The Contribution of Salivary *p*H in Periodontal Health Assessment

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Intensive studies in last decades set that saliva shows a great potential as a diagnostic fluid and offers advantage over serum and other biological fluids by an economic and noninvasive collection method for monitoring of systemic health and disease progression. The purpose of this report is to provide further information regarding whether salivary pH is a feasible means of differentiating between stages of periodontal disease. This study involved a total of 111 patients who were divided into three equal groups (n=37): group A was represented by patients clinically healthy by this point of view, group B comprised patients with generalized chronic gingivitis and group C included patients with chronic periodontitis. Patients were examined and the plaque (Pl) and gingival index (GI) were calculated, after which the unstimulated saliva was collected. The pH of saliva samples was determined and the results were interpreted using statistical technique for comparing the variance ANOVA. The most alkaline salivary pH was determined for the clinically healthy patients 7.3589 ± 0.08205, while the most acidic one is for the patients with generalized periodontitis (6.7256 ± 0.04377, for p-value < 0.001). Our study showed that there are significant changes in pH between healthy patients and those with chronic gingivitis and generalized chronic periodontitis.

Keywords: salivary pH, chronic gingivitis and generalized chronic periodontitis

Periodontal disease is one of the two major dental diseases that affect human populations worldwide at high prevalence rates. Recent studies establish that in recent decades in industrialized countries in Western Europe and the US, periodontal diseases are the cause of more than 50 % of loose of teeth, surpassing the incidence of dental caries [1].

Many current studies argue that anaerobic gram negative bacterial flora plays an important role in the initiation and progression of marginal periodontal inflammation. In this regard, pathogenic bacteria develop a biofilm that is stuck on the tooth surfaces near the gingival neck but also in the gingival sulcus. So, a true ecosystem develops which is incriminated for the initiation and development of gum diseases [2, 3].

Recent studies provide that the *p*H of periodontal pockets have a great potential in the development of pathogenic microorganisms in these sites. Thus, *Porphyromonas gingivalis* develops between *p*H 6.5 to 7, *Prevotella intermedia* develops between *p*H 5-7 and *Fusobacterium nucleatum* develop between *p*H 5.5 to 7 [4, 5].

Easy diagnosis of periodontal disease and the identification of patients at risk is a current challenge for clinicians. Since periodontal disease is an irreversible disease, early diagnosis is imperative. Furthermore, it has been shown that untreated periodontal disease can lead to systemic disorders such as cardiovascular disease and diabetes. By monitoring host response to microbial infection, people who have in the past periodontal disease can be identified and prevention measures can be established for the future [5, 6].

The buffering capacity of saliva and salivary pH were the subject of numerous studies that can assess individual susceptibility to caries [7-9].

For the diagnosis of periodontal diseases there are a lot of enzymatic kits for beta - glucuronidase, alkaline phosphatase, cathepsins or secretory immunoglobulins of saliva or fluid gingival sulcus (GCF) in response to inflammation caused by bacteria [10, 11].

Despite these advances it cannot be appreciated yet with a specific predictor or a combination of predictors the transition from reversible chronic gingivitis to the irreversible chronic periodontitis stage. This shift largely depends on the individual immune response and it can be quantified by dosing interleukin C of saliva or by determination in salivary *p*H changes [12, 13].

In our study we intended to find out, by simple methods of analysis, whether in groups of patients with periodontal disease occur salivary *p*H changes depending on the severity of periodontal inflammation.

Experimental part

The study was developed in 2015 in the Department of Preventive dentistry in collaboration with the Department of Biochemistry, Faculty of Medicine of Sibiu. A total number of 111 patients aged 20-58 years who were present at the Dental Clinic for different treatments are included in this survey. They were distributed into three equal groups (n = 37), as follows:

- Lot A: a total of 37 clinically healthy periodontal subjects;
- Lot B, with a total of 37 patients with chronic gingivitis localized to a number greater than 6 teeth;
- Lot C, with a total of 37 patients with generalized chronic periodontitis.

