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INTENSITY OF DENTAL CARIES AND BACTERIAL STRAINS S. MUTANS, S. SOBRINUS, S. SALIVARIUS, S. MITIS IN SALIVA OF CHILDREN WITH PERMANENT DENTITION

ИНТЕНЗИТЕТ НА ДЕНТАЛЕН КАРИЕС И БАКТЕРИСКИТЕ СОЕВИ S. MUTANS, S. SOBRINUS, S. SALIVARIUS, S. MITIS ВО ПЛУНКА НА ДЕЦА СО ТРАЈНА ДЕНТИЦИЈА

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Abstract

Introduction: Dental caries is one of the most common chronic multifactorial disease affecting the human population. The appearance of a caries lesion is determined by the coexistence of three main factors: acidogenic and acidophilic microorganisms, carbohydrates derived from the diet, and host factors. Caries develops as a resultof an ecological imbalance in the stable oral microbiome. *Streptococcus mutans*, other *streptococci* belonging to the non-mutans streptococci group, Actinomyces, and Lactobacillus species play a key role in this process. **The aim** of our research is to detect the bacteria *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus salivarius*, and *Streptococcus mitis* in saliva and to analyze the interdependence between dental status and the degree of dental caries activity with each examined parameter separately. **Material and method:** The study included 71 children (26 females and 45 males) aged 12 years. We selected the 12-year-old age group based on WHO recommendations, which identify this age for global monitoring of dental caries and apply only to children with permanent dentition. **Results:** The data relating to the detection of *Streptococcus mutans* in the saliva of children with permanent teeth showed that in the experimental group of 40 children (56.30%), *Streptococcus mutans* was isolated in 28 children (39.40%) and in 12 children (16.90%)it was not isolated from the saliva. In the presented distribution of data relating to the detection of *Streptococcus mutans* in the saliva of children with permanent teeth, there is a significant difference between the two groups (p <0.01). **Conclusions:** *Streptococcus mutans* and *Streptococcussobrinus* are considered the main etiological factors in the development of dental caries. **Keywords:** *Streptococcus mutans*, dental caries, saliva, permanent dentition.

Апстракт

Вовед: Забниот кариес е едно од најчестите хронични и мултифакторни заболувања кај човечката популација. Појавата на кариесната лезија е определена од коегзистенцијата на три главни фактори: ацидогени и ацидофилни микроорганизми, јаглехидрати добиени од исхраната и фактори поврзани со носителот. Кариесот се развива како резултат на еколошка нерамнотежа во стабилниот орален микробиом. *Streptococcus mutans*, други стрептококи од т.н. група на не-*Mutans streptococci, Actinomyces* и *Lactobacillus*, играат клучна улога во овој процес. **Целта** на ова истражување е детектирање на бактериите *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus salivarius*, *Streptococcus mitis* во плунката и анлизирање на меѓузависноста на денталниот статус и степенот на активност на денталниот кариес со секој испитуван параметар поодделно. **Материјал и метод:** Во истражувањето се вклучени 71 дете (26 од женски и 45 од машки пол) на возраст од 12 години. Возрасната група од 12 години е избрана според препораките на СЗО која ја препорачува оваа возраст при глобален мониторинг на забен кариес, а кои се однесуваат само на деца со трајна дентиција. **Резултати:** Податоците кои се однесуваат на детекцијата на *Streptococcus mutans* во плунката на децата со трајни заби, покажаа дека во експерименталната група од вкупно 40 деца (56,30%), кај 28 од нив (39,40%) е изолирана е *Streptococcus mutans*, додека кај 12(16,90%) истата не е изолирана. Во прикажаната дистрибуција на податоци кои се однесуваат на детекцијата на *Streptococcus mutans* во плунката кај децата со трајни заби, постои значителна разлика помеѓу двете групи (p <0,01). **Заклучоци:** *Streptococcusmutans* и *Streptococcussobrinus* се сметаат за главен етиолошки фактор за појава на забен кариес. **Клучни зборови:** *Streptococcus mutans*, дентален кариес, плунка, трајна дентиција.

