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ORIGINAL ARTICLE

Association Between Saliva Quantity and Content Parameters with Caries Intensity Levels: A Cross-Sectional Study Among Subcarpathian Children

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Abstract

Objective: To evaluate saliva quantity and content parameters among children of 7 and 12 years old, who permanently living on the territory of Subcarpathia with the registered territory-associated fluoride deficiency in the water, and their association with the caries status of pediatric patients. **Material and Methods:** The study sample was formed of 48 children (22 of 7 years old and 26 of 12 years old). The content of calcium in the oral liquid was determined by the o-cresolphthalein complexone method. Estimation of concentration rate and fluoride activity in the oral liquid was carried out by using the ion-selective electrode ELIS-131 F and ionometer EV-74. The content of inorganic phosphorus in saliva was determined using the phosphorus reaction with molybdic acid. **Results:** Among all study samples, 18.8% were registered with low caries intensity level (DMF = 1.55 ± 0.16), 33.3% with moderate caries intensity level (DMF = 3.94 ± 0.29), and 47.9% with high caries intensity level (DMF = 9.05 ± 1.11). During the comparison of calcium content and mineralization coefficient values between children with low and high caries intensity levels registered difference was statistically significant ($p<0.05$), while for salivary flow rate parameter such difference was no significant ($p>0.05$). Between children with normal salivary flow rate, and children with a lowered salivary flow rate there was no statistical difference in such parameters as fluoride concentration, calcium content, phosphorus content and calcium-phosphorus balance ($p>0.05$). **Conclusion:** Caries intensity levels were more statistically associated with parameters of calcium content in saliva and related mineralization coefficient, rather than with the average salivary flow rate.

Keywords: Dental Care for Children; Dental Caries; Saliva; Fluorides.

Introduction

Saliva, as a specific type of biological liquid and a unique medium of the oral cavity, plays a direct role in the development of dental caries among individuals of different ages [1,2]. That is why variations of salivary flow rate and its content modulations remain subjects of different caries-predicting models, which due to some aspects of their formulations diverge with each other by the meaning of different saliva-associated parameters impact on demineralization and remineralization processes of hard dental tissues [2-8]. Based on that, there is a relevant need for detailed stratification analysis of saliva quantity and quality parameters that influence the risk of caries development and its progression considering different individual patterns.

Due to the previously obtained results hyposalivation itself was considered as a risk factor of caries development, but there is still ongoing discussion about which of two possible mechanism plays more significant role in tooth demineralization process: decrease of salivary flow itself with insufficient cleaning of tooth surfaces, or reduction of microelements content caused by lowered volume of secreted saliva, which restricts conditions for adequate remineralization process [2,4,5,9-11].

Moreover, age and environment-related aspects also influence the level of caries intensity and specific saliva-associated parameters, thus broaden several indicators that should be considered in complex caries-prediction models [11-13]. Taking into account that pattern of caries development starts to form in childhood and most variations of saliva content occur during the first decade of life, it is logical that the target population for caries prognosis based on saliva parameters should be represented by pediatric patients [13-15].

All the facts mentioned above argument the need for providing relevant studies aimed at the evaluation of different saliva quantity and content parameters among children of different age groups related to some normal conditions of permanent living, and their association with different caries intensity levels registered.

The objective of this study was to evaluate saliva quantity and content parameters among children of 7 and 12 years old, who permanently living on the territory of Subcarpathia with the registered territory-associated fluoride deficiency in the water, and their association with the caries status of pediatric patients.

Material and Methods

Study Design and Sample

In this cross-sectional study, patients sample was formed from individuals out of the number of dental patients from School Dental Clinic (Ivano-Frankivsk Secondary School #23) during a routine preventive examination. The sample was formed of 48 children (22 of 7 years old and 26 of 12 years old).

