SALIVARY PARAMETHERS AT CHILDREN WITH PRIMARY TEETH AT AGE OF 5 YEARS WITH AND WITHOUT CARIES

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Summary: The study we included 74 examinees (43 experimental and 31 in control group) of both genders at the age of 5 years.

The purposes of our study is to determine the relationship of some salivary parameters in group subjects with primary teeth, with and without caries.

Results. The differences in values of the examined parameters between the two groups of examinees were not significant for calcium (p = 0,61), about the total proteins in the saliva of children in the control group this difference was significantly higher (p = 0,000) compared to the value of total proteins in the saliva of children in the experimental group, the value of total antioxidant capacity in the saliva of children in the control group was significantly higher (p = 0,000) compared to the value of total antioxidant capacity in the saliva of children in the experimental group. The value of IgG and IgA in the saliva of children in the control group was also significantly higher (p = 0,000) compared to the value of IgA in the saliva of children in the experimental group.

The value of the OHI index in the saliva of children in the experimental group for Z = -3,02 and p<0,01 (p = 0,00) was significantly higher than the value of the OHI index in the saliva of children in the control group.

The distribution of data for magnesium does not deviate from the normal distribution and was tested with t-test whereby the value of the magnesium in the saliva of children in the control group was higher than in the experimental group, but the difference for t = 0,25 and p> 0,05 (p = 0,81) was not significant.

The examined relationship between the presence of dental caries in children with primary dentition as a dependent parameter and values of calcium, magnesium, total proteins, total antioxidant capacity, immunoglobulin A, immunoglobulin G and OHI index in saliva as independent parameters, was for R = 0,74 and p <0,001 (p = 0,000) and also existence of very strong significant correlation.

Conclusion The results obtained in our study show that wide range of ingredients which are present in the saliva may provide relevant indicators with clinical diagnostic and applicative significance, that make saliva an excellent medium for this type of researches.

Key words: dental caries, salivary parameters, oral hygiene

1. INTRODUCTION

Dental caries represents localized posteruptive pathological process that causes destruction of hard dental tissues. It is multifactorial disease, appearing as result of interaction of multiple factors in the oral medium, such as the existence of a receptive host organism, cariogenic microorganisms and suitable substrate. [1,2]

The nutrition, oral hygiene or consumption of fluoride are variable factors that have more or less effect in each case. While traditional prevention is mainly based on strengthening of protective factors, modern strategies for prevention (based on the specific plaque hypothesis) are increasingly being directed to the condition of the aggressive factors, especially the production of bacterial acid. [3]

Saliva is a complex mixture of fluids that surrounds oral tissues and originates from major and minor salivary glands and nonglandular sources such as cell fluid, oral microorganisms and dead cells. [4]

The consistency of the saliva can vary from very liquid, dense, sticky or foamy, depending on its composition, particularly from the amount of proteins in the saliva, which mainly determine its density or foamy consistency.[5]

Different authors have investigated the relationship between the composition of saliva and the cariogenic activity, its beginning and progression.[6]

The most important organic component of saliva are proteins and glycoproteins of the which most dominant are prealbumins, albumins, alpha 1-acid glycoprotein, beta-lipoprotein, lactoferrin, transferrin, immunoglobulins IgA, IgG, IgM, the amylase enzyme and others.[7]

The phosphates from the saliva have an important anticarious role by participating in the composition of the

salivary systems, maintaining the stability of the mineral content of teeth in the process of demineralization and remineralization in the oral cavity.[8,9,10]

Antibacterial and antiviral activity of saliva is mainly accomplished by immunoglobulin class IgA, IgG and rarely with IgM. Considering that saliva is in permanent contact with all tissues in the oral cavity and that contains components with a protective role, maintaining the health of the oral mucosa and other oral tissues, involves constant presence of these elements in physiological conditions. Salivary immunoglobulins are necessary for the preservation of all oral tissues and organs, because they have enormous antimicrobial activity.[11,12]

Based on the available data from literature, we can conclude that dental caries is a disease for which there is undoubted interest in professional and scientific circles for further researches. Considering these findings, the objectives of our research were to determine the relation of some salivary parameters and dental caries in a group of participants with primary teeth with carious lesions and children without caries.

2. MATERIALS AND METHODOLOGY

2.1. Explored materials

In this study 74 examinees of both genders at the age of 5 years were included, of which 43 examinees comprised the experimental group with dental caries and 31 examinees were included in the control group who were without caries , randomly selected from the primary schools in Stip .