Introduction

Dental caries is one of the most common chronic multifactorial disease affecting the human population. The appearance of a caries lesion is determined by the coexistence of three main factors: acidogenic and acidophilic microorganisms, carbohydrates derived from the diet, and host factors. Caries develops as a result of an ecological imbalance in the stable oral microbiome. Oral microorganisms form dental plaque on the surfaces of teeth, which is the primary cause of the caries process and exhibits characteristics of the classic biofilm. *Streptococcus mutans*, other *streptococci* belonging to the so-called non-mutans streptococci group, *Actinomyces,* and *Lactobacillus* species, play a key role in this process[1]. Dental caries remains the most common chronic disease in children and the exact etiology is not fully understood. Although *Streptococcus mutans* is an important factor in the initiation and progression of caries, its presence is not always associated with the disease. The existence of caries in which *Streptococcus mutans* counts do not correlate with caries experience, poses a challenging problem[2]. Dental caries is an infectious and transmissible disease caused by a group of phenotypically similar bacteria collectively known as mutans streptococci, which are the main bacterial components responsible for the initiation and development of caries. The earliest time at which cariogenic *mutans streptococci* can appear and act is during tooth eruption, which requires exposed hard tooth surfaces, streptococcal colonization, and their multiplication., which are the main bacterial components responsible for the initiation and development of caries. The earliest time at which cariogenic *mutans streptococci* can appear and act is during tooth eruption, which requires exposed hard tooth surfaces, streptococcal colonization, and their multiplication[3]. Current concepts of the etiology of dental caries have focused on the fermentation of carbohydrates by cariogenic bacteria into organic acids. Plaque bacteria produce a variety of metabolic end products that may vary depending on the diet. In the presence of fermentable carbohydrates, the most common organic acids produced are lactic, formic, and acetic acids. These acids cause a drop in plaque pH, resulting in tooth demineralization and the creation of an environment favorable for further growth of *mutans streptococci*. In addition to acid production, *mutans streptococci* possess a wide range of virulence factors that are responsible for the overall cariogenic potentialof dental plaque[4,5,6,7,8].

Microorganisms play an important role in the development and progression of caries. Bacteria in the oral cavity are in microbial balance, but the risk of caries increases if the number of certain bacteria (*Streptococcus mutans, lactobacilli*) increases significantly while protective factors do not function normally. It has been established that the largest number of cariogenic bacteria belongs to the group of streptococci, especially *Streptococcus mutans* and Streptococcus sobrinus as well as lactobacilli. Mutans streptococci are considered significant determinants of plaque cariogenicity and are associated with the initial development of caries, while the number of *lactobacilli* increases during the progression of caries[9,10]. The aim of our research is to detect the bacteria *Streptococcus mutans, Streptococcus sobrinus, Streptococcus salivarius,* and *Streptococcus mitis* in saliva and to analyze the interdependence between dental status and the degree of dental caries activity with each examined parameter separately.

Material and method

To achieve the set goal, children were randomly selected from primary schools in the city of Shtip. The study included 71 children (26 females and 45 males) aged 12 years. We selected the 12-year-old age group based on WHO recommendations, which identify this age for global monitoring of dental caries and apply only to children with permanent dentition[11]. Dental examinations were performed using portable 60 W white-blue light bulbs and sterilized periodontal probes No. 5, as well as a mirror. To avoid visual fatigue, a maximum of 15 children were examined per day. The examinations were performed after verbal consent was obtained from the subjects and their parents, and they were divided into two groups: a control group (31 subjects without caries, fillings, or extractions; DMFT=0) and an experimental group (40 subjects with caries, fillings, and tooth extractions).

In the study, we used widely accepted index of the presence or absence of carious process, the Klein – Palmer index, designated as DMFT, which is the sum of Decayed (carious), Missing (extracted) and Filled (filled) teeth. We registered clearly visible lesions on the surfaces of the teeth as caries, while changes in transparency and initial demineralization with an intact surface and without cavitation were recorded as healthy tooth. According to the data obtained from the clinical examination, we determined the intensity (presence or absence of dental caries, which were recorded and interpreted them as follows[12]:

a. 0.0 - 0.9 - very low caries risk
b. 1.0 - 2.4 - low caries risk
c. 2.5 - 3.8 - moderate caries risk
d. 3.9 - 5.5 - high caries risk
e. ≥ 5.6 - very high caries risk

The assessment of *Streptococcus mutans, Streptococcus sobrinus, Streptococcus salivarius,* and *Streptococcus mitis*

in saliva was performed by applying saliva to sterile paper No. 50, which was held with sterile beaked-ended tweezers and placed under the tip of the child's tongue for 1 min to soak in saliva. Each paper was then transferred to sterile Eppendorf tubes with sterile tweezers, which were transported to a portable freezer at a temperature of -80°C. Undiluted saliva samples with sterile swabs were plated on Mitis Salivarius agar (Fluka, a medium with sucrose, glucose, tryptan blue, and crystal violet) which is recommended for the isolation of mixed streptococcal cultures, such as *Streptococcus mitis, Streptococcus mutans, Streptococcus salivarius, Enterococcus faecalis,* etc. The plates were incubated under microaerophilic conditions (5-10% CO_2) for 48 hours at 37°C. The characteristic colony morphology and standard microbiological techniques were used to identify the isolates. Specific characteristic of Mitis Salivarius agar include the appearance of blue colonies due to absorption of tryptan blue dye. *Streptococcus salivarius* is characterized by large, pale-blue, smooth colonies with a diameter of 1-5 mm, which resemble "chewing gum" due to the production of levan from sucrose. Colonies of *Streptococcus mutans* were raised, convex, pale-blue and granular, resembling "ground glass". Sometimes the surface of the colony had a "mesh-like" appearance, due to the synthesis of glucan from sucrose. *Streptococcus mitis* forms small, tiny colonies. Suspect colonies were sub-cultured on blood agarand finally identified with the Vitek 2 system, especially *Streptococcus salivarius*. DNA isolation of *Streptococcus mutans* and *Streptococcus sobrinus* from saliva samples was done with the genomic ChargeSwitch®Forensic DNA Purification Kit, (Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad).