Inclusion of children into the study sample was carried up due to the next criteria: 1) age of a child 7 or 12 years old (such age groups were used due to the need for compliance with the WHO

guidelines for clinical-statistical study designs); 2) permanent living of a child on the territory of Subcarpathia (Ivano-Frankivsk, Ukraine), which is characterized with the registered territory-associated fluoride deficiency in the water; 3) the absence of any associated general medical condition or complementary contraindications that would restrain possibility of child to take part in the clinical survey; 4) informed consent of parents that allowed their child to take part in clinical study.

Exclusion criteria were the next: 1) age of the patient out of 7-12 years range; 2) facts of child previously living out of Subcarpathia region for more than 6 months; 3) presence of oral alterations that could influence remineralization and demineralization processes in dental hard tissues; 4) intake of medications that affect the state of microelements in the child's organism; 5) parent's refusal to sign informed consent form or personal child's rejection to take part in clinical examination.

Data Collection

Each individual was preliminary examined to find out the initial level of salivary flow rate and initial level of caries intensity. Clinical assessment and saliva collection procedures were provided based on the School Dental Clinic by a trained dentist, who previously participated in epidemiological studies.

The amount of saliva was determined two times a day: on an empty stomach and 2 hours after meals, and the average rate of salivation was calculated. The salivary flow rate with the levels lower than 0.25 ml/min was considered as hyposalivation, and with the rates over 0.25 ml/min as a normal value of unstimulated saliva secretion rate [16]. In the received fraction of saliva content of minerals was studied with the use of biochemical analytical methods.

Caries intensity levels were calculated by DMF index with the next interpretation: levels lower than 1.2 – very low caries intensity, levels in the range 1.2-2.6 – low caries intensity, levels in the range 2.7-4.4 – moderate caries intensity, levels greater than 4.4 – high caries intensity. Further research due to the caries intensity results was provided just among children with low caries intensity parameters (lower than 2.7 by DMF index) and high levels of caries intensity (greater than 4.4 by DMF index) to find out the objective cause of the difference between them [17,18]. Also, children with moderate caries intensity were not included for the further evaluation of salivary flow rate, overall rate of saliva secretion per day, calcium content and concentration in saliva, and mineralization coefficient, because such results were already represented in previously published studies dedicated to the registration of dental status among children of Subcarpathia region in Ukraine [19,20].

The children were conveniently classified into the next several pairs of groups: 1) children with normal level of salivation (greater than 0.25 ml/min for unstimulated saliva secretion) and children with registered hyposalivation (lower than 0.25 ml/min for unstimulated saliva secretion) for the evaluation of fluoride concentration parameters, calcium and phosphorus content levels, and calcium/phosphorus ratio; 2) children with low level of caries intensity (lower than 2.7 by DMF

index) and children with high level of caries intensity (greater than 4.4 by DMF index) for the evaluation of salivary flow rate, overall rate of saliva secretion per day, calcium content and concentration in saliva, and mineralization coefficient calculated by the ratio of salivary flow rate to the concentration of calcium in saliva.

The content of calcium in the oral liquid was determined by the o-cresolphthalein complexone method. The solution of cresolphthalein complexone with calcium in an alkaline medium forms a complex of red-violet color, which intensity is proportional to calcium concentration. The content of calcium in the oral liquid was expressed in mmol/l. A photometric set of Calcium Ars-DaC-Lg (DAC-SpectroMed Company, Chisinau, Republic of Moldova) was used for the evaluation of calcium content in saliva [21].

To individualize the results of the natural mineralization properties of saliva, we used the method of assessing the mineralizing potential of the oral fluid by studying the calcium homeostasis. After the evaluation of the salivation rate and the concentration of calcium content in the saliva expressed in mmol/l, the latter parameter was converted to mg/% of calcium considering the total amount of saliva secreted during the day. By multiplying the rate of salivation on the concentration of calcium in the saliva, we also calculated the mineralization coefficient, according to which we can determine whether there is enough calcium in the oral fluid to perform complete mineralization of teeth in each particular case.

