

CORRELATION OF THE CHEMICAL COMPOSITION OF ENAMEL AND ORAL FLUID WITH INTACT TEETH AND CERVICAL CARIES

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ABSTRACT

INTRODUCTION: Caries is a widespread polyetiological disease. Oral fluid properties and composition play an important role in its development and progression.

OBJECTIVES: The aim of this study was to present the ability of different voxel sizes in identifying osseous defects of mandibular condyle using CBCT images.

MATERIAL AND METHODS: We determined Ca^{2+} , PO_4^{3-} , and SO_4^{2-} using spectrophotometric method, Na^+ , K^+ , Al by atomic emission spectrometry, Mg^{2+} , Zn by atomic absorption spectrometry, Cl^- using chloride-selective electrode of pH-meter in oral fluid of 22 patients divided into two groups: case DMFT = 1.45 ± 0.48 and control DMFT = 0. Correlation of oral fluid and cervical enamel chemical composition of 19 extracted teeth was made.

RESULTS: The values of molar coefficients were high in intact teeth enamel: P/Ca by 10%, Mg/Ca by 82%, and K/Na by 50%. The following values were lower: Ca/P by 10%, Ca/Cl by 16%, and Mg/P by 43% ($p \leq 0.05$). The distinctive feature of correlation of oral fluid and enamel chemical composition in the control group patients was the presence of average Mg^{2+} bond strength: direct with Cl^- , K^+ , and Na^+ , and inverse with Ca^{2+} and PO_4^{3-} ($p < 0.0001$). The correlation between molar coefficients of oral fluid and enamel of patients with intact hard tissues was distinguished by the presence of high direct correlation of $\text{Mg}^{2+}/\text{Ca}^{2+}$ with $\text{PO}_4^{3-}/\text{Ca}^{2+}$, moderate inverse correlation of $\text{Ca}^{2+}/\text{Cl}^-$ with $\text{PO}_4^{3-}/\text{Ca}^{2+}$, and the absence of moderate correlation of $\text{Mg}^{2+}/\text{Ca}^{2+}$ with $\text{Ca}^{2+}/\text{PO}_4^{3-}$ ($p < 0.0001$).

CONCLUSIONS: Revealed correlations may be used to diagnose cervical caries and control the effectiveness of its prevention in terms of mixed saliva over time.

KEY WORDS: dental caries, saliva, spectrometry, scanning electron microscope, analytical chemistry.

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INTRODUCTION

Dental caries is a widespread polyetiological disease, and cervical form of this pathology represents a significant part of all clinical cases diagnosed in the group of 30-50 years of age [1, 2]. Since the teeth are in constant contact with saliva, the composition and properties of oral fluid (OF) play an important role in the onset and

progression of this disease [1, 3-6]. The intensity of caries is in negative correlation with the indicators of mineral metabolism in OF [7]. The mineralizing properties of saliva determine the resistance to caries after teeth eruption, contribute to the maintenance of their physical and chemical integrity, and the leading role belongs to calcium and phosphorus, which are the main structure-forming elements of enamel hydroxyapatite [3, 5-9].

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Enamel is capable of reversible isomorphic replacement of its ions with OF ions without destroying the crystal structure and abrupt changes in properties. It minimizes its demineralization and enhances remineralization, which is carried out by saliva supersaturated with ions relating to tooth enamel [10]. Based on macro- and microelement state of hard tissues of the teeth, one can assess their mineralization [11]. Saliva is a perspective substance for early detection of diseases of the oral cavity and assessment of the effectiveness of prophylactic agents applied [12]. Therefore, it is necessary to study the chemical composition of both tooth tissues and biological fluids, which wash the teeth to prevent demineralization processes [13]. If there is a correlation between the indicators for OF composition, the mineral state of enamel can be established.

OBJECTIVES

The purpose of the study was to determine the chemical composition and the level of OF mineralization index in patients with cervical caries and clinically intact hard tissues as well as to find correlations between the indicators of cervical enamel and OF, and identify their possible role in the development of cervical caries of teeth.