Results

A total of 71(100.00%) children comprised the study population with permanent dentition. Of these, 40 (56.30%) belong to the experimental group, in which 16 (22.50%) children were female and 24 (33.80%) children were male. The data relating to the detection of *Streptococcus mutans* in the saliva of children with permanent teeth showed that in the experimental group, out of a total of 40 (56.30%) children, *Streptococcus mutans* was isolated in 28 (39.40%) children, and in 12 (16.90%) children it was not isolated from the saliva.In the control group, out of a total of 31(43.70%) children, *Streptococcus mutans*

Tabele 1. Group / *Streptococcus mutans*

			Streptococcusmutans		Total
			Yes	No	
Group	Experimental	Count	28	12	40
		% of Total	39,4%	16,9%	56,3%
	Control	Count	12	19	31
		% of Total	16,9%	26,8%	43,7%
Total		Count	40	31	71
		% of Total	56,3%	43,7%	100,0%

Tabele 2. Group / *Streptococcus sobrinus*

			Streptococcussobrinus		Total
			Yes	No	
Group	Experimental	Count	30	10	40
		% of Total	42,3%	14,1%	56,3%
	Control	Count	12	19	31
		% of Total	16,9%	26,8%	43,7%
Total		Count	42	29	71
		% of Total	59,2%	40,8%	100,0%

Tabele 3. Group / *Streptococcus salivarius*

			Streptococcussalivarius		Total
			Yes	No	
Group	Experimental	Count	35	5	40
		% of Total	49,3%	7,0%	56,3%
	Control	Count	10	21	31
		% of Total	14,1%	29,6%	43,7%
Total		Count	45	26	71
		% of Total	63,4%	36,6%	100,0%

was isolated from the saliva of 12 (16.90%) children and in 19 (26.80%) children *Streptococcus mutans* was not isolated from the saliva. In the presented data distribution relating to the detection of *Streptococcus mutans* in saliva in children with permanent teeth, there is a statistically significant difference between the two groups for Pearson's chi-square=6.95 and p<0.01(p=0.008) (Table 1).

The data relating to the detection of *Streptococcus sobrinus* in saliva in children with permanent teeth for the experimental group of 40 (56.30%) children, in 30 (42.30%) children *Streptococcus sobrinus* was isolated from the saliva and in 10 (14.10%) *Streptococcus sobrinus* was not isolated. In the control group of 31 (43.70%) children, in 12 (16.90%) children *Streptococcus sobrinus* was isolated from the saliva and in 19(26.80%) children *Steptococcus sobrinus* was not isolated from the saliva.In the presented distribution of data relating to the detection of *Streptococcus sobrinus* in saliva in children with permanent teeth, there is a statistically significant difference between the two groups, with Pearson's chi-square=9.53 and p<0.01 (p=0.002) (Table 2).

The data relating to the detection of *Streptococcus salivarius* in the saliva of children with permanent teeth indicate that in the experimental group of 40 (56.30%) children, *Streptococcus salivarius* was isolated from the saliva of 35 (49.30%) children and was not isolated in 5 children (7.00%). In the control group of 31 (43.70%) children, *Streptococcus salivarius* was isolated from the saliva of 10 (14.10%) children and was not isolated in 21 (29.60%).In the presented distribution of data relating to the detection of *Streptococcus salivarius* in the saliva of children with permanent teeth, with Pearson chi-square=22.96 and p <0.001 (p=0.000), there is a statistically significant difference between the two groups (Table 3).

The results of the detection of *Streptococcus mitis* in the saliva of children with permanent teeth indicate that in the experimental group of 40 (56.30%) children, *Streptococcus mitis* was isolated from the saliva of 37 (52.90%) children and *Streptococcus mitis* was not isolated from 2 (2.90%) children. In the control group of 31 (43.70%) children, *Streptococcus mitis* was isolated from the saliva of 8 (11.40%) children and *Streptococcus salivarius* was not isolated from the saliva of 23 (32.90%) children.