Estimation of concentration rate and fluoride activity in the oral liquid was carried out by potentiometric method using the ion-selective electrode ELIS-131 F and ionometer EV-74 (Biomer Co. Ltd., Novosibirsk Region, Russia). The preliminary calibration of the electrode was carried with the use of a calibration curve, reflecting the correspondence of the fluoride concentration to the ionomer potential [22].

The content of inorganic phosphorus in saliva was determined using the phosphorus reaction with molybdic acid, which resulted in the formation of phosphoric-molybdic acid. Phosphoric-molybdic acid in the ascorbic acid recovery process provides the establishment of a complex with the blue color. The intensity of the color is proportional to the concentration of inorganic phosphorus expressed in mmol/l. Dac-Spectromed Set (DAC-SpectroMed Company, Chisinau, Republic of Moldova) was used for the evaluation of inorganic phosphorus content in saliva [21].

Data Analysis

Correlation analysis was used to determine the relationship between two or more random variables. It was used to establish a correlation between the intensity of caries, salivation rate, and content of minerals in saliva among children of 7 and 12 years old. The Pearson correlation coefficient (r) measures the degree of linear dependence between two interval variables. For the application of the Pearson correlation coefficient, the following conditions must be met: two comparative variables must be obtained at least in the interval scale or ratio scale, which defines the degree in which two variables is proportional to each other; the distribution of variables should be

close to normal; the number of variables in the comparable variables set should be the same. The value of the correlation coefficient does not depend on the scale of the measurement. The reliability of the differences in mean values (p) was estimated in accordance with the generally accepted statistical method, using the Student's T-criterion. Data were considered reliable at a significance level of 0.95, which is $p<0.05$. All statistical analysis was provided in Microsoft Excel software (Microsoft Office 2016, Microsoft Corp., Albuquerque, New Mexico, USA) [23].

Ethical Aspects

The study protocol was approved by the Ethics Committee of Uzhhorod National University. All children and their parents voluntarily agreements to take part in the clinical survey.

Results

Among all study samples, nine persons were registered with low caries intensity level (DMF = 1.55 ± 0.16) and 23 with high caries intensity level (DMF = 9.05 ± 1.1), while 16 children were characterized with caries intensity values in the range of 2.7-4.4, which corresponds to the moderate level. During the comparison of all estimated salivary parameters between children with low and high caries intensity levels registered difference was statistically significant ($p<0.05$), except parameter of salivary flow rate ($p>0.05$) (Table 1).

Table 1. Comparison of salivary parameters between children with low and high caries intensity.

Parameters	Low Caries Intensity	High Caries Intensity	p-value
DMF	1.55 ± 0.16	9.05 ± 1.1	<0.05
Salivary Flow Rate (ml/min)	0.35 ± 0.04	0.38 ± 0.04	>0.05
Calcium Content in Saliva (mmol/l)	1.08 ± 0.1	0.72 ± 0.1	<0.05
Calcium Content (mg/%)	4.32 ± 0.5	2.88 ± 0.35	<0.05
Calcium Content per Day (mg)	21.8 ± 2.4	15.8 ± 1.8	<0.05
Calcium Content (mg/min)	0.015 ± 0.001	0.011 ± 0.001	<0.05
Mineralization Coefficient	0.38 ± 0.03	0.27 ± 0.03	<0.05

Further analysis was dedicated to the evaluation of calcium, fluoride, and phosphorus content in saliva among children with different salivation rate. It was interesting to note that between children with normal salivary flow rate (26 persons – 54.17%) and children with lowered salivary flow rate (22 persons – 45.83%) there was no statistical difference in such parameters as fluoride concentration, calcium content, phosphorus content and calcium-phosphorus balance ($p>0.05$) (Table 2).

Table 2. Comparison of salivary content parameters between children with normal and low salivary flow rate.