For this study we obtained the agreement of the Commission of Ethics Faculty of Medicine and in addition, the patients who agreed to participate in this evaluation signed the inform consent. After anamnesis we removed from study patients presenting following conditions: diabetes mellitus, diagnosed cancer lesions, respiratory infections, and patients with cardio - vascular medication, *big* smokers, patients with malocclusion and patients who had less than 2 functional teeth in each sextant of the dental arch.

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All patients were examined on the stomatological chair using a halogen light bulb.

For the periodontal examination we used the periodontal probe following the internationally criteria recommended by WHO [14, 15]. The data obtained were recorded in individual sheets and served in calculating the plaque index (PI) and gingival index (GI) using Silness - Loe method.

In the group of healthy patients were included patients with clinically healthy gums, with no bleeding on probing having a gingival sulcus smaller than 3 mm.

In the patients with chronic gingivitis group were included patients with clinical signs of gingival inflammation without epithelial insertion loss and a gingival sulcus smaller than 5 mm. The clinical criteria for the award of gingival inflammation scores were:

0 - For healthy gums,

1- Mild inflammation of the gums edemas, no bleeding on probing,

2- Moderate inflammation of the gums and a tendency to spontaneous bleeding.

In the group C (patients with generalized chronic periodontitis) were included patients with periodontal pockets greater than, or equal to 5 mm, with bleeding on probing at least 30 % of examined teeth [14].

Saliva was collected in the morning, between 9-11 a.m., in single use sterile recipients, at least two hours after brushing. The subjects were asked to rinse mouth with water to remove food residue and wait at least 10 min after rinsing to avoid sample dilution before collecting saliva.

Salivary *p*H measurement was carried out immediately after collecting to remove changes that may occur over time. For this we used a digital pH meter with a single electrode (Mettler Toledo Seven Compaq PH - AutoInc. USA).

For statistical assessment of the results we used the software for the Social Sciences (SPSS) version 17.0 for Windows (Chicago, IL, USA). We calculated the average values of pH, of PI and GI indices, together with the standard error of the mean (± SEM).

As a statistical method we used parametric analysis method ANOVA single factor intergroup (One Way ANOVA) with Scheffe correlation test. The value of p was considered significant in terms of statistical p-values < 0.05.

Results and discussions

After centralizing the results presented in table 1, we noticed that the most alkaline pH is 7.3589 ± 0.08205 and appears in group A, which represents the healthy patients, and the acid *p*H of 6.7256 ± 0.04377 is present in group C, representing patients with generalized chronic periodontitis.

Regarding the PI and GI indices it can be also observed an apparent increase of the *p*H values depending on the severity of periodontal inflammation.

Graphical representation of *p*H values in the three groups is shown in figure 1.

For the PI index the application of the Scheffe test indicates the following average variance values represented in table 3.

Table 1
THE AVERAGE VALUES OF *p*H AND PI AND GI INDICES IN STUDIED GROUPS

Oral health status (n=37)	**pH** ± SEM	**PI** ± SEM	**GI** ± SEM
A	7.3589 ± 0.08205	0.7456 ± 0.05204	0.2741± 0.02350
B	7.0919 ± 0.07041	1.1796 ± 0.04201	1.5644± 0.03528
C	6.7256 ± 0.04377	2.3770 ± 0.03926	2.6019± 0.03456

Table 2
STATISTICAL COMPARISON OF *p*H VALUES IN THE STUDIED GROUPS

(I) Oral health status	(J) Oral health status	pH		
		Mean Difference (I-J)	Std. Error	P (signification)
A	B	0.26704*	0.09524	0.024
	C	0.63333*	0.09524	0.000
B	A	-0.26704*	0.09524	0.024
	C	0.36630*	0.09524	0.001
C	A	-0.63333*	0.09524	0.000
	B	-0.36630*	0.09524	0.001

* The mean difference is significant at the 0.05 level.

Table 3
VARIANCE VALUES OF THE PI INDEX

(I) Oral health status	(J) Oral health status	PI		
		Mean Difference (I-J)	Std. Error	P (signification)
A	B	-0.43407*	0.06332	0.000
	C	-1.63148*	0.06332	0.000
B	A	0.43407*	0.06332	0.000
	C	-1.19741*	0.06332	0.000
C	A	1.63148*	0.06332	0.000
	B	1.19741*	0.06332	0.000

*. The mean difference is significant at the 0.05 level.

