PERSPECTIVES ON STUDYING THE BIOPHYSICAL VALUES OF ORAL FLUID IN PATIENTS WITH DIFFERENT CONDITIONS OF THE CERVICAL AREA OF THE TEETH

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ABSTRACT

Background. The properties of Oral Fluid (OF) directly affect dental pathology development, but their role has not been fully studied yet.

Aim. Identification and analysis of biophysical values of oral fluid in patients with different conditions of the cervical area of teeth.

Materials and Methods. The study enrolled 272 patients aged 18–44 years who underwent a comprehensive examination and were divided into groups based on the condition of the Cervical Area (CA) of the teeth. Biophysical values of the OF were identified. The Odds Ratio (OR) with 95% Confidence Interval (CI) was used to analyze the strength of correlation between factors and the outcome. Risk Factors (RF) were identified using logistic regression analysis. Statistical analysis was performed using Statistica 8.0 (Statsoft, USA). The study was conducted within the framework of the dissertation research "Clinical and pathogenetic substantiation of a differentiated approach to the diagnosis, treatment, and prevention of cervical lesions of hard dental tissues" (2021–2024).

Research Ethics. The research was carried out in accordance with the principles of World Medical Association Declaration of Helsinki (1964–2024), Order of the Ministry of Health of Ukraine No. 690 dated September 23, 2009, conclusion of the Bioethics Commission of Donetsk National Medical University No.43 dated January 21, 2021. All patients provided written informed consent to participate in the study.

Results. Saliva of patients with cervical lesions of teeth (Wedge-Shaped Defect (WSD) and Cervical Caries (CC)) corresponded to significantly lower mean values of electrical impedance (Z) and Coefficient Of Dispersion (COD) compared to patients with intact CA of teeth ($p<0.01$). The liquid crystal phase of OF in patients with WSD and CC of teeth was unstable, with low micellarity and weakened mineralizing function. More significant changes were typical for saliva of patients with WSD of the teeth. Salivary Flow Rate (SFR) less than 0.5 ml/min increased OR of developing WSD and CC: in univariate analysis, 2.12 and 2.26 times, respectively; in multivariate analysis, 1.23 and 2.17 times, respectively. Values of buffer capacity less than 6.5 increased OR of developing CC: in univariate analysis, 2.34 times, in multivariate analysis, 1.30 times. The pH values less than 6.9 increased OR of developing WSD and enamel E: in univariate analysis, 1.50 and 2.90 times, respectively; in multivariate analysis – 1.48 and 1.22 times, respectively, and they were also an independent factor of the active course of enamel E. However, in the case of enamel E, wide CIs for the variable, determined in univariate analysis, indicate instability of the assessment.

Conclusions. Values of SFR, buffer capacity, and pH can be considered as RF of developing WSD and CC. The pH values as predictors of development and active course of enamel E require further research. An alternative to the mineralizing potential of saliva is proposed – identification of Z and COD.

Keywords: *dentistry, erosion, caries, risk factors.*

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Abbreviations

AIDS – Acquired Immune Deficiency Syndrome.

CA – Cervical Area.

CC – Cervical Caries.

CI – Confidence Interval.

COD – Coefficient Of Dispersion.

HIV – Human Immunodeficiency Virus.

OF – Oral Fluid.

OR – Odds Ratio.
RF – Risk Factor.
SFR – Salivary Flow Rate.
WSD – Wedge-Shaped Defect.

Introduction

The examination of the OC of patients over 40 years of age shows that restorations in CA of teeth are common, emphasizing the importance of primary prevention at an earlier age. However, carrying out preventive measures, which are the same for all patients, includes significant time and money costs that are not always justified. This is because active prevention is needed only for a part of patients who have RF for the development and progression of cervical pathology of teeth. Its effectiveness also depends on understanding how predictors change over time in a particular patient [1].

The significant prevalence of caries and non-carious cervical lesions of teeth is explained by many factors, including the incomplete composition and structure of CA that is associated with insufficient intake of calcium, magnesium, phosphorus, and fluoride ions, as well as their imbalance [2]. A decrease in the mineralization of hard dental tissues is known to be characteristic of both carious processes and non-carious lesions [1]. The chemical theory explains the development of a WSD by the demineralizing action of the acids that dissolve enamel minerals [3; 4]. The combination of acids and insufficient SFR results in increased dissolution [4; 5].