In all participants in this study, clinical and laboratory examinations were conducted. This research was realized according to the recommendations for activities resulting from the basic criteria for oral and dental health assessment recommended by WHO in 2006.[13]

This survey was consisted of clinical and biochemical examinations.

2.2. Clinical examinations

2.2.1.Clinical assessment of dental health

Clinical assessment was used for dividing of the participants into two groups:

-group of participants with caries- experimental group

-group of participants without caries-control group

The assessment of dental health was conducted according to generally accepted index for presence or absence of dental caries, the Klein - Palmer index, which takes into consideration all morbidity components of kep-index.

2.2.2. Determination of the index of oral hygiene (OHI-„Oral Higiene Index „)

For assessment of the oral hygiene habits and the presence of soft plaques we used the simplified method of Greene Vermillion which was noted by the following formula:

$$OHI - y = \frac{\sum \text{of six diagnosed tooth surfaces}}{\text{Number of assessed teeth}}$$

The scoring according to Greene-Vermillion index was conducted in the following way:

0 points = 0 extremely good oral hygiene (without soft plaques)

1 point = 0.1 - 0.9 good oral hygiene (soft plaques localized only in the gingival third of the tooth)

2 points = 1.0 - 1.9 poor quality of oral hygiene (soft plaques that cover more than one third and less than two-thirds of the area of the crown surfaces)

3 points = 2.0 - 3.0 very poor oral hygiene (soft plaques cover more than two-thirds of the crown surfaces)

2.3. Laboratory examinations

2.3.1. Collecting samples of saliva

Collecting samples from the saliva of examinees was performed in the morning and at least one hour after the meal and brushing of the teeth. The was not performed if the examinees were during the process of treatment, in preparation for sealing or after injection of local anesthetic.

2.3.2. Biochemical analyses

2.3.2.1. Assessment of the calcium in saliva

The principle of the methodology for determining of the calcium in saliva is based on the fact that calcium ions from the saliva sample, in an alkaline environment react with O-cresolphthalein complex thus forming a complex with purple color that absorbs light maximally 530 nm wavelength. The intensity of color is proportional to the concentration of calcium ions in the sample.

2.3.2.2. Assessment of the magnesium in saliva

The principle of the method for determining magnesium in saliva is based on the fact that in an alkaline environment magnesium ions from the saliva sample react with xylidil blue diazonium salt to form a complex with purple red color that absorbs light at maximally 530 nm. The intensity of color is proportional to the concentration of magnesium ions in the sample.

2.3.2.3. Assessment of the total proteins in saliva

Principle method is by taking pyrogallol in combination with molybdenum, in acidic environment, which forms pyrogallol red-molybdate complex which interacts with base amino groups of protein molecules in the saliva sample, forming a blue-purple complex which absorbs light at maximum of 600 nm. The intensity of color is proportional to the concentration of proteins in the saliva sample.

2.3.2.4. Assessment of IgG and IgA in saliva

The principle of the immunoturbidimetric methodology for determining IgG, IgM and IgA is based on the fact that the proteins in saliva form immune complexes through specific immunochemical reaction with specific common anti-IgG, IgM and IgA antibodies. These complexes blur the sample. The amount of blurring is proportional to the concentration of immunoglobulins in the sample. The result is evaluated through a standard curve.

2.3.2.5. Assessment of the antioxidant capacity of the saliva

The principle of the method for determining TAC appears as result to the 2,2-azino-di- [3-ethyl-benzthiazoline sulfonate] substance (ABTS), which incubated with peroxidase and hydrogen peroxide generates radical cations, $ABTS^{+}$, with relatively stable blue-green color, which intensity is measured at 600 nm. Antioxidants that are present in the saliva sample, which is added, suppress the level of production of this color that is proportional to the concentration of antioxidants in the sample.

2.3.4. Statistical processing

Data analysis is performed in statistical program Statistica 7.1 for Windows, using the following methods:

1. The analysis of the series with attributive characteristics are determined with % of structure

2. At the series with numerical characteristics was performed Descriptive Statistics (Mean;

Std.Deviation;±95,00%CI; Minimum; Maximum);

3. Distribution of the data is tested by: Kolmogoro-Smirnov test; Lilliefors test;

The significance is determined for p <0,05. The data are presented in tables and in graphics.

