzer Random Access A-15 (Biosystems, Spain). A calcium–arsenazo reagent (Biosystems, Spain) was used to determine the level of total calcium in oral fluid. A magnesium reagent (Biosystems, Spain) was utilized to obtain the concentration of magnesium. The phosphorus level was determined using phosphorus reagents (Biosystems, Spain). Ionized calcium, potassium, sodium, and pH levels were determined by an ion-selective method using an Easylyte Calcium Na/K/Ca/pH electrolyte analyzer (Medica Corp., USA), using flow-through ion-selective electrodes. The CFE index and its components were used to assess the intensity of caries.

Data was statistically analyzed using the statistical software package Stata v.18 (Stata Corp., Texas, USA). Caries prevalence was presented as proportions with 95% confidence interval (CI) calculated using Wilson's method. The descriptive statistics for boys and girls were presented separately. Comparisons of proportions were made using the Pearson chi-square test. The CFE index and its components were presented as arithmetic means ( $M$ ) with 95% CI. Considering that the distribution of the CFE index and its components has a pronounced right-sided asymmetry, a Poisson regression analysis was conducted. The sodium, potassium, magnesium, phosphorus, ionized calcium, and total calcium concentrations and pH levels were presented as  $M$  (95% CI) to ensure comparability with those in other studies. Intergroup differences were assessed using the nonparametric Mann – Whitney test. To level out the effect of outliers and asymmetry, the concentrations of all mineral components of oral fluid and pH were divided into tertiles for the main analysis. The relationship between the intensity of the caries process and tertiles of sodium, potassium, magnesium, phosphorus, ionized calcium, and total calcium concentrations and pH levels was examined using Poisson regression analysis. The lower tertile, corresponding to the lowest concentrations, was used

as the reference category for all independent variables. As a measure of effect, relative risk (RR) with a 95% CI was applied, indicating how many times the mean value of the response variable (CFE, C, F, E) will be higher in the second and third tertiles of each of the independent variables compared to the reference category. To assess the trend and increase sensitivity, regression analysis was repeated with the introduction of tertiles by quantitative variables. The analysis of statistical interactions did not reveal a modifying effect of sex on the relationship between the pH of the oral fluid, concentrations of the studied elements, and CFE index components; hence, the analysis was performed without stratification by sex, which significantly increased the analysis sensitivity and reduced the probability of beta errors.

The study was approved by the ethics committee of the Northern State Medical University (protocol no. 08/11-18; November 28, 2018).

## RESULTS AND DISCUSSION

The prevalence of caries in the sample population did not differ by sex and was 87.8% in boys and 93.3% in girls ( $p = 0.221$ ). On average, boys had 1.8 decayed teeth per person versus 1.4 in girls ( $p = 0.021$ ). No differences were found in the number of filled ( $p = 0.167$ ) and extracted ( $p = 0.981$ ) teeth. Table 1 presents the mean values and 95% CIs for sodium, potassium, magnesium, phosphorus, and total and ionized calcium levels. Comparisons revealed significant differences by sex only for potassium concentration ( $p = 0.004$ ).

The sodium level in oral fluid was directly proportional to the CFE index ( $p = 0.040$ ). A more pronounced relationship was noted in the number of decayed teeth ( $p < 0.001$ ). Adolescents with oral sodium concentrations in the middle and upper tertiles had 39% and 80% more decayed teeth, respectively.

No statistically significant relationships were observed between potassium level and CFE index or its components, except a trend towards a decrease in the number of extracted teeth with an increase in potassium concentration ( $p = 0.070$ ). Additionally, adolescents with the highest potassium level in oral fluid had 2.4 times fewer teeth extracted than in the reference group; however, the results did not reach the level of significance.

Inversely proportional relationships were found between magnesium level and the number of filled teeth ( $p = 0.028$ ); this amount in adolescents with the highest magnesium concentrations was on average 22% less than in the reference group. Moreover, in the same group, the CFE index was 16%



**Table.** Means and 95% confidence intervals for concentrations of minerals in oral fluid among 15–17-year-old adolescents in Nenets Autonomous Area, mmol/l

**Таблица.** Средние арифметические (*M*) и 95 % ДИ концентраций минералов в ротовой жидкости 15–17-летних подростков в Ненецком автономном округе, ммоль/л

Indicator	Boys		Girls		<i>p</i> =
	<i>M</i>	95% CI	<i>M</i>	95% CI	
Sodium	12.6	12.1–13.1	11.8	11.5–12.2	0.280
Potassium	20.8	19.3–21.1	18.3	17.5–19.1	0.004
Magnesium	0.33	0.23–0.43	0.26	0.20–0.33	0.446
Phosphorus	3.94	3.65–4.24	3.63	3.37–3.88	0.286
Ionized calcium	0.15	0.13–0.18	0.14	0.12–0.17	0.855
Total calcium	0.40	0.36–0.45	0.38	0.34–0.43	0.843

lower, whereas the test for trend did not reach the level of significance ( $p = 0.054$ ). Oral phosphorus levels were inversely associated with the number of filled teeth ( $p = 0.037$ ), and adolescents with oral phosphorus levels in the middle and upper tertiles had 27% and 19% fewer filled teeth than those with the lowest concentrations of phosphorus in oral fluid. Total oral fluid calcium was directly proportional to the CFE index ( $p = 0.019$ ) and number of filled teeth ( $p = 0.001$ ). Adolescents with the highest total calcium concentration in the oral fluid had a 19% higher CFE index, which was due to a higher (39%) number of filled teeth. Further, adolescents with average total calcium concentrations (middle tertile) had one-third fewer decayed teeth than those in the reference group. No significant trends were noted for ionized calcium; however, adolescents with average levels of ionized calcium (middle tertile) had 54% higher levels of CFE because they had 85% more filled teeth.

