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Bacterial Response to The Xylitol at Different Levels of The Oral Medium.

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ABSTRACT

Stomatology science is in a constant search for new assets that can actively participate in preventing oral caries. One of the key factors in that trend is control over the carbohydrate intake. The aim of this study is to check whether the use of the sucrose substitute - Xylitol, affects the colonization of Str. Mutans over the salivary and plaque levels and if it has a protective caries effect.The study was conducted on a group of 90 subjects, of which, the test group consisted of 60 subjects, and the control 30 subjects. The Xylitol is applied three times a day in a 10% solution of aqua pro injectione and xylitol for oral rinsing, suggested by Muhlemann, three times a day, after every meal. Colonization of Str. Mutans is noted over salivary and plaque levels, with a mature plaque and stimulated saliva. The gathered data showed strong caries protective effect of the xylitol in both researched levels, the investigated group with a drastic drop of the colonization of Str. mutans from the beginning to the end of the research period. Sucrose substitute xylitol shows a beneficial protective caries effect, lasting six months after ceasing application.

Keywords: dental caries, Streptococcus mutans, xylitol

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INTRODUCTION

Dental caries is a multifactorial, infective disease, influenced by numerous factors in the oral medium. It is an ecological model based on the irreplaceable ecologic factors by the Keyes triad, in which, all factors have equally important roles and their simultaneous action over a prolonged period of time, causes demineralization of the dental enamel, resulting in caries.

Preventing dental caries is complex and sees action over multiple fronts, starting with dental plaque control, level of cariogenic microorganisms control, and even transcanine sector dental fissure filling, and of course, application of means and methods boosting the process of natural remineralization.

The cariogenic microorganisms participation is undeniable and of crucial importance, because of their ecological characteristics, the ability to produce acids and endure in very low pH value conditions in the dental biofilm. Thus, great importance is put on two essential processes - demineralization and remineralization - DEM and REM, as a natural phenomenon, both of which act dynamically on the dental surface. Equilibrium disturbance between these two processes benefiting DEM, creates conditions for a strategic quantity mineral loss, mostly Ca and P of the crystal structure of the hydroxylapatite, the ultimate product of which is dissolution and destruction of the dental enamel. Weakened, it is affected by masticatory forces, and can't sustain its integrity. The ultimate product is open cariogenic lesion, demanding restoration treatment.

Nevertheless, in a balanced ecosystem, the prolonged and deep downfall of dental plaque pH values, caused by frequent sugar consummation, are rare and/or quickly neutralized, and the plaque pH value is restored to normal levels. Biochemical processes in the plaque, create conditions which sometimes favor REM, and sometimes DEM, but they are usually regulated by plaque and rarely reach the critical pH value of 5,5-5,0, resulting in demineralization. Microflora of such plaque is stable and none of the dental plaque inhabitants have ecological value advantage over the others.

However, the balance between nonpathogenic and pathogenic cariogenic microorganisms is fragile and can easily benefit pathogens. In the long run, changes in abiothic components in an ecosystem, such as increased sugar intake in diet and/or drop in salivary secretion and buffer capacity, cause changes in the microbiotic composition of plaque, causing it to become cariogenic. Increasing the carbohydrate intake, especially sucrose, causes creation of additional acids, which can surpass the saliva capacity to remove acidic products, similarly suffers the saliva neutralization power/plaque buffer capacity, resulting in frequent plaque acidification. Sustained reduction of salivary secretion, especially combined with an increased sugar intake, causes the same outcome. As a result to increased frequency of plaque acidification, the ecological advantage benefits bacteria that is resistant to acids, and causes the enrichment of viable bacterial cells in the plaque with acidogenic and aciduric microorganisms. The thoroughly proven high acidic tolerance of mutans streptococcus pH-4, lactobacillusand other non-mutans streptococcus with low pH, endows selective growth advantage leading to increased acidogenic and intensive selection of these bacteria.

In the fight to overwhelm the caries process, the need to control these organisms is evident, even more than the mechanical removal of dental biofilm, in order to stop the causal link on several crucial spots.

In a modern, preventive model, the steps are taken to eliminate or suppress certain microorganisms from the bacterial flora of the individual. In the dental caries case, these steps help us find agents that can suppress the number of Str. mutans colonies in the saliva and dental plaque.