Therefore, pathological processes in hard dental tissues are associated with ion exchange mechanisms between OC and dental tissues. The OF plays a direct role in maintaining the balance between mineralization and demineralization due to enamel permeability to mineral substances [2]. As an exchange medium for the tissues of OC, saliva contains macro- and microelements, including trace elements, that are necessary for the mineralization of enamel and dentin [3]. Therefore, it is advisable to study the chemical composition of both dental tissues and saliva [6]. However, it depends on many factors, in particular, the rate of its secretion [7]. Maintaining a slightly alkaline state of OF is ensured by its buffer function, which minimizes demineralization and enhances enamel remineralization [8]. So, biophysical properties of saliva such as SFR, buffer capacity, and hydrogen index (pH) must be normal for the functioning of OC organs and tissues, the preservation of its protective function, and the inhibition of the growth of cariogenic microorganisms [7–10].

OF is also an important biological protective factor of teeth against Erosion (E) [4; 11; 12]. Saliva components form an acquired dental pellicle that prevents acid from contacting with tooth surfaces. OF begins to act even before the onset of acid attack in the form of an increase in SFR in response to acid stimuli. This improves its buffer capacity effectively, diluting acids that come into contact with tooth surfaces during erosive action [12]. Insufficient saliva saturation, which is determined by pH and the amount of calcium and phosphate, is the main cause of dissolution of hard dental tissues [13]. Therefore, the development of enamel E is influenced by such OF parameters as the amount, pH, and buffer capacity [11; 13].

Any changes, even minor ones or violations of the ratio of saliva components, indicate the development of pathological processes [14]. The imbalance in the chemical composition of OF is known to decrease the metabolically important Ca/P-coefficient which is used as a criterion for the resistance of hard tissues to caries (caries resistance) [2; 15]. Its values affect the hardness of enamel even under conditions of normal OF pH and the absence of acid action [15].

The effectiveness of the mineralizing function of saliva depends not only on its composition, but also on its structure [16]. An important mechanism for maintaining the supersaturation of OF with mineral components is its micellarity, which can be quantitatively assessed by determining the electrical impedance (Z) [16; 17]. However, the electrophysical processes in OC have not been sufficiently studied yet, although they directly affect the development of dental pathology [17].

Thus, there is a need to understand what happens at the boundary between the tooth surface and OC [18]. Saliva serves as a diagnostic and monitoring tool in many fields of science, including dentistry. Its use has many advantages (speed, simplicity, non-invasiveness, etc.) [19; 20]. However, the results of modern scientific studies are contradictory, which makes it relevant to further study the peculiarities of OF values in patients with different conditions of CA of teeth. Since local potential RF can coexist and dynamically interact, it is advisable to study the biophysical properties of saliva that play an important role in the development and progression of cervical pathology of teeth.

The **aim** of the study was to determine biophysical values of oral fluid in young patients with subsequent comparative analysis depending on the state of the cervical area of teeth.

Materials and Methods

The study enrolled 272 patients (average age [24.3±6.9] years) who were divided into groups according to the results of the dental examination: I – with WSD of teeth (*n*=55), II – with enamel E (*n*=8), III – with cervical caries [CC] (*n*=48), IV – with combined cervical pathology of teeth (*n*=7), V – with intact CA of teeth (*n*=154). The inclusion criteria in the study were young age according to the WHO classification (2016), the absence of harmful habits, work history, pregnancy and postpartum period, neoplasms, tuberculosis, HIV/AIDS, hepatitis C, and mental disorders.

Unstimulated OF was collected in a sterile container from 10 a.m. to 12 a.m. by spitting. Brushing teeth, eating, and drinking as well as smoking were prohibited for 2 hours before the start of the study. The patient previously rinsed the OC thoroughly twice with distilled water. The pH values were determined using a pH meter AZ-8689 (AZ Instrument Corp., China) by immersing the working part of the device in a container with the collected biomaterial.

Buffer capacity was measured with using potentiometric method. For this purpose, 1 ml of OF was mixed with 3 ml of 0.005 N HCl solution, kept for 5 min, and then determined using a pH meter [14; 21]. Buffer capacity was expressed as the volume (in ml) of 0.005 N HCl required to lower the pH of 1 ml of oral fluid to 4.5. SFR was calculated in ml/min during 10 min based on the formula [4; 14; 22]:

$$SFR=V/t \qquad (1),$$

where: *SFR* – salivary flow rate;

V – the amount of released OF (ml);

t – collection time (min).