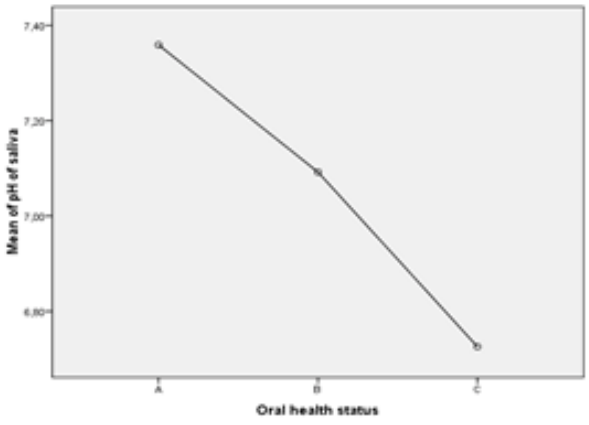

Fig. 1. Graphical representation of pH variation depending on the state of oral health.

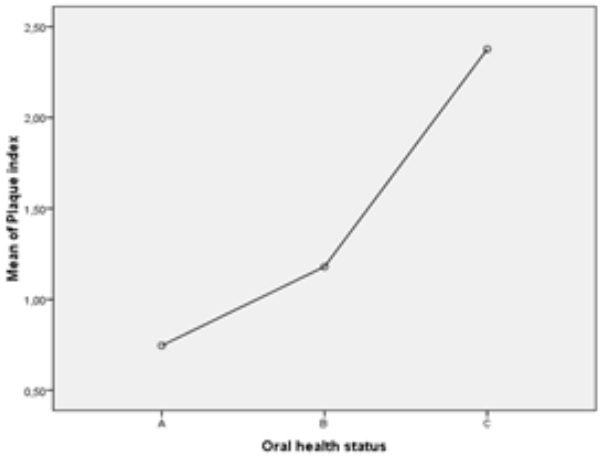

Fig. 2.Graphical representation of PI index variance

Table 4
VARIANCE VALUES OF THE GI INDEX

(I) Oral health status	(J) Oral health status	GI		
		Mean Difference (I-J)	Std. Error	P (signification)
A	B	-1.29037*	0.04465	0.000
	C	-2.32778*	0.04465	0.000
B	A	1.29037*	0.04465	0.000
	C	-1.03741*	0.04465	0.000
C	A	2.32778*	0.04465	0.000
	B	1.03741*	0.04465	0.000

Graphical representation of the variance of the PI index is shown in figure 2.

The Scheffe test for the gingival index (GI) provides the results presented in table 4.

The graphic representation of the variance of the GI index is shown in figure 3.

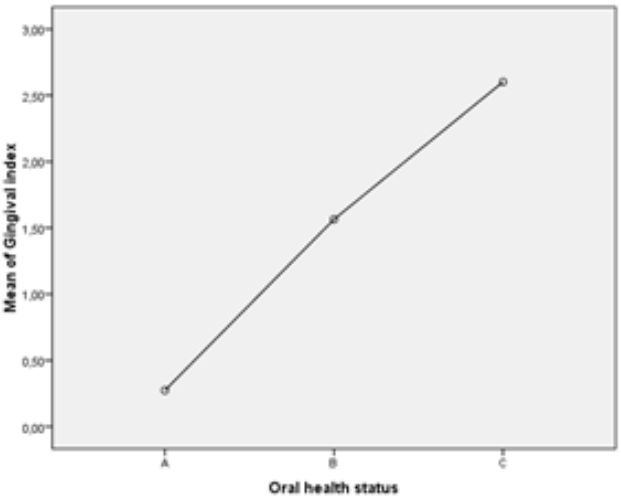

Fig. 3.The graphical representation of the variance GI index values

The amount of saliva secreted by an adult is normally between 500-1500 mL, during 24 h. Resting salivary flow normally varies between 0.3 – 0.5 mL/min and the salivary pH is between 6.7 – 7.6.