Suppressing the Str. mutans colony number as an initiator for dental caries, is possible with sucrose substitutes such as sugary alcohols - xylitol. Xylitol is 5-carbohydrate alcohol with the chemical name xylo-pentane. Its empirical formula is $C_5H_{12}O_5$. It belongs in the functional category - sweeteners. Manufactured by different types of cellulose, from different sources- trees, clams, fruits and vegetables - xylan hemicellulose. Also, it is produced as a normal intermediate in the human carbohydrate metabolism. The human body produces 5-15 grams of xylitol daily, mainly in the hepatocytes. Its caloric value is around 2,4 kcal/gram. Chemical characteristics of xylitol allow its diet inclusion without any significant food taste modifications, having a self-cooling effect, as a result of the negative effect on heat during dissolution. It's considered as hypoacid, essentially nontoxic and non-irritable material. Xylitol is universally accepted as a substitute that does not undergo glycolysis and it is insulin nondependent, meaning that diabetics are allowed to use it. It

does not metabolize in acids, leading to food with non-fermenting carbohydrates, and sweetened with xylitol, being called caries free- does not cause caries.

Therefore, the aim of this study is to check whether xylitol influences the colonization of Str. mutans and if it has a caries protective effect.

MATERIALS AND METHODS

Xylitol is implemented as a 10% solution in a sterile, apyrogenic water- aqua pro injectione, as suggested by Muhlemann, used for mouth rinsing.[1] Thus gaining the absolute, local influence of xylitol, without side effects. The daily intake of xylitol is 9 grams, divided in three daily rinses of the oral medium, after every major meal.

The research was conducted on 90 subjects, acting in usual manner, without using fluoride prophylaxis, or any other caries preventive substance over the sixmonths research period.

Xylitol influence was analyzed on saliva and dental plaque levels. The analysis were conducted on mature plaque, one basic and two control. The first was one month after application, the second was six months later.

The methodology of this study is in accordance with the world science standards and uses the Orion diagnostica Dentocult Strip Mutans test Jensen et Brathall, 1989 test, requiring stimulated saliva, and plates with a special microorganism growth medium. The method enables simultaneous use of both saliva as a medium and the dental biofilm, thus enabling insight in the numbers of the mentioned microorganisms on both levels.

Result evaluation of the material is conducted after 48 hours incubation. This method ensures visual and numerical data as a research parameter, in this case before and after application of the polyol xylitol. The number of formed colonies is denoted with CFU-Colony Forming Units, on levels marked as 1, 2 and 3 or index 1 with less then 100.000 bact/ml saliva; index 2 with more then 100.000 bact/ml saliva; and index 3 with more then 1.000.000 bact/ml saliva.

On the plaque level, the colonization is checked with selective Mitis salivarius agar surfaces by Difco.

Statistical analysis with Student t-test, Mann-Whitny U-test, Wilcoxon test, ANOVA and Friedman test were made.

RESULTS

Table 1 shows the results related to colony formation of Str. mutans in saliva and dental plaque. Basic values at the begging of investigation for colony formation of Str. mutans show the near-equal values for categories 1 and 2 (25 and 21), while the number of subjects under category 3 is somewhat lower. It's evident and interesting that at the first control examination, one month after xylitol application, the number of index 1 subjects rises from 25 to 33, in the second control examination, six months after xylitol application, the same number rises to 49, meaning there is reduction in Str. mutans colonization, from the start of polyol application.

Index 2 falls from 21 subjects at the basic examination before polyol application, to 10 at the second control examination, meaning the bacterial colonization in the saliva has decreased during polyol application.

Index 3 noted a drastic decline, from the basic 14 subjects with this colonization index, in the first control examination the CFU value for Str. mutans falls to 6, to follow in the second control examination 6 months after application, the same value falls to 1 subject.

The data shows a strong decline in bacterial colonization during and at the end of the research period.

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Table 1: CFU values for salivary and plaque Streptoccocus mutans

Numbers of bacteria	Before application		I control		II control	
	saliva	plaque	saliva	plaque	saliva	Plaque
< 100 000	25	32	33	34	49	55
> 100 000	21	26	21	25	10	4
> 1 000 000	14	2	6	1	1	1

The same parameter (CFU Str. mutans) for dental plaque notes the following situation: at the basic initial examination, index 1 had 32 subjects; 1 month after polyol application the number of subjects with the same index was 34, at the end of the research period, the number of subjects with index 1 rose to 55. This underlines the fact that the dental plaque sees a decline in bacterial colonization. Index 2 at the basic examination had 26 subjects, at the first research examination 25, just to have their number drastically drop at the second control examination, when index 2 had only 4 subjects. This means that the index 2 subjects showed a drastic decline as well. Index 3 subjects included 2 subjects at the basic examination, the number fell to 1 at the first control examination, but it stayed the same at the second control examination.

Table 2: CFU colonies before application of sorbitol/xylitol

Examined level	Rank sum of CFU colonies sorbitol	Rank sum of CFU colonies xylitol	U	p-level	Significance
saliva	1402	2783,5	906	0,8439	No signif.
plaque	1477,5	2708,5	878,5	0,6662	No signif.