Parameters	Normal Salivary Flow Rate	Lowered Salivary Flow Rate	p-value
F concentration (mg/l) - C_F	0.21 ± 0.06	0.27 ± 0.01	>0.05
Ca (mmol/l)	0.31 ± 0.05	0.27 ± 0.04	>0.05
P (mmol/l)	3.42 ± 0.45	3.74 ± 0.51	>0.05
Ca/P	0.090 ± 0.01	0.072 ± 0.01	>0.05

The subsequent stage of research carried out an evaluation of saliva-associated parameters not only among children with different DMF-index values but also considering the age of children. Among 7 years old children, three persons were registered with low caries intensity level by DMF index (2.3 ± 0.36) and nine children with high caries intensity parameters (10.9 ± 1.02). The average salivary flow rate among 7 years old children with low caries intensity was 0.42± 0.06 ml/min, average calcium content – 0.81± 0.07 mmol/l, average mineralization coefficient – 0.34 ± 0.04. Distribution of those parameters among 7 years old children with high caries intensity was next: average salivary flow rate was 0.34 ± 0.04 ml/min, average calcium content – 0.56 ± 0.06 mmol/l, average mineralization coefficient – 0.19 ± 0.02. The registered difference of average saliva calcium content and average mineralization coefficient between 7 years old children with low and high caries intensity levels was statistically significant ($p>0.05$), while the difference in average salivary flow rate was statistically insignificant ($p<0.05$).

Among 12-year-old children, six persons were registered with low caries intensity level by DMF index (average value – 0.8 ± 0.09) and nine children with high caries intensity parameters (7.2 ± 0.82). The average salivary flow rate among 12 years old children with low caries intensity was 0.37 ± 0.04 ml/min, average calcium content – 1.14 ± 0.13 mmol/l, average mineralization coefficient – 0.42 ± 0.05. Distribution of those parameters among 12 years old children with high caries intensity was next: average salivary flow rate was 0.26 ± 0.03 ml/min, average calcium content – 1.08 ± 0.09 mmol/l, average mineralization coefficient – 0.28 ± 0.03. A statistically significant difference between 12 years old children with low and high caries intensity was noted during comparison of average salivary flow rate and average mineralization coefficient ($p<0.05$), while the difference in saliva calcium content was not statistically proven ($p>0.05$).

Discussion

Saliva remains a medium consisting of components and markers, which can be effectively used for possible caries prognosis with different levels of reliability [6,7,24]. Moreover, the diagnostic value of saliva is augmented by the possibility of its time- and cost-effective analysis, the non-invasive methodology of collection and relatively accurate obtained results [1,2]. But the variation of caries-prediction models and their nonagreement with each other entangles the selection of those saliva-associated parameters, which can be effectively used in clinical practice as veridical and consistent criteria for caries impact-analysis [4,6,11,13].

Systematic review dedicated to the questions of saliva flow rate, pH levels, buffering capacity and total calcium content difference among caries-free and caries-active children (7-14 years old) found out next: 1) caries-free group of children characterized by higher levels of calcium content in saliva; 2) saliva flow rate, pH and buffering capacity parameters lowering in caries-active group of children analogically to total calcium level in the same group (by results of two analyzed studies); 3) linear association between salivary flow rate, pH and buffering capacity among caries-free or caries-affected group of children was not established [25]. Even though in our study, we did not have a

control group of caries-free children, but we also found that children with lower caries intensity are characterized by higher calcium content in saliva. Moreover, we also established an absence of statistical association between salivary flow rate and fluoride, calcium, and phosphorus concentrations, nevertheless, there is a need for further research to an argument such as statistical fact among study samples with a more significant number of participants. Inversely to the results previously described [25], we had not noticed any statistical dependence between salivary flow rate and caries intensity parameters. Such nonagreement with systematic review results could be augmented by the fact that we analyzed a specific group of 7 and 12 years old children, who were permanently living on the territory of Subcarpathia with the registered territory-associated fluoride deficiency in the water. Also, the disparity with evidence of systematic review could occur because of differences between the design of our study and designs of studies included in the systematic review; besides, it should be considered that present research characterized by relatively small study sample, and this fact is could influence received statistical outcome.