Saliva fulfils numerous functions in the oral cavity. One of these important roles is to provide lubrication necessary to the oral surfaces which helps speaking, chewing and swallowing. Another important role of saliva is maintaining the integrity of soft and hard tissues in the oral cavity [17, 18].

By its buffering capacity and function of clearance, saliva intervenes in the adjustment of the *p*H in the oral cavity. The last one influences many biological processes ranging from demineralization / remineralization to the influence in microbial ecology of the oral cavity niches.

Both the quantity and composition of saliva depends on numerous factors including circadian rhythm, salivary gland health, diet, medication, age, sex and general individual health [19]. The use of salivary analyses offers great diagnosis potential through the identification of biomarkers, whose their presence could confirm or disprove either local inflammatory (periodontal disease) or systemic inflammations developed in various diseases like cardiovascular diseases, atherosclerosis, breast cancer, diabetes or even pregnancy [19, 20].

Analyzing the results of Scheffe test, we find that the difference of salivary pH means between groups with oral health status A and respectively C is 0.63333, significant at $p < 0.001$ for the Scheffe test, in both cases less than the critical threshold of 0.05, which allows us to say with a risk of error of less than 5% that the first group C (having chronic generalized periodontitis) has a more acidic *p*H than group A with subjects clinically healthy.

Similarly, the difference of salivary *p*H means between groups with oral health status B and respectively C is 0.36630, significant at $p = 0.001$ for the Scheffe test. Because P (the significance) is less than the critical threshold of 0.05, the subjects from the group C (with chronic generalized periodontitis) have a more acidic *p*H than subjects from the group B (with chronic gingivitis).

The *p*H levels of the group of patients with gingivitis are more alkaline than patients with periodontitis. This can be explained by the fact that sub gingival plaque bacteria are able to neutralize the active *p*H of saliva, because these predominantly anaerobic bacteria degrade nitrogen components in simple peptides or amino acids by proteolysis. These components are able to raise the *p*H followed by the absolution of local gingival sulcus liquid from these periodontal pockets in saliva contributing to a more alkaline *p*H of saliva. The slightly alkaline local *p*H may partly be explained by the predisposition to calculus over and under the gum in these patients.

In their studies, Takahashi et all [4] found correlations between the *p*H of the periodontal pockets and severity of gingival inflammation demonstrating a preference for growth of bacteria and for promoting an acid environment. And in our current study, we also found more acidic pH in saliva both in group B patients (chronic gingivitis) and those in groups C (generalized chronic periodontitis).

Fujikawa et all [21] reported correlations between the *p*H of periodontal pockets and the *p*H of saliva. They reported a significant change of the *p*H to alkaline in the deep periodontal pockets where the proteolytic anaerobic bacterial flora remodeling occurs.

Unlike Baliga S et all [13] who found significant values for a more alkaline salivary *p*H in patients with gingivitis than for patients with periodontitis or even healthy ones, we found in our study a more acidic *p*H of saliva both for patients with chronic gingivitis and for those with generalized chronic periodontitis.

We found statistically significant correlations between the *p*H of saliva plaque index (PI) and gingival index (GI) and periodontal health status. This can be explained in part by the fact that resting salivary flow may contain a smaller

amount of periodontal pocket fluid in the, which doesn't significantly alters is the *p*H of saliva.

The data obtained by us, which highlights that those patients with chronic gingivitis don't have a more alkaline pH in comparison with those with chronic periodontitis were obtained by other researchers too, who found a random *p*H value between 2-9 in the periodontal pockets [22]. These results can be explained in part by the fact that the physiology of the process of inflammation of the deep periodontal can have multiple and complex causes. The different values of *p*H in these cases suggest different stages of periodontal disease which coincide with the periods of lysis or repair of periodontal fibers.

Conclusions

Although the limited technics we used in this research, our results can be compared with the results of other studies in which more sophisticated techniques were applied. These results show that the *p*H of saliva can be used in most cases as a biomarker for periodontal disease, where there are significant differences between chronic gingivitis and generalized chronic periodontitis.

As a result, we conclude that there is a need of more, complex studies and larger groups in order to determine the limits between clinical stages of chronic inflammation from reversible to irreversible, dependent besides salivary *p*H of numerous individual parameters.

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