The Comparative Evaluation of Salivary Biomarkers (Calcium, Phosphate, Salivary *p*H) in Cario-resistance Versus Cario-activity

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The aim of this study is to evaluate the existence of a causal relationship between salivary parameters: calcium, inorganic salivary phosphate, salivary pH and the dental caries in study subjects. We also analyzed the relationship between these parameters and the dental status in cario-resistant and cario-active patients.

Key words: calcium, phosphate, salivary pH, dental status

Due to the presence of calcium and phosphate, the human saliva has a great potential in the remineralisation of carious enamel, a potential which is quite constant in the same subject and different from one subject to another. The mineral component of enamel, dentin and cement is the hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. In a neutral *p*H, the hydroxyapatite is in a balance with the oral environment, saturated in Ca^{2+} and PO_4^{3-} [1].

The demineralisation process is reversible if the *p*H is neutral and if there are enough Ca^{2+} and PO_4^{3-} in the liquid environment [2].

The remineralisation process consists in the precipitation of calcium and phosphate as insoluble Calcium Phosphate which, when present in the saliva, is brought to the demineralised enamel in incipient defects resulted from surface demineralisation [3].

The optimal and correct remineralisation is impaired by certain organic substances in the saliva, like mucin whose affinity for the hydroxyapatite is known and together they form a surface pellicle which perturbs the remineralizing ionic transport. The fluoride ions facilitate the profound penetration of remineralizing ions into the demineralised defects [4].

A good oral hygiene and high buffer abilities are frequent in cario-resistant subjects versus cario-active ones who have unsatisfactory oral hygiene and buffer ability of the saliva.

The decrease of salivary *p*H is opposed by the buffer ability of the saliva. A long persistence of a low *p*H can generate overwhelmed salivary buffer systems, with the precipitation of mucins and salivary proteins on the dental surface and the formation of a mucin bacterial plaque which initiates the carious process [3, 4].

We proposed the evaluation of a causal relationship between salivary parameters: calcium, inorganic salivary phosphate, salivary *p*H and the dental caries in study subjects. We also analyzed the relationship between these parameters and the dental status in cario-resistant and cario-active patients.

Experimental part

Materials and method

The research was conducted between 2006 and 2011 on 516 child and teenager subjects from I-XII grades which were submitted to a dental examination in the school dental office. The obtained data (from alimentation questionnaire, extra-oral and oral examination, radiologic and laboratory data and the salivary concentrations of calcium, phosphate and *p*H) were registered in the speciality observation charts.

The saliva samples were collected during the clinical examinations from each patient for the biochemical assessment of salivary *p*H, salivary inorganic phosphates and total salivary calcium.

The saliva collection preceded the diagnosis procedures and was done after the therapy measures, by chewing a cotton roll for 5 min. The cotton roll was centrifuged and 1-2mL of saliva was collected, permitting the biochemical evaluations.

Following the literature data, we aimed therefore the correlations between the salivary concentrations of calcium, phosphate and pH to the carious lesions observed in 516 subjects [5].

Calcium Evaluation

For the evaluation of the salivary calcium we used the Calcium-Arsenase III test, Konelab method. In neutral environment, in the presence of arsenase III, the calcium ions form a coloured compound. The intensity of the colour is proportional to the calcium ions concentration.

In order to verify the values, we evaluated the salivary calcium also by the Hitachi 912: *CALCIU SYS1* METHOD, equivalent for the quantitative evaluation of calcium in serum, plasma and urine on the Hitachi 912 system [6].

Salivary *p*H evaluation

It is generally admitted that the saliva has a slightly acid reaction, with mean values of 6.5-7.5. The assessment of salivary *p*H was conducted with a paper band – standard *p*H indicator. The band was placed in the oral cavity in contact with saliva and the obtained colour was compared to a standard colour scale; the obtained values were registered in the personal charts of each patient.

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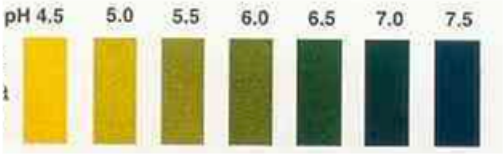

Fig.1 The colour scale for the pH indicator

Inorganic Phosphate Evaluation

The buffer systems protect the oral tissues. This protection also requires a constant salivary *p*H. The UV test was employed for the evaluation of salivary buffer systems. For comparison, we also evaluated the serum, plasma and urine phosphor on the Hitachi 912 system.

We evaluated the oral health status, correlated to the mean values of salivary parameters: salivary calcium, phosphate and *p*H.

The study was a prospective one, including patients between 6 and 18 years old, structured on age groups (6-11 years old, 12-14 years old and 15-18 years old) and dental status groups (cario-resistant and cario-active subjects).