Physicochemical properties of saliva as pH, calcium, and phosphorus content, buffering capacity, and *Streptococcus mutans* have a relevant association with caries activity [4]. Some authors also have found that persons with "higher salivary calcium may have more number of intact teeth" [26]. The conclusions of both these studies are reconciled with our results since we also have found a statistical relationship between higher calcium content in saliva and lower values of caries intensity ($p<0.05$).

Even though the association between saliva remineralization potential and presence of caries lesions was established, the overall statistical difference of Ca/P coefficient among children with normal a level of salivation and hyposalivation was not significant ($p>0.05$). Such findings could be augmented by the insufficient quantity amount of study sample and by the possible perspective role of other saliva-associated factors in the process of caries development. For example, the use of Human Oral Microbiome Identification Next Generation Sequencing method in previous researches helped to registered a statistically higher abundance of such microorganisms as Neisseria, Haemophilus, and Fusobacterium in saliva comparing healthy and caries-affected group of individuals [27]. Such a way diversity of microbial content of saliva may influence not only the regions of bacterial plaque accumulated at the tooth surface but also saliva parameters itself, which in turn additionally could change saliva remineralization potential and indirectly affect caries-sensitive zones [28].

Also, summarizing the results of the provided study, we can conclude that regardless of the salivation rate, the degree of calcium content in the saliva is more critical for preserving a low level of caries intensity. Previously provided studies have found that the mineralizing potential of the oral fluid in children with high caries intensity is lowered either due to the decrease in the synthesis of calcium or because of the difficulties of its receipt by the hard dental tissue [5,11,12,25,26].

Limitations of the present study are associated with the relatively small study sample and with the need to expand many parameters that primary should be in the focus of research.

Specifically, it would be reasonable to provide further research among the study sample with the objective of their diet evaluation and detailed sugar content assessment. Also, changes in microbiome profile and other saliva biomarkers should be considered for analysis simultaneously with registering variations of saliva calcium, fluoride, and phosphorus content. Another limitation was associated with the fact, that children with moderate caries intensity were not included for the further evaluation of salivary flow rate, the overall rate of saliva secretion per day, calcium content and concentration in saliva, and mineralization coefficient. Such a design of the study was augmented because such results were already represented in previously published studies dedicated to the registration of dental status among children of Subcarpathia region in Ukraine [19,20]. But in future studies with the higher number of participants, there is a need to provide differential analysis of saliva- and caries-associated parameters among children with low, moderate and high caries intensity levels to find out valid and statistically proven dependencies among different parameters.

Considering the age of children in the study sample, their specific place of permanent living and results of saliva analysis, we can suspect that decrease in the mineralization among them apparently was provoked due to a violation of the calcium synthesis process, and also, but in a lower manner of dependency, due to the hyposalivation. To establish exact levels of importance for these two factors, we need to provide further research with a higher amount of study sample in more isolated conditions due to the formulated aim.

Conclusion

The use of saliva content parameters can provide new possibilities in caries prediction modeling, considering that saliva-quality criteria are more prognostically reliable that quantity-associated values. Results obtained from children of 7 and 12 years old, who permanently were living on the territory of Subcarpathia with the registered territory-associated fluoride deficiency in the water, helped to summarize that in those specific conditions of research caries intensity values were more statistically associated with parameters of calcium content and related mineralization coefficient, rather than with average salivary flow rate. Moreover, no statistical significance was established between concentration levels of fluoride, calcium, and phosphorus among children with normal and decreased indicators of salivary flow rate.

Authors' Contributions: LB contributed to the conception and data design, performed the data collection, analysis, and interpretation and revised the manuscript, AK performed the data collection, and revised the manuscript, MGK contributed to the data analysis and interpretation, and wrote the manuscript. YZ critically revised the manuscript, and provided scientific guidance in data representation.

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