3.RESULTS

Total of 74 children (100.00%) constituted the group of children with primary dentition. Out of them,43 children (58.10%) were included in the experimental group, from which 18 (24.30%) children were female and 25 (33,80%) children were male. The control group was composed of 31 (41.90%) children, 17 (23.00%) children were female and 14 (18.90%) children were male. In the displayed distribution of the children by gender (table 1), for Pearson Chi-Square = 1,22 and p> 0,05 (p = 0,27) there was no significant difference between the two groups.

Table 1. Group & Gender

			Gender		
			Female	Male	Total
Group	Experimental	Count	18	25	43
		% of Total	24,3%	33,8%	58,1%
	Control	Count	17	14	31
		% of Total	23,0%	18,9%	41,9%
	Total	Count	35	39	74
		% of Total	47,3%	52,7%	100,0%

3.1. Control group

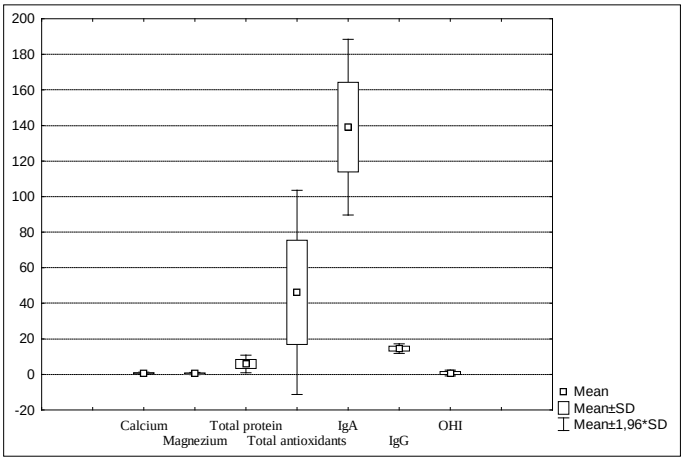
The descriptive statistics of the values of calcium, magnesium, total proteins, total antioxidants, IgG, IgA, OHI in the saliva of children with primary teeth is shown in Table 2 and graph 1.The values of calcium vary in range of 0,65 ± 0,33 mmol / L; ± 95,00% CI: 0,52-0,77; minimum value is 0.20 mmol / L and the maximum value is 1.50 mmol / L.

Magnesium values vary in the range of 0,51 ± 0,33 mmol / L; ± 95,00% CI: 0,39-0,63; minimum value is 0.03 mmol / L and the maximum value is 1.25 mmol / L.

The total proteins vary in interval 5,89 ± 2,53 g / L; ± 95,00% CI: 4,96-6,82; minimum value is 1.30 g/L and the maximum value is 9.80 g/L.

Table 2. Descriptive statistics

Parameters	Valid N	Mean	Confidence -95,00%	Confidence +95,00	Minimum	Maximum	Std. Dev.
Calcium (mmol/L)	31	0,65	0,52	0,77	0,20	1,50	0,33
Magnesium (mmol/L)	31	0,51	0,39	0,63	0,03	1,25	0,33
Tot.proteins (gr/L)	31	5,89	4,96	6,82	1,30	9,80	2,53
VAC (mmol/L)	31	46,16	35,41	56,90	8,30	122,10	29,29
IgA (mgr/mL)	31	139,07	129,82	148,31	16,10	155,50	25,20
IgG mgr/mL)	31	14,51	14,00	15,01	11,90	18,20	1,37
OHI	31	0,81	0,50	1,11	0	2	0,83



Graph 1. Descriptive statistics

The values of total antioxidants vary the interval between 46,16 ± 29,29 mmol / L; ± 95,00% CI: 35,41-56,90; minimum value is 8.30 mmol / L and the maximum value is 122.10 mmol/L.

IgA values vary in the range 139,07 ± 25,20 milligrams / mL; ± 95,00% CI: 129,82-148,31; minimum value is 16.10 milligrams /mL and the maximum value is 155.50 milligrams / mL.