MATERIAL AND METHODS

Twenty-two patients (11 men, 11 women) without somatic pathology (mean age, 23.39 ± 5.61 years) participated in clinical and laboratory studies. According to the results of clinical examination, two groups (11 pts./11pts.) were formed to accomplish assigned tasks: case – DMFT (decayed missing filled teeth) = 1.45 ± 0.48 ((ICDAS (International Caries Detection and Assessment System) = group 3 (localized enamel breakdown [without clinical visual signs of dentinal involvement] seen when wet and after prolonged drying); control: DMFT = 0 ((ICDAS = group 0 (sound tooth surface: no evidence of caries after 5 sec. of air drying)). The absence of bad habits, diseases of periodontal tissues and oral mucosa, orthopedic and orthodontic structures in the oral cavity were the criteria for inclusion. The work was performed in accordance with the principles of WMA Declaration of Helsinki “Ethical Principles for Medical Research involving Human Subjects”, order No. 690 of the Ministry of Health of Ukraine (dated September 23, 2009) and approved by the Bioethics Commission of Donetsk National Medical University. Before being involved in the survey, all the participants were provided with written informed consent.

The material for laboratory studies was unstimulated OF, which was collected from 10 till 12 o'clock. Brushing teeth, eating, drinking, and smoking were forbidden 2 hours before the beginning of the research. Previously, the oral cavity was rinsed twice thoroughly with distilled water. 20 ml of saliva was collected into a sterile plastic

test tube with a lid and was examined in the laboratory of the Department of Chemical Metrology of Kharkov National University. Spectrometry method was applied to determine phosphate ions, ionized calcium, and SO_4^{2-} (PO_4^{3-} with ammonium molybdate at $\lambda = 340$ nm, Ca^{2+} with o-cresolphthalein at $\lambda = 570$ nm, SO_4^{2-} with methylene blue at $\lambda = 650$ nm). The emission variant of atomic emission spectrometry was used to detect potassium and sodium (for K^+ $\lambda = 766.5$ nm, monochromator slit width is 0.5 nm; for Na^+ $\lambda = 589.0$ nm, monochromator slit width is 0.1 nm). Inductively coupled plasma atomic emission spectrometry ($\lambda = 308.22$ nm) was used to evaluate aluminum. Magnesium and zinc were defined using atomic absorption spectrometry (for Mg^{2+} , $\lambda = 285.2$ nm, amperage – 5 mA, photoelectron multiplier – 1.3 kV, monochromator slit width – 0.1 nm; for Zn^{2+} , $\lambda = 213.9$ nm, amperage – 5 mA, photomultiplier – 1.3 kV, monochromator slit width – 0.1 nm). Chloride ions were identified using an ELIS 131 Cl chloride selective electrode, with an EVL-1 ME reference electrode connected to a pH meter. The samples for the content of K^+ , Na^+ , and Mg^{2+} were diluted with bidistilled water by 1,000 times. The molar coefficients were calculated as the ratio of the amounts of chemical analytes in the supernatant. The level of OF mineralization potential was calculated as the ratio of the rate of salivation to the concentration of ionized calcium, where values of 0.5 and higher were regarded as high intensity of mineralization, and values less than 0.5 as decreased [9]. The salivation rate was determined in ml/min according to the formula: the amount of taken OF/collection time [14].

We performed the correlation of chemical composition of the oral fluid and the enamel of cervical region of 19 teeth of both jaws, which were removed for clinical indications (12 clinically intact, 7 with cervical caries) in patients between 25 and 54 of age. The teeth were extracted for orthodontic indications. JSM-6490 LV focused beam electron microscope (scanning) with system of energy-dispersive X-ray microanalysis INCA Penta FETx3 (OXFORD Instruments, England) was used. The chemical composition of 162 areas of the cervical enamel was determined as a percentage of weight amounts of carbon, oxygen, calcium, phosphorus, sodium, magnesium, sulfur, chlorine, zinc, potassium, and aluminum [15]. Replication measurements were averaged in one sample before statistical analysis. The study was conducted at the base of Donetsk Institute of Physics and Technology of the National Academy of Sciences of Ukraine.

STATISTICAL ANALYSIS

Statistical analysis was performed using Statistica 12.0 computer program (3BA94C4ED07A). To verify the presence of a relationship between the variables, the correlation analysis was carried out (Pearson's parametric correlation method) based on the determination

of parametric Brave-Pearson coefficient (r), with 95% confidence level. The reliability of obtained results was assessed using Student's t -test, and the correlation between the indicators based on Student's t -test was determined with Z -test (Fisher's Z -test). The differences were considered statistically significant at $p \leq 0.05$. The significance of the differences between the groups was evaluated based on an analysis of variance.

RESULTS

The comparative analysis of the content of chemical analytes in OF was carried out at the first stage (Table 1). Since the indicators changed in opposite directions in the groups, it became possible to increase the significance of the differences by calculating their molar ratios (Table 2).

