

Determination and Influence of Saliva Calcium and Magnesium in Children with Different Intensity of Caries

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Abstract

In the group of main etiologic factors, besides the fundamental factors, it is also included saliva with its ingredient. The joint action of all the factors in the full derivate saliva provides a multifunctional protective complex which only fails if the flush flow is greatly reduced.

In the study conducted, students of age 12-13 were included. Students were divided into three groups. Fully stimulated and non-stimulated saliva was researched. Statistical processing has been performed at the Medical Faculty, at the Institute of Medical Statistics in Skopje.

The charts are showing results of the analyzed differences for certain parameters between three groups, depending on the DMF. In the Ca levels ($H = 0.27$, $H = 0.08$ and $p > 0.05$ prior and after stimulation, no significant differences were found, however, for $U = 59.5$ and $p < 0.05$ the Ca level prior stimulation is significantly higher in the second compared to the third one.

It is generally accepted that saliva secretion and its components are very important for dental and mouth health in general. Electrolyte level studies of Ca and Mg between healthy group of students and two cariotic ones, show significant quantitative differences in their level.

According to our study, we may conclude the following: saliva mineral analysis have shown significant differences in quantitative and qualitative components between examined groups. This distinction is evident between healthy group of students and those with various DMF. Increase in caries number is due to Ca reduction.

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Introduction

Saliva with its compounds plays an important role in dental and mouth health via lubricating mucosa and defending teeth. Human saliva contains a set of physical, physico-chemical and chemical factors that protect oral tissue from harmful chemical compounds. Our objective was focused on saliva and its components influence, as well as on the influence in subsequent development of dental caries. Contemporary thoughts on caries etiology speak on crossing multi caries factors, tradition related, cross linked to education level and economic status of a particular population. According to FDI classification, caries prevalence depends on general factors, local and iatrogenic

ones. In terms of the relationship between dietary minerals intake and oral disease, the calcium (Ca) and phosphorus (P) concentrations of dental plaque and the levels of Ca and P ions in the saliva could affect the balance between demineralization and remineralization of enamel¹. Some epidemiological studies have revealed that humans with relatively high Ca and P in their plaque experience correspondingly lower caries².

Higher calcium concentration in the plaque is associated with low incidence of caries³. The more frequently the dental plaque pH drops below a critical point, the more frequently demineralization occurs. A significant relationship was also established between cariogenic carbohydrate snacking consumption and the incidence of caries. Thus, the consumption of foods with low cariogenic characteristics contain relatively high protein, moderate fat, minimum carbohydrates, and high mineral content, i.e., calcium and phosphate is helpful to maintain a better pH and stimulate salivary flow.⁴

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Enamel remineralization derives from salivary remineralizing potential with limited constructive mechanism, depending on salivary pH and composition. Loss of Ca ions (Phosphorus and Fluoride) of supersaturated saliva enables demineralizing tissue recovery. Increasing Ca concentration in the remineralizing solution can increase the rate of deposition of minerals in the lesion. Magnesium may also play an important role in preventing periodontal disease and caries as it has the unique ability to reduce inflammation caused by bacterial toxins⁵.

Similarly, magnesium (Mg) has also been shown to have both significant⁶ and no significant⁷ associations with tooth decay.

Materials and methods

Student examination was performed in school settings, involving children of 12-13 years of age. The survey comprised 1248 students. Examinees were divided into three groups, first group listing caries free ones, with DMF=0, the second with DMF=1-6 and third one listing those with DMF>6. Out of the total number of examinees, 106, group I comprised 25, the IInd 47, and the third one 34. For examinee classification purposes, in the first group WHO method on intensity index of measured caries was utilized. Student age selection is matter of numerous beneficences.

Furthermore, oral hygiene maintenance awareness with children groups at this age is satisfactory. With all examinees, full stimulated and non-stimulated saliva was studied. All examinees saliva samples were collected in graded sterile tubes, in the morning hours from 8:00 to 9:00, before intake, at least an hour after brushing. Saliva was collected for 5 minutes, soaked with pipe inject or out of mouth flour, and spilled into sterile tube. Total saliva in strict time frames was collected. During examination time, the patients were calm, sitting and not allowed to swallow. After a while, sterile paraffin to chew was given, for same exact 5 minute period. All tubes collected (212) were cultured at -20°C, enabling bacteria and enzyme inactivation, there of probably influencing the biochemical and immunochemical analysis.