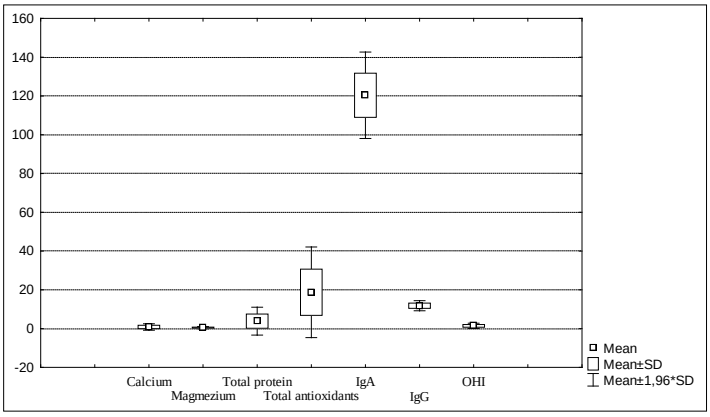
IgG values vary in the interval between 14,51 ± 1,37 milligrams / mL; ± 95,00% CI: 14,00-15,01; minimum value is 11.90 milligrams / mL and the maximum value is 18.20 milligrams/mL.

The values of OHI index vary in the range 0,81 ± 0,83; ± 95,00% CI: 0,50-1,11; the minimum value is 0 and the maximum value is 2.

3.2. Experimental group

Descriptive statistics of the values of calcium, magnesium, total proteins, total antioxidants, IgG, IgA, OHI in the saliva of children with permanent dentition is shown in table 3 and graph 2.

The values of calcium vary in the range 0,86 ± 0,85 mmol / L; ± 95,00% CI: 0,60-1,12; minimum value is 0.20 mmol / L and the maximum value is 4.70 mmol / L.



Graph 2. Descriptive statistics

Table 3. Descriptive statistics

Parameters	Valid N	Mean	Confidence -95,00 %	Confidence +95,00	Minimum	Maximum	Std. Dev.
Calcium(mmol/L)	43	0,86	0,60	1,12	0,20	4,70	0,85
Magnesium (mmol/L)	43	0,49	0,40	0,58	0,07	1,19	0,30
Tot.proteins (gr./L)	43	3,87	2,74	4,99	0,00	15,60	3,67
VAC (mmol/L)	43	18,76	15,09	22,43	5,10	55,20	11,93
IgA (mgr/mL)	43	120,37	116,87	123,87	100,90	154,00	11,37
IgG (mgr/mL)	43	11,82	11,41	12,24	9,80	15,20	1,34
OHI	43	1,44	1,23	1,66	0	2	0,70

Magnesium values vary in the range of 0,49 ± 0,30 mmol / L; ± 95,00% CI: 0,40-0,58; minimum value is 0.07 mmol / L and the maximum value is 1.19 mmol / L.

The total proteins vary in interval 3,87 ± 3,67 g / L; ± 95,00% CI: 2,74-4,99; minimum value is 0.00 g / L and the maximum value is 15.60 g / L.

The values of total antioxidants vary the interval 18,76 ± 11,93 mmol / L; ± 95,00%CI: 15,09-22,43; minimum value is 5.10 mmol / L and the maximum value is 55.20 mmol / L.

IgA values vary in the range 120,37 ± 11,37 milligrams / mL; ± 95,00% I: 116,87-123,87; minimum value is 100.90 milligrams / mL and the maximum value is 154.00 milligrams / mL.

The values of IgG vary in the interval 11,82 ± 1,34 milligrams / mL; ± 95,00% CI: 11,41-12,24; minimum value is 9.80 milligrams / mL and the maximum value is 15.20 milligrams / mL.

The values of OHI index vary in the range 1,44 ± 0,70; ± 95,00% CI: 1,23-1,66; the minimum value is 0 and the maximum value is 2.

1.1 Difference / Control group & Experimental group

The differences in the values of calcium, magnesium, total proteins, total antioxidants, IgG, IgA, OHI in the saliva of children between the control group (without dental caries) and the experimental group (with dental caries) are shown in table 4.

The value of the calcium in saliva at children from the experimental group is higher than in control group, but the difference for Z = -0,50 and p> 0,05 (p = 0,61) is not significant.

The value of total proteins in the saliva of children in the control group for Z = 2,88 and p<0,01 (p = 0,00) is significantly higher than the value of the total proteins in the saliva of children in the experimental group.

The value of total antioxidant capacity in saliva at children in the control group for Z = 4,50 and p<0,001 (p = 0,000) is significantly higher than the value of total antioxidant capacity in the saliva at children in the experimental group.

The IgA value in saliva at children in the control group for Z = 5,21 and p <0,001 (p = 0,000) is significantly higher than the value of IgA in saliva at children in the experimental group.