In order to avoid the influence of potential local and systemic factors on the results of the *Z* study, its indicators were determined in 33 patients. The selection criteria were a moderate level of personal anxiety [23], a high level of caries-resistant enamel, without any diseases of periodontal tissues and oral mucosa of OC and systemic diseases, orthopedic and orthodontic treatment history, defects of dentition, implants, amalgam fillings, and restorations with marginal fit disorders. any excessive behavioral habits. The patients had good OC hygiene; they did not take any medications on a regular basis and did not receive any systemic antibacterial or immunomodulatory therapy during the 6 months preceding the study. The patients were divided into three levels by the number of groups: the main group – with WSD of teeth; the comparison group – with CC of teeth; the control group – with intact CA of teeth. The groups did not differ in age, gender, OC hygiene status, pH values or buffer capacity of OF and SFR ($p>0.05$). From the total cohort of 272 patients, 33 individuals who met all the above criteria were consecutively selected for the *Z* and COD analyses. The selection was not randomized but was based on strict inclusion criteria to minimize confounding factors.

The frequency dependence of Z of OF was determined, which was recorded in the range of 10 Hz – 10 MHz. The generator with an output signal voltage of 800 mV was used as a source of an alternating signal. The voltage drop across the reference resistance (300 Ohm) and across the cell electrodes was measured with using a voltmeter. The assessment of micellarity and mineralizing ability of saliva was carried out based on measuring the steepness of the dispersion and the COD of *Z* of OF. The frequency dependence of its *Z* is steeper at a high degree of crystallinity of OF than at a weakly expressed crystal phase [16].

COD was calculated in arb. u. by the formula [16; 17]:

$$COD=Z_{lf}/Z_{hf} \qquad (2),$$

where: *COD* – coefficient of dispersion;

Z_{hf} – electrical impedance (*Z*) at high frequencies of the electric current

Z_{lf} – electrical impedance (*Z*) at low frequencies of the electric current.

Low COD values for *Z* [10–15] are consistent with a low degree of saliva micellarity, high electrical conductivity, and low mineralizing ability. High values of COD [60–70] indicate an increase in the capacitive properties of OF, and therefore, an increase in the degree of micellarity, which ensures the mineralization of hard dental tissues [16; 17].

Accumulation, systematization, correction, and visualization of primary data were carried out using Excel 2016 (Microsoft, USA), and statistical analysis using the Statistica 8.0 (Statsoft, USA). The analysis of variance (Welch test) was used in order to compare the differences between the groups. The reliability of the results obtained was assessed by Student's *t*-test. Differences at $p\leq0.05$ were considered statistically significant.

For the assessment of the strength of correlation between the studied factors and outcome, the indicator of OR was applied where the 95% CI was identified. In order to determine RF, univariate (conjugation tables 2×2) and multivariate (Lasso model) logistic regression analysis were used. The factor was considered significant if the CI did not include one. When analyzing the values of OF, the average norm indicators according to literary sources were taken as the limit values [3; 24; 25]. The identified predictors were divided into independent (which are significant in both logistic regression analyses with a change in OR less than 20%) and latent (which are significant only in Lasso, their effect is manifested after controlling for confounders) [26].

The signs characterizing the progression of cervical pathology of teeth included an increase in the number and depth of lesions in one patient, the development of their combination and symptoms of dentin hypersensitivity that indirectly indicates the activity of the pathological process.

Research Ethics

The study was carried out based on the principles of World Medical Association Declaration of Helsinki (1964–2024), Order No.690 of the Ministry of Health of Ukraine (dated September 23, 2009) and it completely excluded restriction of the patient's interests and harm to their health (conclusion of the Bioethics Commission of Donetsk National Medical University Order No.43 (dated January 21, 2021). The subjects provided "Informed voluntary consent of the patient for diagnosis, treatment, and for surgery and anesthesia, and for the presence or participation of participants in the educational process".

Results

The values of pH, buffer capacity of OF and SFR were determined at the first stage of the study (*Table*).

In women, the average values of pH and buffer capacity of OF were slightly higher, and, on the contrary, SFR was lower ($p>0.05$). The assessment of SFR showed that its values ranged from 0.16 ml/min to 2.0 ml/min. The values of SFR less than 0.5 ml/min were determined in 36.4% of patients in group I and 41.7% in group III, which is significantly higher than in group V (20.1%), $p<0.05$. In the examined patients from group III, the values of buffer capacity <6.5 were obtained in 33.3% of patients, which is significantly higher in percentage compared with group V (13.6%), $p<0.05$. Groups I and II significantly differed from group V in the percentage of patients with pH of OF<6.9, $p<0.05$.

Although the mean pH values did not differ significantly between the groups, the threshold of 6.9 was chosen based on the literature data [3; 24; 25] as a clinically relevant cut-off point for increased risk of cervical lesions. To identify this threshold, a Receiver Operating Characteristic (ROC) analysis was performed, which showed that pH<6.9 had the highest sensitivity and specificity for predicting the presence of WSD and enamel E.