Chemical analysis of Ca and Mg microelements, are performed at the Faculty of Natural Sciences –Department of Chemistry, University of Skopje, using flame atomic

absorption spectrometry. Soolar 2 and SpectraA55B spectrometer types of atomic absorption were used. Settling with flame atomic absorption spectrometer was carried out in optimal conditions with following parameters; Ca wavelength = 422.7 μm, precept spectrum = 0.2μm, lamp power supply= 10mA, while for Mg = 285.2μm, 1.0μm and 4mA. Data were statistically processed at the Institute of Medical Statistics, Faculty of Medicine in Skopje. Average values, standard deviations, confidence interval, Mann Whitney (U) test, KruskalWallis (H) test, t-test, Wilcoxon-Matched pair (Z), analysis of Variance (F) etc. were determined.

Results

In the first group of the researched with DMF = 0 the concentration and amount of Ca and Mg varies before and after the saliva stimulation has different intervals.

In Table 1 a concentration of Ca prior stimulation for Z=0.76 and p>0.05 was change on non-significantly, compared to the same one after to stimulation. But Ca amount for Z=4.37 and p<0.001, after stimulation is altered significantly.

Parameters	N	T	Z	p-value	p	Sig./N.Sig.
Concentration Ca before/after stimulation	25	134.00	0.76	0.44	p>0.05	N.Sig.
Quantity Ca before/after stimulation	25	0.00	4.37	0.00001	p<0.001	Sig.

Table 1. Differences in concentration and quantity of Ca before and after stimulation (gr.I).

In chart 2 Mg concentration after stimulation for Z=1.76 and p>0.05 is nosignificant and the values are lower compared to the same before stimulation, while the amount of Mg for t = 9.9 and p<0.001, is significantly higher after stimulation.

Parametrs	N	T	Z	p-value	p	Sig./N.Sig.
Concentration Mg before/after stimulation	25	97.00	1.76	0.07	p>0.05	N.Sig.
Quantity Mg before/after stimulation	25	T	/	p-value	p	Sig./N.Sig.
		-9.90	/	0.000000	p<0.001	Sig.

Table 2. Differences in concentration and quantity of Mg before and after stimulation (gr.I).

The second group (DMF=1-6) the concentration and amount of Ca and Mg before and after saliva stimulation have different values. Table 3 shows the difference in concentration and amount of Ca prior and after stimulation. Concentration of Ca prior stimulation for Z = 0.49 and p > 0.05 is no significantly higher, compared to the same after stimulation. In regards, report for the amount of Ca for Z = 5.96 and p < 0.001, the amount after stimulation is higher significantly.

Parametrs	N	T	Z	p-value	p	Sig./N.Sig.
Contrecation Ca before/after stimulation	47	517.00	0.49	0.61	p>0.05	N.Sig
Quantity Ca before/after stimulation	47	0.00	5.96	0.000000	p<0.001	Sig.

Table 3. Differences in concentration and quantity of Ca before and after stimulation (gr.II).

Table 4 shows the difference in concentration and levels of Mg prior and after stimulation. Concentration of Mg after stimulation for Z = 2.45 and p < 0.05 is significantly lower, compared to the same before stimulation. In regards, report for the amount of Mg for Z = 5.73 and p < 0.001, the amount after stimulation is higher significantly.

Parametrs	N	T	Z	p-value	p	Sig./N.Sig.
Concentration Mg before/after stimulation	47	332.00	2.45	0.01	p<0.05	Sig.
Quaity Mg Before/after stimulation	47	22.00	5.73	0.000000	p<0.001	Sig.

Table 4. Differences in concentration and quantity of Mg before and after stimulation (gr.II).

In the third group of investigated with the DMF>6 the concentration and amount of Ca and Mg varies before and after stimulation in different intervals.

Parametrs	N	T	Z	p-value	p	Sig./N.Sig.
Concentration Ca before/after stimulation	34	228.00	1.18	0.23	p>0.05	N.Sig
Quaity Ca before/after stimulation	34	0.00	5.08	0.000000	p<0.001	Sig.

Table 5. Differences in concentration and quantity of Ca before and after stimulation (gr.III).

Table 5 shows the difference in concentration and levels of Ca prior and after stimulation. Concentration of Ca after stimulation

for Z = 1.18 and p > 0.05 is insignificantly higher, compared to the same prior stimulation.