The IgG value in saliva of children in the control group for Z = 6,17 and p <0,001 (p = 0,000) is significantly higher than the value of IgG in saliva of children in the experimental group.

The value of the OHI index in the saliva of children from the experimental group for Z = -3,02 and p<0,01 (p = 0,00) is significantly higher than the value of OHI index in the saliva of children in the control group.

Table 4. Difference / Control group & Experimental group

Parameter	Rank Sum Conrol	Rank Sum Experiment.	U	Z	p-level	Valid N Control	Valid N Experiment.
Calcium(mmol/L)	1116,50	1658,50	620,50	-0,50	0,61	31	43
Tot.proteins (gr/L)	1425,00	1350,00	404,00	2,88	0,00	31	43
VAC (mmol/L)	1573,50	1201,50	255,50	4,50	0,000	31	43
IgA (mgr/mL)	1638,50	1136,50	190,50	5,21	0,000	31	43
IgG (mgr/mL)	1726,00	1049,00	103,00	6,17	0,000	31	43
OHI index	887,00	1888,00	391,00	-3,02	0,00	31	43

The value of magnesium in the saliva at children in the control group is higher than in the experimental group, but the difference for t = 0,25 and p> 0,05 (p = 0,81) is not significant.

Table 4.1 Difference / Control group & Experimental group

Parameter	Mean Control	Mean Experim.	t-value	df	p	Valid N Con.	Valid N Exp.	Std. Dev. Con.	Std.Dev. Exp.
Magnesium (mmol/L)	0,51	0,49	0,25	72	0,81	31	43	0,33	0,30

3.3. dmf index / Analyzed parameters

The results in table 5 show the examined relation between the presence of dental caries in children with primary dentition as dependent parameters and values of calcium, magnesium, total proteins, total antioxidant capacity, IgA, immunoglobulin G, OHI index in saliva as independent parameters.

For R = 0,74 and p <0,001 (p = 0,000) in the examined relation it is determined existence of very strong significant correlation.

The greatest impact to the examined relation has the immunoglobulin G (Beta = -0,33), immunoglobulin A (Beta = -0,27), the total antioxidant capacity (Beta = -0,25), calcium (Beta = 0,20), OHI index (Beta = 0,18), magnesium (Beta = -0,16) and the weakest is the impact of the total proteins (Beta = -0,15).

For single unit increasing of immunoglobulin G value in the saliva of children the value of dmf index is significantly decreased for 0,51 (B = -0,51) / p <0,01 (p = 0,003), at constant values of the other analyzed parameters.

For single unit increasing of immunoglobulin A value in the saliva of children the value of dmf index is significantly decreased for 0,04 (B = -0,04) / p <0,01 (p = 0,007), at constant values of the other analyzed parameters.

For single unit increasing of total antioxidant capacity value in the saliva of children, the value of dmf index is significantly reduced for 0,03 (B = -0,03) / p <0,01 (p = 0,009), for constant values of the other analyzed parameters.

For single unit increasing of calcium value the in saliva of children, the value of calcium is significantly increased for 0,87 (B = 0,87) / p <0,05 (p = 0,02), for constant values of the other analyzed parameters.

For a single unit increasing of the OHI index value, the value of dmf index significantly increases for 0,65 (B = 0,65) / p <0,05 (p = 0,04), for constant values of other parameters analyzed.

For a single unit increasing of the magnesium value in saliva of children, the value of dmf index insignificantly decreases for 1,54 (B = -1,54) / p> 0,05 (p = 0,06), for constant values of the other analyzed parameters.

For a single unit increasing of the total proteins value in the saliva of children, the value of kep index insignificantly decreases for 0,13 (B = -0,13) / p> 0,05 (p = 0,10), for constant values of the other analyzed parameters.