Table 3: CFU colonies after 1 month of application of sorbitol/xylitol

Examined level	Rank sum of CFU colonies sorbitol	Rank sum of CFU colonies xylitol	U	p-level	Significance
saliva	1291	2895	795	0,2582	No signif.
plaque	1301,5	2884,5	805,5	0,2971	No signif.

Table 4: CFU colonies after 6 months of application of sorbitol/xylitol

Examined level	Rank sum of CFU colonies sorbitol	Rank sum of CFU colonies xylitol	U	p-level	Significance
saliva	1349	2837	853	0,5190	No signif.
plaque	1299,5	2886,5	803,5	0,2894	No signif.

Results for the significance of differences between values for CFU of MS in saliva and plaque for the control and investigated group are given on Table 2, 3 and 4. There are no significant differences between the groups, which means that sorbitol (hexitol) and xylitol (pentitol) have almost equal influence with lowering the colonization with Str.mutans in saliva and dental plaque.

Table 5: CFU coloniesbefore application of sorbitol/xylitol

Examined level	Rank sum of CFU colonies sorbitol	Rank sum of CFU colonies xylitol	U	p-level	Significance
plaque	1386,5	2799,5	890,5	0,740	No signif.

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Table 6: CFU colonies after 1 month of application of sorbitol/xylitol

Examined level	Rank sum of CFU colonies sorbitol	Rank sum of CFU colonies xylitol	U	p-level	Significance
plaque	1336	2850	840	0,451	No signif.

Table 7: CFU colonies after 6 months of application of sorbitol/xylitol

Examined level	Rank sum of CFU colonies sorbitol	Rank sum of CFU colonies xylitol	U	p-level	Significance
plaque	1570	2661	786	0,227	No signif.

CFU values for Str. mutans in plaque isolated on Difco plates showed no significant differences in colonization with MS in both groups (U=890,5 and p>0,05) before application of sorbitol/xylitol, after 1 month and after 6 months. (Tables 5, 6 and 7)

On Table 8 we can see that there are significant differences (p<0,001) between the groups for CFU of MS on plaque level in different periods of time.

Table 8: Significance for CFU of MS from the begging till the end in group with xylitol

Parameter	Wicoxon test		
	Z-value	p-level	Significance
Before appl/I control	3,621	0,0002	Sig.
I control/II control	5,908	0,0000	Sig.
Before appl/II control	6,334	0,0000	Sig.
	Friedman ANOVA		
Before appl/I control/II control	Chi.Sqr=91,32	P<0,001	Sig.

DISCUSSION

When talking about evaluating the caries protective effect of the polyol - xylitol, we can't evade the importance of it's influence over the macrobiotic flora in the oral medium, as an important link in the causal chain of caries. The use of polyol solutions containing xylitol and the review of the Str. mutans presence in the saliva and dental plaque is an attempt to participate in modern preventive measures leaning towards caries prevention, striving to indefinitely stop the cariogenic causal effect on a specific place.

The results of our research show that the xylitol selectively influences, for us, the most important bacterial type for initiating the cariogenic process, Str. mutans. The data gained in our research states that regular presence of xylitol in the oral medium, makes the ecosystem unsuitable for Str. mutans. Thus, creating the opportunity in the ecosystem, to be replaced with another type of streptococcus or other less acidogenic bacterial types, or bacteria that does not produce acids.

The research was conducted in order to affirm which bacterial types grow influenced by xylitol. It showed that during its regular, habitual use, less virulent xylitol resistant species appeared, compared to the species that existed before the habitual application of xylitol.

The attitude that xylitol influences the reduction of Str. mutans population is shared with other authors which conducted similar research. It's interesting that from the whole streptococcus genus, the greatest sensitivity is manifested in the mutans species - growth inhibition is 80%. [2]Str.salivarius et sanguis are weakly inhibited, while growth inhibition is not noted in Actinomyces and Lactobacillus.

Ly, Milgrom and Rothen [3] in 2006 state that xylitol causes a drop in Str. mutans levels in plaque and saliva and influences the reduction of dental caries in young individuals, mothers and children compared to transfer mothers. This data supports and confirms the results of our study.

The fact that the predisposition towards dental caries and risk level for its initiation and development on relation mother and child with microorganismstransferis proved, according to Nakaiet al. [4], an important caries prevention strategy for children includes measure which interfere the Str. mutans transmission. The study confirms that early mother xylitol exposition by chewing gums, has a valuable positive effect in MS transmission from mother to child, or, the xylitol MS colonization reduction effect leads to significantly lower risk of dental caries in children, relating the MS transfer from mother to child. The results from this Japanese study are similar to those conducted in the Nordic countries, and to our pleasure correlate to the results of our study.

Makinen et al.[5,6] state that the levels of mutans are lower in children that use xylitol, underlining the attitude that there is a dose of dependent effect.