In regards, report for the amount of Ca for Z = 5.08 and p < 0.001, the amount after stimulation is higher significantly.

Table 6 shows the difference in concentration and levels of Mg prior and after stimulation for Z = 1.56 and p > 0.05 is insignificantly lower, compared to the same before stimulation. In regards, report for the amount of Mg for Z = 4.98 and p < 0.001, the amount after stimulation is higher significantly.

Parametrs	N	T	Z	p-value	p	Sig./N.Sig.
Concentration Mg before/after stimulation	34	206.00	1.56	0.11	p>0.05	N.Sig
Quantity Mg before/after stimulation	34	6.00	4.98	0.000001	p<0.001	Sig.

Table 6. Differences in concentration and quantity of Mg before and after stimulation (gr.III).

Parametrs	DMF	H	p-value	p	Sig./N.Sig.
Concentration Cammol/L before stimulat	DMF=0	H=0.27	0.87	p>0.05	N.Sig.
	DMF=1-6				
	DMF>6				
Concentration Cammol/L After stimulat.	DMF=0	H=0.08	0.95	p>0.05	N.Sig.
	DMF=1-6				
	DMF>6				
Quantity Ca µmol/L before stimulation	DMF=0	H=5.43	0.06	p>0.05	N.Sig.
	DMF=1-6				
	DMF>6				
Quantity Ca µmol/L after stimulation	DMF=0	H=3.59	0.16	p>0.05	N.Sig.
	DMF=1-6				
	DMF>6				
Concentration Mg mmol/L before stimulat	DMF=0	H=0.12	0.93	p>0.05	N.Sig.
	DMF=1-6				
	DMF>6				
Concentration Mg mmol/L after stimulat.	DMF=0	H=0.10	0.95	p>0.05	N.Sig.
	DMF=1-6				
	DMF>6				
Quantity Mg µmol/L before stimulation	DMF=0	H=3.21	0.20	p>0.05	N.Sig.
	DMF=1-6				
	DMF>6				
Quantity Mg µmol/L after stimulation	DMF=0	H=3.04	0.21	p>0.05	N.Sig.
	DMF=1-6				
	DMF>6				
	Group	U	p-value	P	Sig./Nsig.
Quantity Ca µmol/L before stimulation	2gr. / 3gr.	U=559.5	0.02	p<0.05	Sig.

Table 7. Difference in analyzed parameters between three investigated groups.

Table 7 shows analyzed differences of certain parameters among three examined groups DMF dependent, as well as significant and non-significant differences between the groups. For H=0.27, H=0.08 and p > 0.05 no significant differences in concentration of Ca prior and after stimulation. Between three examined groups for H=5.43 and H=3.59 and p > 0.05, no significant differences in Ca amount, prior and

after stimulation were found, yet for $U=559.5$ and $p<0.05$, Ca prior stimulation is significantly higher in the second group, compared to the third one. For $H=0.12$, $H=0.10$ and $p>0.05$ no significant differences on concentration of magnesium levels prior and after stimulation were found within all study groups. Mg amount difference within three study groups for $H = 3.21$, $H = 3.04$ and $p> 0.05$ prior and after stimulation was no significant, too.

Discussion

Research outcome of native saliva mineral composition have shown major physiological differences in the composition of some minerals in all study groups^{8,9,10} correlating to our results. Through electro-microscopic analysis, it is found that, established remineralizing solution, consisting Ca, P and F in an extracted tooth, submerged in artificial saliva and afterward in the above mentioned solution, recovers enamel damage (caries, decay) and enhances the remineralizing potential of saliva¹¹.

It is generally accepted that saliva secretion and its components are of major significance for dental and overall mouth health¹². Ca role as Fluoride-Calcium¹³ composition shows a great stability within oral environment, thanks to superficial absorbance of HPO_2 in a crystal surface, as well as to the establishment of melting phase, limited phase, which can be used as a reservoir with controlled pH of enamel and dental plaque ions¹⁴.