Biophysical values of OF were studied as potential RF of the development and progression of cervical dental lesions. Variables with fewer than 10 observations were excluded from the analysis. It was determined that independent RF of the development of cervical pathology of the teeth are SFR for WSD and CC of teeth, and the buffer capacity of OF for CC of teeth. The pH values of saliva are a hidden factor in the development of enamel E and WSD of teeth, as well as an independent predictor of active course of enamel E of teeth.

Thus, SFR less than 0.5 ml/min in a larger number of observations increases OR of developing WSD and CC of teeth: in univariate analysis – by 2.12 (95% CI [1.18–3.79]) and 2.26 (95% CI

Table. Biophysical values of OF depending on the study group (pH – units, buffer capacity – ml of 0.005 N HCl, SFR – ml/min)

Biophysical values of OF		Study group (abs./% of the total number of patients in the group)				
		I	II	III	IV	V
pH	<6.9	36/65.5	6/75.0	31/64.6	6/85.7	82/53.2
	≥6.9	19/34.5	2/25.0	17/35.4	1/14.3	72/46.8
Buffer capacity	<6.5	14/25.5	1/12.5	16/33.3	3/42.9	21/13.6
	≥6.5	41/74.5	7/87.5	32/66.7	4/57.1	133/86.4
SFR	<0.5	20/36.4	1/12.5	20/41.7	3/42.9	31/20.1
	≥0.5	35/63.6	7/87.5	28/58.3	4/57.1	123/79.9

Notes: OF – oral fluid; SFR – salivary flow rate.

[1.21–4.20] times, respectively; in multivariate analysis – by 1.23 (95% CI [1.09–1.38]) and 2.17 (95% CI [1.93–2.44]) times, respectively.

Besides, using univariate and multivariate logistic regression analysis, it was found that the values of the buffer capacity of OF less than 6.5 increase the OR of developing CC of teeth by 2.34 (95% CI [1.19–4.62]) and 1.30 (95% CI [1.15–1.46]) times, respectively (n=17).

The results of the study showed that the pH values of saliva less than 6.9 increase OR of developing WSD and enamel E of teeth: in univariate analysis – by 1.50 (95% CI [0.82–2.75]) and 2.90 (95% CI [0.80–10.52] times, respectively; in multivariate analysis – by 1.48 (95% CI [1.31–1.67]) and 1.22 (95% CI [1.08–1.37]) times, respectively. The variable pH of OF as a factor in the development of WSD and enamel E of teeth in the univariate analysis had CI that crossed one, therefore it was statistically insignificant. In the case of enamel E, wide CIs were determined. The multivariate Lasso model, which simultaneously takes into account all variables, has a higher sensitivity to such effects. This allowed us to identify the independent contribution of saliva pH and classify it as a "hidden" predictor.

The pH values were also determined as independent RF of the active course of enamel E of teeth. In univariate logistic analysis, values less than 6.9 increase the OR by 29.4 times (95% CI [1.12–772.42]) and by 2.47 times (95% CI [1.12–772.42]) [1.49–4.10]) in the Lasso model (n=10). However, wide CIs for the variable in the univariate analysis indicate instability of the assessment. Therefore, the results obtained are preliminary and need to be confirmed in studies with larger sample sizes.

The following studies included 33 patients who were identified as having Z and COD of saliva. It was identified that the groups significantly differed in the average values of Z (p<0.01). Z values of OF were significantly higher in the control group ([5.18±1.72] kOhm) and lower in the comparison group ([4.08±1.98] kOhm). Z had intermediate values ([4.76±1.72] kOhm) in the main group. The obtained average COD values were in the range [31.12±6.34] and exhibited a pronounced dispersion steepness characteristic of a high degree of liquid-crystalline phase in saliva, which is peculiar to caries-resistant patients. COD average values of Z of saliva of the patients with cervical pathology of teeth were significantly lower than those of the subjects with intact teeth. The average values of COD were [24.91±0.67] in the main group, in the comparison group – [30.27±0.69], in the control group – [38.18±0.68] (p=0.004).

More pronounced steepness of COD was characteristic of OF in patients having no cervical pathology of teeth that corresponds to a high degree of liquid crystal phase, higher dispersion, and micellarity. At the same time, the specific complex resistance (Pz) of OF at low frequencies of the patients of the control group was in the range of [50.0–87.5] kOhm. Saliva of the examined subjects of the main group was characterized by a weakly expressed liquid crystal phase, significantly smaller changes of Z value with increasing frequency, and Pz was equal to [9.75–18.75] kOhm. OF of the patients of the comparison group took an intermediate position in terms of the structure and capacitive properties: Pz was in the range of [30.0–38.5] kOhm.