Table 5. dmf index / Analyzed parameters

Regression Summary for Dependent Variable: dmf ; R= 0,74 ; F(7,66)=11,63 p<0,000						
	Beta	Std.Err. of Beta	B	Std.Err. of B	t(66)	p-level
Intercept			15,09	2,07	7,28	0,000
Calcium (mmol/L)	0,20	0,09	0,87	0,37	2,34	0,022
Magnesium(mmol/L)	-0,16	0,09	-1,54	0,81	-1,91	0,060
Tot.proteins(gr/L)	-0,15	0,09	-0,13	0,08	-1,68	0,098
VAC (mmol/L)	-0,25	0,099	-0,03	0,01	-2,67	0,009
IgA (mgr/mL)	-0,27	0,10	-0,04	0,01	-2,79	0,007
IgG (mgr/mL)	-0,33	0,11	-0,51	0,16	-3,11	0,003
OHI index	0,18	0,09	0,65	0,30	2,12	0,038

4. DISCUSSION

One of the main components of oral fluid, which enables the dynamic equilibrium between the oral tissues and organs, resulting with preservation of the integrity of soft and hard tissues in the oral medium is the saliva.[18] According to Core[14] , the saliva as biological environment is similar to other tissue fluids such as blood, lymph, liquor that are centrally regulated and present part of the general humoral protection of the organism with its protective mechanisms. The saliva has many other functions such as excretory, neutralizing acid and base ingredients of the food.[15]

The values that we obtained for the tested parameters between the two examined groups differed from each other, but we want to emphasize that the difference for each parameter is not significant.

Namely, the differences for the values of examined parameters between two examined groups for calcium value is not significant (p = 0,61).

The results obtained for the values of total proteins in saliva, the total antioxidant capacity in saliva, the value of IgA and IgG at the children in the control group significantly higher (p = 0,000) compared to the value of total proteins in the saliva at children in the experimental group.

The distribution of data for magnesium values does not deviate from the normal distribution and was tested with t test whereby the magnesium value in the saliva of children in the control group is higher than in the experimental group, but the difference for t = 0,25 and p> 0,05 (p = 0,81) is not significant.

We want to emphasize that increasing of the antioxidant activity of saliva is associated with increased protein suspension and cariogenic activity that is not the case with our participants[16] . However, several authors suggest that the differences in the total antioxidant capacity (TAC) between caries active participants and participants without caries is not significant.[17,18]

In contrast to this, available evidence implicate involvement of the oxidative stress in the caries process[19,20] , our results indicate that the total values for antioxidant capacity in the saliva may be of relevance for determinating the susceptibility to caries process.

Our researches does not correspond to the researches of Dipanshu et al[21] which aim of study was to estimate the total antioxidant capacity (TAC) in unstimulated saliva in 100 healthy children at the age of 3-5 years divided into two

groups, control and study group established on the absence or presence of caries and to connect them at individual level with TAC with dmft and the age. The results at the average level TAC in the saliva of children in the study group were significantly increased ($p < 0.001$), and in significant linear regression between TAC and DMFT ($p < 0.001$) but also insignificantly between TAC and the age ($p = 0.078$).

Similar results obtained Tulunoglu et al[22] which examined the antioxidant capacity in saliva and serum in children with and without caries and have found that the antioxidant capacity is significantly increased in the both media ($p < 0.001$) in participants with increased DMFT. The total antioxidant capacity of saliva has positive correlative relation with caries, respectively, with increasing of the dmf values the level of antioxidant capacity also increased.

Karshan[23] and Tulunoglu et al. [22] noted elevated levels of calcium among the participants without caries which is not in accordance with the results obtained in our research.

The OHI index value in the saliva in children from the experimental group was significantly higher than the value of the same index in children from the control group.

The obtained values for maintaining of the oral hygiene suggest about poor and insufficient oral hygiene in both examined groups.

It is generally known fact that poor oral hygiene leads to the presence of dental plaque that represents non-mineralized organized substance of microorganisms in the organic matrix of mucopolysaccharides adherent to the surfaces of teeth. The amount of plaque and the number of bacteria in saliva are directly related to the threat of caries in each individual. This coating at the surface of the teeth is difficult to be removed, it is transparent and invisible and can be removed only by mechanical cleaning, but with staining it becomes visible.
In general, the results for the examined relation between the presence of dental caries in children with primary dentition as dependent parameter and values of calcium, magnesium, total proteins, total antioxidant capacity, IgA, immunoglobulin G and OHI index in saliva as independent parameters, indicated the existence of very strong significant correlation ($R = 0,74$ and $p < 0,001$).

Conclusion

The obtained results suggest to the conclusion that a wide range of ingredients present in the saliva may provide relevant indicators with clinical diagnostic and applicative significance, which make saliva remarkable medium, because its collection is noninvasive and collection process is relatively easy, without any stress, the transportation to the laboratory is easy and is much safer medium for contamination compared to blood and other body fluids.

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