According to Makinen [7], habitual use in relatively small daily quantities of chewing gum containing xylitol, in young individuals, can be treated as an important caries preventive procedure, such as combining the treatment in daily care centers and home conditions. Chewing gum containing xylitol significantly reduces the Str. mutans growth in saliva and dental plaque, the two most important parameters associated with dental caries.

Marwaha et al. [8], also confirm the reduction of present salivary Str. mutans levels, but it is insignificant to the dose and frequency of sugar-free chewing gum intake. Therefore, it is advised to limit the dose of four chewing gums daily, instead of eight as in the previous advice.

Trahan et al. [9,10], in the Turku study, conducted in Finland attempted to explain the xylitol action mechanism as a caries preventive sweetener. The xylitol sensitive mutans species accumulate toxic xylitol phosphate during the intake process.

Evaluating the data gained in the Finland study in 1991 [9, 10], shows a significantly lower Str. mutans number in the proximal zones to the molars in habitual xylitol consumers. Our results and the resulting opinion are in correlation to the previously stated, therefore the plaque specimens taken from our subjects are from the interproximal chambers of the molar region teeth.

When xylitol is the only sweetener, it significantly reduces the quantity of plaque and levels of Str. mutans, which benefits the opinion that it is capable to modulate plaque and its microbiotic composition.

There are findings that relate to the xylitol influence on the sucrose metabolism. These findings show that xylitol does not influence this process, but it causes glucose translocation over the bacterial membrane, casing a blockade to the glucose-attaching proteins, and affects the phospho-transferase or the permeases system. Thus, it blocks glycolysis (bacteria can't metabolize it), resulting in an inability to create acidic products which would lead to a pH value drop near critical levels, creating conditions to initiate the carious process.

Xylitol seems to be the only sugary alcohol in its inhibitory effect on glycolysis in oral bacteria, especially in Str. mutans. The inhibitory effect is explained with the xylitol intake with the established fructose specific PTS system and the following intracellular accumulation of xylitol 5 phosphate. In "in vivo" conditions, this mechanism leads to a reduction in acid formation by glucose and reduction in Str. mutans numbers.

The majority of studies conducted in the world, research the xylitol effect in a chewing gums.A minority, amongst which is our study, research in a rinsing water shape, and a part of them, such as the Nayak et al.[11], research xylitol as a xylitol syrup application. This type of xylitol carriers is indicated in children with early childhood caries, because of the possibility to develop permanent teeth caries, compared to children without early childhood caries. This way of xylitol administration is safe and acceptable in young children. Such xylitol administration, twice daily in syrup, a combined dose of 8 grams, is observed as an effective caries prevention. Studies show that the anti-caries effects are influenced by the xylitol.

As to the rinse water, outstanding results were archived when xylitol is combined with chlorhexidine. The streptococcus colonization level effects are greater during the early biofilm development stage, and are greater when these two substances are combined, rather than when the xylitol is used as an only ingredient. These newfound synergy effects can be used in high risk patients or to reduce MS transmission from mother to child.

The reduced plaque adhesiveness caused by decreased polysaccharide formation, is suggested as a xylitol inhibitory mechanisms over Str. mutans by Soderling et al. Also they sees a drop in salivary and plaque levels of mutans influenced by xylitol.[12,13,14]

There is no doubt that xylitol has interesting effects on a bacterial cell metabolism. (Assev et al.[15]).

Makinen's [5, 6, 7] attitude which circulates in professional and scientific circles, that the use of xylitol as an active agent in preventive child program, can be compared to children immunization to polio, smallpox and tetanus. If taken into account the facts benefit, in a way, conditioning the oral medium with noncariogenic flora during teeth eruption and the possibility to form an environment of commensal bacterial flora which minimizes caries risk, and in accordance to the proven xylitol effects, is evident that this thesis obtains its confirmation.

CONCLUSION

- Gathered data for the level of Str. mutans presence in the saliva, speaks of a great fall in their colonization during and at the end of the research period.
- Str. mutans colonization in dental plaque, during the research period, also shows significant and rapid fall, meaning that the xylitol affects suppressive equally on the mutans colonization in both researched levels, on the salivary and dental plaque levels.
- Lowered Str. mutans colonization, as a crucial bacteria to initiating dental caries, points out to the strong caries protective effect of xylitol.
- The ability to substitute sucrose with xylitol in everyday diet secures a significant control on carbohydrate intake.
- Carbohydrate intake in everyday diet significantly lowers the ability to create conditions to initiate dental caries growth.
- 6.Quality oral medium conditioning with nonpathogenic microorganisms, lowers the risk of hard teeth substance ailments.
- Xylitol, as a proven caries protective agent is implemented in different confectionery products, toothpaste, chewing gum, etc., therefore a quality promotion of its effects to the greater public is needed.

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