The trend between pH value and the number of decayed teeth was expected ( $p = 0.067$ ), and adolescents with the highest pH levels had 25% fewer decayed teeth. However, the results were not statistically significant.

Notably, a high level of caries prevalence is recorded in NAD adolescents aged 15–17 years. Considering the predominance of filled teeth in the structure of the CFE index, adolescents receive the necessary dental care through an organized visit to a dentist [9]. Girls may be more motivated to maintain oral health than boys; thus, they have fewer decayed teeth.

The dynamics of sodium and potassium in oral fluid during the day is variable and depends on several factors, namely, the time of day, food consumed, and regulation of adaptive hormones when living in a harsh climate [17, 18]. Since the results were not corrected for these factors, what exactly influenced the increase in potassium levels in the boys could not be determined. Despite the absence

of significant sex differences in the concentrations of other studied ions, increased levels were registered in boys compared to girls. This is due to the regulation of sex hormones in boys [19]. Thus, the sodium and potassium levels in the oral fluid may indirectly indicate the functional state of adaptive hormones of the adrenal cortex under the influence of various environmental factors and state of the body, providing a response to changing environmental conditions. The level of these minerals is inversely proportional to the amount of corticosteroids and catecholamines in the blood [18].

More decayed teeth when oral sodium levels increase was probably due to several reasons. First is the consumption of foods high in salt and flavor enhancers, namely, crisps, snacks, fast food, and sausage products, in adolescence [20, 21]. Excessive consumption of salty foods causes a feeling of thirst, and according to some studies, adolescents tend to replace clean drinking water with sweet carbonated drinks, tea with sugar, and juices, which do not normalize the water–salt balance [8]. These factors induce a shift in the electrolyte balance and an increase in electrolyte concentration.

Second, during adolescence, the hormonal system develops, which affects the regulation of the salivary glands. In the sympathotonic type, the rate of salivation was found to be decreased, which may change the concentration of cations [22]. Moreover, a decrease in the rate of salivation creates a cariogenic situation in the oral cavity, along with other factors.

Magnesium exhibits significant antibacterial efficiency by disrupting the adhesion of microorganisms to the surface of the teeth, and phosphorus promotes remineralization. This presumably explains the lower number of filled teeth that is caused by the fact that the teeth are more mineralized and are exposed to caries less often.

With redox balance in saliva, proteins are rich in proline, and stearins bind to calcium, preventing its precipitation and promoting formation and maintenance of the structure of minerals in enamel [23]. During therapeutic dental treatment, antioxidant system activity is disrupted and oxidative stress occurs, which can lead to calcium level changes in the oral fluid. For example, in children with a predominance of composite fillings in their teeth, an increase in the reactive form of oxygen was noted at 1.5 years from the moment of their inserting, which reduced the level of calcium in the oral fluid [12]. In contrast, in the presence of glass ionomer cement fillings in teeth, redox balance is achieved 3 weeks after treatment [24]. As part of compulsory health insurance for children, fillings made of glass ionomer cement are predominantly inserted in teeth with unformed roots and chemically cured composites in fully formed teeth. Our study did not consider the material used to fill the teeth; therefore, which fillings in the oral cavity prevail in NAD adolescents and whether their quality could affect the calcium levels in the oral fluid could not be determined. Clearly, the high content of some inorganic components in the oral fluid of the sanitized oral cavity can explain the maximum value of its pH level [25].

Research under conditions of the far north can significantly contribute to the study of the dental health of the young population of this region and make adjustments to the development of preventive measures. The advantage of the present study is the sufficient sample and international method of examination, which is beneficial for qualitatively interpreting data obtained. Additionally, the method of collecting oral fluid, being noninvasive and relatively informative, is preferable in children and adolescents. Oral fluid indicates the state of the oral cavity homeostasis and can characterize some features of the adolescent body. Data processing was applied using modern biostatistical methods to obtain the study results.

Factors that could hypothetically influence the course of the study include violation by adolescents of the conditions for collecting oral fluid (lack of a fasting break or brushing their teeth), belonging to the indigenous or alien inhabitants of the far north (this factor was not considered during the study), and intake of vitamin and mineral complexes that can affect the mineral composition of oral fluid.

## CONCLUSION

In the NAD, adolescents aged 15–17 years have a high level of caries prevalence. Furthermore, statistically significant connections were found between the mineral composition of the oral fluid and CFE index and its components. Obtaining more complete clinical and laboratory data requires more

in-depth studies of the mineral composition of oral fluid, considering confounding factors such as nutrition and type of filling material.

## ADDITIONAL INFORMATION

**Authors' contribution.** Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

**The contribution of each author.** M.A. Gorbatova — development of the general concept, data collection and analysis, writing the article; T.N. Yushmanova — planning and design of the study, writing the article; A.A. Simakova — study design, article writing; G.A. Antonova — data collection, article writing; P.A. Pochinkova — data collection, article writing; L.L. Shagrov — laboratory analysis of biological material; Yu.M. Zvezdina — laboratory analysis of biological material; N.I. Pechinkina — laboratory analysis of biological material; A.M. Grzhibovsky — statistical data analysis, article writing.

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