Obtained results indicated the absence of significant differences in the content of chemical analytes and molar coefficients in OF ($p > 0.05$). Mineralization of mixed saliva was significantly higher (0.93 ± 0.29) in the control group than in the case group (0.67 ± 0.26) by 1.39 \pm 0.08 times ($p = 0.006$). This indicator was in high directly proportional correlation with the coefficient of dispersion of OFs' impedance and the rate of salivation in all patients ($p \leq 0.05$) [14]. High inverse correlation was found between the coefficient of OF impedance variance and the amount of Cl^- ($r = -0.767$, $p = 0.006$) in the control group [14].

The data on chemical composition of the cervical enamel of teeth with caries and intact hard tissues is presented in a previous work [15]. Inverse proportional correlation was shown in the cervical region of the samples of the main and control groups: high – between carbon and oxygen ($r = -0.7311$), carbon and phosphorus ($r = -0.7573$); moderate – between oxygen and aluminum ($r = -0.5288$), $p < 0.0001$. Directly proportional correlation was determined: high – between calcium and phosphorus ($r = 0.7635$); moderate – between aluminum and carbon ($r = 0.5819$), $p < 0.0001$.

The results of molar coefficients calculations in the investigated groups are presented in Table 3.

Significant differences were determined in the values of Ca/P, P/Ca, Ca/Cl, Mg/Ca, Mg/P, K/Na ($p \leq 0.05$). The ratios of chemical analytes were large in the enamel of the samples with clinically intact hard tissues: P/Ca – by 10%, Mg/Ca – by 82%, K/Na – by 50%; lower: Ca/P – by 10%, Ca/Cl – by 16%, Mg/P – by 43% ($p \leq 0.05$). High correlation was found between molar coefficients in the cervical enamel: very high directly proportional correlation between Mg/Ca and Mg/P ($r = 0.9806$); moderate inversely proportional correlation between Mg/Ca and Na/Mg ($r = -0.5087$), and Mg/P and Ca/Mg ($r = -0.6618$), $p < 0.0001$.

The correlation analysis was performed to verify the presence of a relationship between the chemical composition of OF and cervical enamel at the second

stage of the study. The results of the revealed correlation between the indicators of moderate and high strength are presented in Table 4.

The distinctive feature of the relationships in the control group was the presence of moderate degree of Mg^{2+} correlation: directly proportional correlation with Cl^-

TABLE 1. Chemical analytes in OF, $X \pm m$

Chemical analytes	Case group	Control group	p -value
K^+ , mmol/l	22.20 ± 5.10	24.60 ± 5.20	0.378
Na^+ , mmol/l	11.80 ± 10.70	18.30 ± 15.70	0.183
Mg^{2+} , mmol/l	2.90 ± 5.30	6.50 ± 7.90	0.421
Ca^{2+} , mmol/l	0.90 ± 1.00	1.10 ± 0.60	0.757
PO_4^{3-} , mmol/l	3.90 ± 1.70	2.70 ± 1.30	0.125
Cl^- , mmol/l	22.40 ± 6.50	19.20 ± 5.80	0.461
SO_4^{2-} , mmol/l	0.0020 ± 0.0010	0.0008 ± 0.0011	0.207
Zn^{2+} , mg/l	0.04 ± 0.02	0.04 ± 0.02	0.464
Al^{3+} , mg/l	0.0030 ± 0.0001	0.0030 ± 0.0001	0.802

Data presented as mean \pm standard deviation

TABLE 2. Molar ratios of chemical analytes in OF, $X \pm m$

Molar coefficients	Case group	Control group	p -value
Na^+/K^+	0.58 ± 0.56	0.75 ± 0.62	0.437
$\text{Na}^+/\text{Mg}^{2+}$	14.83 ± 18.23	9.44 ± 10.06	0.183
$\text{Ca}^{2+}/\text{PO}_4^{3-}$	0.28 ± 0.21	0.45 ± 0.29	0.137
$\text{PO}_4^{3-}/\text{Ca}^{2+}$	13.95 ± 21.88	3.09 ± 1.76	0.115
$\text{Ca}^{2+}/\text{Mg}^{2+}$	1.32 ± 1.73	0.85 ± 0.97	0.344
$\text{Ca}^{2+}/\text{Cl}^-$	0.05 ± 0.06	0.06 ± 0.03	0.757
Al/Zn	0.008 ± 0.003	0.009 ± 0.006	0.629
$\text{Mg}^{2+}/\text{Ca}^{2+}$	4.53 ± 6.09	9.71 ± 17.00	0.353
$\text{Mg}^{2+}/\text{PO}_4^{3-}$	0.88 ± 1.59	3.43 ± 5.72	0.170