The comparative evaluation was made on cario-active subjects versus cario-resistant subjects for each salivary parameter, on age and gender groups.

The statistical analysis was conducted using SPSS 10 Software, parametric tests (independent samples T test), non-parametric tests (Man – Whitney test and Chi – square test) and association tables.

Results and discussions

The Evaluation of the Oral Health Status in School Age Population (6-18 Years Old)

The most common evaluation method of the carious morbidity degree is the DMF Index, for the permanent dentition on 28 teeth, without wisdom teeth. DMF Index translates into D – Decayed, M – Missing and F – Filled teeth.

This Index corresponds to the sum of unhealthy teeth [11].

Based on individual DMF values, according to the Lehnert classification, we observed a number of 384 of cario-active subjects and 132 cario-resistant subjects.

The carious intensity index was calculated for the included subjects with values of DMF-T=3.77 and DMF-S=5.85, respectively. The values correspond to those from the literature, comparable to the values obtained in similarly economic countries such as Turkey, Hungary, Czech Republic and Belarus. Contrary, in the Nordic countries the carious intensity index is much lower (DMF-T=2.8 in Finland) [8-10].

The Salivary Parameters in Cario-active and Cario-resistant in Both Genders

The difference in salivary calcium values between the cario-active and cario-resistant groups is obvious. We also observed remarkable differences between the mean *p*H and salivary phosphate values.

The lower value of the buffer ability in cario-active subjects than in cario-resistant subjects is clear.

The mean results of the parameters show, both in female and male subjects, significant higher values for the cario-resistant subjects (p=0.000).

The Salivary Parameters in Cario-active and Cario-resistant in Male Subjects

The mean values of the salivary biomarkers are significantly lower in male cario-active subjects than in male cario-resistant subjects.

The Salivary Parameters in Cario-active and Cario-resistant in Female Subjects

The mean values of the salivary biomarkers show a significant difference in female cario-active subjects versus female cario-resistant subjects. The values are significantly higher in patients who present fewer carious lesions.

Table 1
THE FREQUENCY OF CARIO-RESISTANT AND CARIO-ACTIVE SUBJECTS

Total	Cario-active	Cario-resistant
516	384	132
100%	74%	26%

Table 2
THE MEAN VALUES OF SALIVARY pH, PHOSPHATE AND CALCIUM

	Salivary pH	Salivary Phosphate	Salivary Calcium
Cario-resistant	6.99	3.14	1.89
Cario-active	6.47	2.01	1.16

Table 3
THE MEAN VALUES OF SALIVARY pH, PHOSPHATE AND CALCIUM IN MALE SUBJECTS (6-18 YEARS OLD)

	Salivary pH	Salivary Phosphate	Salivary Calcium
Cario-resistant	6.95	3.36	1.74
Cario-active	6.50	2.35	1.20

Table 4
THE MEAN VALUES OF SALIVARY pH, PHOSPHATE AND CALCIUM IN FEMALE SUBJECTS (6-18 YEARS OLD)

	Salivary pH	Salivary Phosphate	Salivary Calcium
Cario-resistant	7.02	2.99	2.00
Cario-active	6.47	1.91	1.15

Table 5
THE MEAN VALUES OF SALIVARY pH, PHOSPHATE AND CALCIUM IN 6-11 YEARS OLD GROUP

	Salivary pH	Salivary Phosphate	Salivary Calcium
Cario-resistant	6.93	3.92	1.40
Cario-active	6.38	3.33	1.14

Table 6
THE MEAN VALUES OF SALIVARY pH, PHOSPHATE AND CALCIUM IN 12-14 YEARS OLD GROUP

	Salivary pH	Salivary Phosphate	Salivary Calcium
Cario-resistant	7.17	2.32	1.84
Cario-active	6.50	1.56	1.04

The Salivary Parameters in Cario-active and Cario-resistant in Both Genders, on the Age Group of 6-11 Years Old

The mean values of the salivary parameters in 6-11 years old group are significantly higher in cario-resistant versus cario-active subjects. The difference between groups was statistically significant for salivary calcium, salivary phosphate and salivary *p*H with p-values of 0.002, 0.002 and 0.036 respectively.

The Salivary Parameters in Cario-active and Cario-resistant in Both Genders, on the Age Group of 12-14 Years Old

The mean values of the salivary parameters in 12-14 years old group are significantly higher in cario-resistant versus cario-active subjects (p=0.000).

The Salivary Parameters in Cario-active and Cario-resistant in Both Genders, on the Age Group of 15-18 Years Old

The 15-18 years old group is characterized by significantly higher values of the biochemical parameters for the cario-active subjects when compared to the cario-resistant patients.