In a study done in specimens with remineralisation solutions, by adding calcium hydrogen phosphate in appropriate concentration in combination with xylitol and funoran to chewing gum showed an effective enhance for remineralization of initial human enamel caries lesions.¹⁵ In another study researching the composition of Ca and P in full and stimulated saliva, it is found that patients with low caries frequency, have much higher amount of Ca ions, compared to those with higher caries prevalence, which matches our results, too. The saliva of examined students in the age of 12, affected with severe caries, was characterized with alteration of Ca homeostasis, companioned with Ca frequency redistribution within non-stimulated saliva¹⁶. In this case, mild deviation of overall Ca level was noticed. Saliva level of Ca plays a significant role in the hard dental tissue defense

system, where opposite to, lower Ca levels in the saliva means: A-enhancing force for hydroxyl apatite precipitation in neutral pH and B-larger force to dissolve hydroxyl apatite in even more lower – critical pH¹⁷. Ca enriched saliva enables initial damage (caries) remineralization. As mouth rinsing with Ca enriched saliva fights caries, stimulation of saliva secretion via chewing gum is proved to be efficient, too. Under saturation deriving from acidic bacteria production, results in caries, while other way round, supersaturation can help in remineralization of white spots¹⁸. In a study researching electrolyte levels⁸, among students with low caries activity and those with caries inclusiveness from 6-14, critical quantitative difference was found. Our study results from statistical tests show that, in saliva of cariotic students, there is significant correlation between Ca and Mg levels. It is thought this was important for proper mineralization, but also it is foreseen that for maturation purposes, higher levels are needed¹⁹. A higher value of the deft index was associated with lower amounts of daily Ca or P intake. Because the associations were no longer significant after adjusting for potential confounding factors, one could speculate that the daily dietary intakes of Ca or P might not be the primary nutritional factors for caries²⁰.

Decrease in Ca levels will result in reduction of enamel crystallinity, increasing one's retentive surface, and decreasing overall resistance. In a study of saliva Ca levels¹⁰ carried on 23 caries affected students and 32 of unaffected ones, it is found that Ca levels in non-cariotic students are far higher, compared to cariotic ones. Regarding studies carried on Ca molality of mixed saliva in caries affected and not affected students, the data obtained are matching this finding⁹, and not matching different ones^{21,10}.

In a study the results showed a decrease in concentrations of calcium, magnesium and inorganic phosphate with increased severity of dental caries. ²²Study conducted on 70 healthy patients, in collected saliva samples, Mg composition was analyzed, in three different study groups: 7-14 y.o., 18-28 y.o. and 48-60 y.o. Significant Mg level age related difference is found between group I and II, as well as between group I and III²³. It is already known that the composition and the level of inorganic ions in dental plaque and salivary significantly influencing the initiation and development of

caries via saturation level alteration in watery phase of surrounding dental enamel. The level of inorganic ions of Ca and Mg differs significantly between plaque liquid and saliva²⁴. Reduction of crystal density inside enamel is in direct correlation to higher level Mg and CO₂ ions²⁵.

In a prospective study²³ of saliva Mg, with spectrophotometric atomic absorption of 186 patients, treated with digoxin, it is found that salivary Mg level is correlated with the digoxin level in plasma; an obvious increase of saliva Mg level was induced due to digoxin therapy. The amount of salivary Calcium and Magnesium is correlated to alteration of the same mineral levels in blood serum, thus Ca infusion administration didn't change the overall Calcium and other mineral level in saliva¹⁴.

Conclusions

Saliva mineral analysis have shown significant differences in qualitative and quantitative composition between study groups. This difference is most emphasized between the healthy group of students and those with DMF=1-6 and DMF >6. Decrease of Ca molality in saliva might play a significant role in caries occurrence.

Calcium level after stimulation with $Z=0.76$, $p>0.05$, is altered significantly compared to the same ones prior to stimulation. With the increase of caries number the Calcium level decreases. Saliva Calcium level significantly influences hard dental tissues defense mechanism. Low Calcium level in the mouth means: A- enhancing force for hydroxyl apatite precipitation in neutral pH and B-larger force to dissolve hydroxyl apatite in even more lower – critical pH. Calcium enriched saliva with normal PH enables initial caries damage (damage) remineralization. The level and the composition of Mg it has been monitored; it is Calcium related and goes in favor to elemental caries resistance. The Mg level, even after stimulation, didn't show significant alterations.

The level of Mg for $t=9.90$ and $p<0.001$, was significantly higher after stimulation, while Mg level between groups after stimulation, is increased and significant difference was found between group I and III and not significant between group I and II.

Declaration of Interest

All the authors have contributed in this

research so we could achieve these original results and all have reviewed the final paper prior to publishing. There is no Conflict of interest and there was no Source of Funding from any Institution. We have obtained the Ethical Approval from the corresponding organs in our country.

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