Data presented as mean \pm standard deviation

TABLE 3. Molar ratios of chemical analytes in cervical enamel, $\pm m$

Molar coefficients	Case group	Control group	p -value
Na/K	55.8706 ± 41.3412	38.2085 ± 41.7515	0.071
Na/Mg	10.2195 ± 14.9372	8.8071 ± 17.5403	0.706
Ca/P	2.0977 ± 0.1962	1.8983 ± 0.1864	$< 0.001^*$
P/Ca	0.4803 ± 0.0403	0.5313 ± 0.0473	$< 0.001^*$
Ca/Mg	307.7388 ± 242.8532	361.8856 ± 201.7616	0.309
Ca/Cl	92.0273 ± 33.7824	76.8978 ± 22.7115	0.032^*
Al/Zn	18.4448 ± 38.3761	19.8389 ± 40.4231	0.879
Mg/Ca	0.0042 ± 0.0019	0.0232 ± 0.0015	0.018^*
Mg/P	0.0086 ± 0.0038	0.0060 ± 0.0026	0.001^*
K/Na	0.0456 ± 0.0644	0.0911 ± 0.0982	0.016^*

Data presented as mean \pm standard deviation

TABLE 4. Correlation between the content of chemical analytes in OF and cervical enamel, r ($p < 0.0001$)

Chemical analytes	Group	K ⁺	Group	PO ₄ ³⁻	Group	Na ⁺	Group	Cl ⁻
Ca ²⁺	I	-0.8817	I	0.9607	I	-0.6410	I	-0.8664
	II	-0.9433	II	0.9781	II	-0.6799	II	-0.9125
Na ⁺	I	0.6343	-	-	-	-	I	0.6177
	II	0.7168					II	0.6926
PO ₄ ³⁻	I	-0.8727	-	-	-	-	I	-0.8286
	II	-0.9545					II	-0.9110
K ⁺	-	-	-	-	-	-	I	0.9203
							II	0.9388

I – case group, II – control group

($r = 0.5554$), K⁺ ($r = 0.6570$), Na⁺ ($r = 0.6463$); inversely proportional correlation with Ca²⁺ ($r = -0.5757$) and PO₄³⁻ ($r = -0.5975$), $p < 0.0001$.

The molar coefficients of the chemical analytes of the cervical enamel and OF in both groups were associated in direct proportion (moderate and high strength): Ca²⁺/P and Na⁺/K⁺, Ca²⁺/Mg²⁺ and Ca²⁺/P, Ca²⁺/Cl⁻ and Na⁺/K⁺, Ca²⁺/Cl⁻ and Ca²⁺/P, Mg²⁺/P and Ca²⁺/P ($p < 0.0001$). The distinctive feature of relationships in the control group was the presence of correlation of inversely proportional moderate strength between Ca²⁺/Cl⁻ and P/Ca²⁺ ($r = -0.5973$), directly proportional high strength between Mg²⁺/Ca²⁺ and P/Ca²⁺ ($r = 0.7055$), and the absence of moderate inversely proportional Mg²⁺/Ca²⁺ with Ca²⁺/PO₄³⁻ ($r = -0.5863$), typical for patients with cervical caries ($p < 0.0001$).

DISCUSSION

From the mineral metabolism point of view, caries is polyelementosis requiring complex pathogenetic treatment, including the normalization of water-salt, phosphorus-calcium metabolism, antioxidants, and immune status of the body [16]. In the case of a pathology development, the relationship between demineralization and remineralization is influenced by the presence of saliva, which ensures the transport of ions on the surface of teeth [17]. Presumably, mineralization/demineralization affects only the outer layer of the enamel, while deeper layers are not affected by these processes [18]. However, these factors can be explained only by the evaluation of chemical composition of the enamel surface of teeth.

Our study involved patients with resistance to caries process and with compensated form of dental caries (DMFT = 1.45 ± 0.48). The level of caries resistance is characterized by certain physical and chemical parameters of OF [7]. There is a high concentration of free and ionized calcium, and a relatively low content of phosphate with a high level of caries resistance [19]. An important role in the onset and progression of the identi-

fied pathology may play the decrease in calcium molarity, which was identified in patients with cervical caries [17]. The increase in the amount of inorganic phosphates is one of the factors that enhance the remineralizing potential of saliva [8]. We have not observed any significant difference in OF chemical composition in the patients with cervical caries and intact hard tissues like other researchers, which could be caused by the compensated form of caries in the main group ($p > 0.05$) [17, 20]. At the same time, other researchers have identified a significant decrease in the number of mineral groups in OF in patients with multiple caries [17, 21]. They showed reduced concentrations of K⁺, Na⁺, Mg²⁺, Ca²⁺, and increased concentrations of PO₄³⁻, Cl⁻, and SO₄²⁻ in saliva of patients with carious pathology; these results are comparable with that of previous studies ($p < 0.05$) [16].