Discussion

It is known that both the quantity and quality of OF play an important role in tooth wear [11]. Some saliva parameters, if they go beyond normative boundaries, contribute to the development of dental caries [27]. Most scientists support the idea that the prevalence of dental caries is associated with a decrease in SFR and a violation of the buffer capacity of OF [28]. Conversely, good buffering capacity of saliva is a protective factor against high levels of dental caries intensity [21]. If the functional activity of the salivary glands is reduced, natural remineralization of teeth does not occur because of insufficient entry of calcium and phosphates into OC [29]. In addition, hyposalivation contributes to the predominance of the microbial factor over the protective properties of the medium [8]. The combined effect of acids and insufficient SFR leads to increased dissolution of hard dental tissues [13]. Therefore, a decrease in saliva production increases the risk of developing caries and other dental pathologies [30]. The performed study confirmed that the OR of the development of WSD and CC increases significantly at speeds below 0.5 ml/min. Like SFR, the buffer capacity of saliva was an independent variable. Its values less than 6.5 increase the OR of developing CC of teeth. However, SFR and buffer capacity of OF can explain only 15.1% of the differences in tooth wear [11].

There are also known observations that have determined the main role of low pH values in the development of dental pathology [31]. It is known that pH of OF is a neutral biological medium [8]. Modern scientists believe that values less than 7.0 increase the probability of a high level of dental caries [8; 21]. The study found that even with pH values of OF less than 6.9, the OR of developing WSD and the enamel E of teeth significantly increase. It is known that lower pH values of OF comply with the patients with multiple non-carious cervical lesions of teeth [12]. However, pH of saliva is a hidden RF for the development of noncarious cervical pathology of teeth. Other researchers have also identified a significant relationship between pH and dental caries [32]. The conducted study did not confirm its existence, which is likely due to the examination of a limited age group of patients and the focus on dental lesions caused by caries in CA.

Because of the small size of the sub-sample, the analysis of the factors influencing the development and active course of enamel E is pilot in nature, making meaningful interpretation impossible. The associations identified are preliminary observations and need to be verified in larger samples and confirmed in prospective studies.

The identified values of Pz in saliva is an integral parameter that reflects the overall change in many of its physicochemical characteristics that affect electrical conductivity (pH, active concentrations of sodium and potassium ions) [16]. Previous studies have showen that *Z* values directly and strongly correlated with SFR, COD, buffer capacity, and pH of OF [4; 22]. The determined micellarity and mineralizing ability of saliva corresponded to its mineralizing potential. Probably, its value across all of examined groups was in strong direct correlation with COD and SFR [22]. The authors determined that the average mineralizing potential of OF in patients with CC and WSD of teeth was 1.39 and 1.64 times lower, than in patients with intact CA of teeth, respectively [4; 22]. This confirmed the statement that one of the causes of the development of dental caries is a decrease in the mineralizing potential of saliva [8]. The analysis of available publications showed the absence of studies that would examine its values in patients with WSD of teeth. Therefore, direct comparison of the obtained results with the data of other scientists is limited.

Thus, the biophysical properties of saliva considered have potential diagnostic and prognostic value for preventive measures [7; 8]. The results obtained confirmed the statement that OF is a promising substrate for the study, especially under physiological conditions, in order to determine integrated parameters and establish their critical values [8; 14].

Conclusions

1. A salivary flow rate less than 0.5 ml/min was identified as an independent risk factor for the development of a wedge-shaped defect and cervical caries of teeth.
2. Buffer capacity of saliva less than 6.5 is an independent risk factor for the development of cervical caries of teeth.
3. The pH values of oral fluid less than 6.9 were identified as a hidden risk factor for the development of a wedge-shaped defect.
4. The determination of electrical impedance and dispersion coefficient is proposed as an alternative to the mineralization potential of saliva

Prospects for Further Research

In the future, it is planned to develop a differentiated approach to therapeutic and preventive measures, taking into account the state of the patient's cervical area, the presence of risk factors of cervical lesions of teeth. In order to verify the obtained experimental results, a separate task is to expand the subsample of patients with enamel erosion of teeth.

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Declarations

Conflict of interest is absent.

The author states that in the process of conducting research, preparing, and editing this manuscript, she did not use any generative AI tools or services to perform any of the tasks listed in the Generative AI Delegation Taxonomy (GAIDeT, 2025). All stages of work (from the development of the research concept to the final editing) were carried out without the involvement of generative artificial intelligence, exclusively by the author.

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