The Salivary Parameters in Cario-active and Cario-resistant in Female Subjects, in the 6-11 Years Old Age Group

The cario-resistant female subjects in the 6-11 years old group presented significantly higher values of the salivary parameters than the cario-active ones (p=0.000).

The Salivary Parameters in Cario-active and Cario-resistant in Male Subjects, in the 6-11 Years Old Age Group

The comparative evaluation in the male subjects of the 6-11 years old group revealed no statistically significant values between resistant and active groups regarding the calcium and the salivary phosphate (p=0.12 and p=0.348, respectively), contrary to the high difference of *p*H values (p=0.009). The number of carious lesions in cario-active subjects was significantly higher than the number in cario-resistant-subjects (p=0.000).

The Salivary Parameters in Cario-active and Cario-resistant in Female Subjects, in the 12-14 Years Old Age Group

The cario-resistant female subjects in the 12-14 years old group presented significantly higher values of the salivary parameters concentrations than the cario-active ones (p=0.000).

The Salivary Parameters in Cario-active and Cario-resistant in Male Subjects, in the 12-14 Years Old Age Group

The mean values of salivary pH, calcium and phosphate in the male subjects of the 12-14 years old group showed statistically significant differences.

	Salivary pH	Salivary Phosphate	Salivary Calcium
Cario-resistant	6.93	2.86	2.42
Cario-active	6.51	1.54	1.22

Table 7
THE MEAN VALUES OF SALIVARY *p*H, PHOSPHATE AND CALCIUM IN 15-18 YEARS OLD GROUP

	Salivary pH	Salivary Phosphate	Salivary Calcium
Cario-resistant	6.99	3.90	1.51
Cario-active	6.33	3.22	1.14

Table 8
THE MEAN VALUES OF SALIVARY *p*H, PHOSPHATE AND CALCIUM IN FEMALE SUBJECTS OF THE 6-11 YEARS OLD GROUP

	Salivary pH	Salivary Phosphate	Salivary Calcium
Cario-resistant	6.89	3.94	1.32
Cario-active	6.49	3.55	1.12

Table 9
THE MEAN VALUES OF SALIVARY *p*H, PHOSPHATE AND CALCIUM IN MALE SUBJECTS OF THE 6-11 YEARS OLD GROUP

	Salivary pH	Salivary Phosphate	Salivary Calcium
Cario-resistant	7.16	2.30	1.78
Cario-active	6.51	1.54	1.05

Table 10
THE MEAN VALUES OF SALIVARY *p*H, PHOSPHATE AND CALCIUM IN FEMALE SUBJECTS OF THE 12-14 YEARS OLD GROUP

	Salivary pH	Salivary Phosphate	Salivary Calcium
Cario-resistant	7.19	2.38	1.99
Cario-active	6.45	1.61	1.04

Table 11
THE MEAN VALUES OF SALIVARY *p*H, PHOSPHATE AND CALCIUM IN MALE SUBJECTS OF THE 12-14 YEARS OLD GROUP

	Salivary pH	Salivary Phosphate	Salivary Calcium
Cario-resistant	6.94	2.88	2.44
Cario-active	6.50	1.54	1.19

Table 12
THE MEAN VALUES OF SALIVARY *p*H, PHOSPHATE AND CALCIUM IN FEMALE SUBJECTS OF THE 15-18 YEARS OLD GROUP

	Salivary pH	Salivary Phosphate	Salivary Calcium
Cario-resistant	6.92	2.83	2.40
Cario-active	6.55	1.54	1.38

Table 13
THE MEAN VALUES OF SALIVARY *p*H, PHOSPHATE AND CALCIUM IN MALE SUBJECTS OF THE 15-18 YEARS OLD GROUP

The Salivary Parameters in Cario-active and Cario-resistant in Female Subjects, in the 15-18 Years Old Age Group

The values of the salivary parameters in 15-18 years old female subjects are significantly higher in cario-resistant subjects than in cario-active subjects ($p=0.000$). The carious lesions number is also lower in resistant subjects than in active ones ($p=0.000$).

The Salivary Parameters in Cario-active and Cario-resistant in Male Subjects, in the 15-18 Years Old Age Group

The values of the salivary parameters in 15-18 years old male subjects are significantly higher in cario-resistant subjects than in cario-active subjects ($p=0.000$).

The DMF Index is negatively correlated with statistic significance to the concentrations of each salivary parameter only for the 15-18 years old age group.

In the 15-18 years old female group, the cario-active subjects are statistically predominant.

The cario-active male subjects are statistically predominant in the 12-14 and 15-18 years old age groups.

Conclusions

The mean values of the salivary parameters (calcium phosphates and *p*H) were statistically higher in cario-resistant subjects when compared to cario-active subjects in both genders in each age group.

We observed a statistically significant positive correlation in male subjects and a statistically significant negative correlation in female subjects.

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