Taking into account the inter-element of synergy and antagonism as well as the complex influence on various physiological parameters of individual chemical elements, a more sensitive indicator of their relationship is the correlation between the ratios of certain element pairs [16, 22]. The imbalance in OF chemical composition has led to a decrease of metabolically important calcium-phosphorus ratio in patients with cervical caries [9]. The ratio of Na⁺ and K⁺ influences the rates of calcium counterflows [23]. Probably, this could explain the significantly lower indices of Na⁺/K⁺ in the main group ($p \leq 0.05$) in the present study. The dynamics of Na⁺/K⁺ coefficient characterizes the activity of sympathetic-adrenal system and the state of thyroid gland. A role of thyroid dysfunction in the development of caries, changes in periodontal tissues, and mineralization has been already proven [24]. The frequency and intensity of caries is higher in children with hypothyroidism than in healthy children, on the basis of changes in OFs' physicochemical and structural properties: a decrease in the rate of salivation, an increase in viscosity with a simultaneous growth of the calcium content, and a decrease in the concentration of inorganic phosphate [7, 24].

The changes in the physicochemical parameters of saliva are associated with disruption of OF mineralization pro-

cesses, which is determined by its mineralization potential [25]. A mineralization potential, value of the dispersion coefficient, and a span of the dispersion of electrical impedance of OF were significantly lower in the patients with cervical caries than in the patients in the control group, which was regarded as the decrease in the intensity of its mineralization, micellarity, and mineralizing ability [14]. Moreover, a mineralization potential correlates with a calcium content of OF [26]. The average indices of mixed saliva mineralization corresponded to its high level in both groups that was explained by participation of patients with resistance to caries process and compensated forms of caries in the study [9]. It has been confirmed that the mineralizing function of saliva changes in case of development of cariogenic situation in the oral cavity, which leads to destabilization of its crystal structure [25].

Teeth enamel with a carious process was characterized by a slightly higher amount of calcium and a lower amount of phosphorus ($p \leq 0.05$), while there was a tendency towards an increase in the amount of magnesium, probably, due to the replacement of a part of calcium atoms in calcium-deficient apatite with magnesium ($p > 0.05$) [15, 22]. The amount of calcium in the enamel determines its development in different types of pathology [27]. In the enamel of cervical region of all samples, the amount of calcium was in a direct relationship of high strength with phosphorus, which confirms the results of other researchers [27].

The magnesium content decreases with the increase of the calcium content, with its direct correlation [17, 18]. Here, we determined an inverse correlation between calcium, phosphorus, potassium, sodium, and chlorine in the enamel of permanent teeth and OF. Other researchers have found a negative correlation between the concentration of phosphorus, sodium, and magnesium in saliva and enamel of deciduous teeth [22]. In their opinion, it indicates the role of OF as a source of ions and/or reflects complex interelement interactions (for example, functional antagonism of Ca^{2+} and P, Ca^{2+} and Mg^{2+}) [22]. Most likely, it is due to the difference in the relationship of saliva and enamel of temporary and permanent teeth.

A number of researchers have determined a strong correlation between the coefficients of Ca^{2+}/P and Ca^{2+}/Mg in OF and hard tissues of deciduous teeth [22]. In our study, we found a similar relationship between the cervical enamel and mixed saliva of the patients with cervical caries and intact hard tissues. The increase in the molar ratio Mg/P in the enamel of the samples with a carious process was the evidence of the partial replacement of calcium with magnesium in the structure of hydroxyapatite [28]. Obtained results show the importance of screening for risk factors for caries development, which begins with the determination of physicochemical parameters of saliva.

CONCLUSIONS

The imbalance of the physical and chemical parameters of oral fluid affecting the level of its mineralization, their correlation with the chemical composition of the enamel is the background, against which it is possible to predict and suspend the development of carious process. Revealed relationships reflect the features of etiopathogenetic processes in the onset and progression of cervical caries. The obtained results can be used as a non-invasive method in pre-clinical diagnosis of the carious process, and as an objective assessment of the effectiveness of prescribed remineralizing therapy.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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