In the presented distribution of data relating to the detection of *Streptococcus mitis* in saliva in children with permanent teeth, with Pearson chi-square=16.14 and p

Tabele 4. Group / *Streptococcus mitis*

			Streptococcusmitis		Total
			Yes	No	
Group	Experimental	Count	37	2	40
		% of Total	52,9%	2,9%	56,3%
	Control	Count	8	23	31
		% of Total	11,4%	32,9%	43,7%
Total		Count	45	25	71
		% of Total	64,3%	35,7%	100,0%

Tabele 5. DMFT index / *Cariogenic bacteria*

Cariogenic bacteria								95% C.I.for EXP(B)	
		B	S.E.	Wald	df	Sig.	Exp(B)	Lower	Lower
Step 1*	*S.mutans (1)*	18,76	28420,71	,000	1	,999	,000	,000	
	S.sobrinus (1)	20,39	28420,71	,000	1	,999	7,180E8	,000	
	S.salivarius (1)	1,18	,89	1,76	1	,185	3,268	,57	**18,82**
	S.mitis (1)	3,37	1,00	11,36	1	,001	29,139	4,10	**207,00**
	Constant	**-3,75**	**,99**	**14,07**	**1**	**,000**	**,024**		

**Variable(s) entered on step 1: S.mutans, S.sobrinus, S.salivarius, S.mitis*

<0.001(p = 0.000), there is a statistically significant difference between the two groups (Table 4).

The data relating to the predictive values of the detected *Streptococcus mutans, Streptococcus sobrinus, Streptococcus salivarius* and *Streptococcus mitis* in saliva in children with permanent teeth for the presence of dental caries (DMFT index) indicate that there is a statistically significant association between the detected bacteria in saliva *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus salivarius, Streptococcus mitis* and the presence of dental caries, with Pearson chi-square=48.13 and p <0.001(p = 0.000).When determining the significance of the contribution of each bacterium to the presence of dental caries, it was determined that the greatest influence has *Streptococcus mitis* (Wald=11.36 /p <0.01(p = 0.001), *Streptococcus salivarius* (Wald=1.76 / p>0.05(p = 0.19), while *Streptococcus mutans* (Wald=0.00 /p>0.05(p = 0.99) and *Streptococcus sobrinus* (Wald=0.00 /p>0.05(p = 0.99) showed no predictive value for the presence of dental caries. Children in whose saliva *Streptococcus mitis* was detected compared to children in whose saliva *Streptococcus mitis* was not detected have a significantly higher probability of the presence of dental caries by 29.14 times (Exp(B)=29.14)(95%CI:4.10-207.00). Children in whose saliva *Streptococcus salivarius* was detected compared to children in whose saliva *Streptococcus salivarius* was not detected have a 3.27-fold (Exp(B)=3.27) insignificantly higher probability of having dental caries (95%CI:0.57-18.82) (Table 5).

Disscusion

Previous experimental and clinical research indicates that the occurrence of dental caries is responsible for several different risk factors that can act independently or in association at the same time[13,14]. Yabao et al. emphasize the relatively well-known etiopathogenesis of dental caries, according to which the cariogenic flora, in the presence of fermentable carbohydrates, produces organic acids that reduce the pH of saliva and disrupt the complex dynamic balance of dental plaque, causing demineralization of enamel, the onset of initial caries, and the formation of cavities in the teeth[15]. Data regarding the intensity of dental caries in children with permanent dentition in the experimental group showed that out of a total of 40 (56.30%) children, 32 (45.10%) children had a low caries risk (1.0-2.4), 1 (1.40%) child had a moderate caries risk (2.5-3.8) and 7 (9.90%) children had a high caries risk (3.9-5.5) compared to the control group, where the subjects were without dental caries and with a very low caries risk. There was a statistically significant difference between the two groups (p<0.001)[16]. The bacterial load in saliva and the amount of plaque are directly related to the occurrence of caries in each individual. Particularly strong indicators are the number of pathogenic *mutans streptococci* present in plaque, saliva, and fissures on the occlusal surfaces of the teeth, which place each patient at high risk for caries. Other important *Streptococcus species* involved in the occurrence of caries are *Streptococcus mitis* and a group of acidophilic *streptococci* that act only at low pH such as *Bifidobacterium* isolated from the white spot lesions of teeth[17]. In the presented distribution of data relating to the detection of *Streptococcus mutans* in saliva in children with permanent teeth, there is a significant difference between the two groups (p<0.01)[18]. *Streptococcus mutans* and *Streptococcus sobrinusare* considered the main etiological factors for the occurrence of dental caries. Oda, using the polymerase chain reaction (PCR) method, compared the levels of these bacteria in 145 patients aged 12 to 20 years and compared them with the occurrence of caries, deter-

mining that children in whom *Streptococcus mutans* and *Streptococcus sobrinus* were isolated had a significantly higher incidence of dental caries than those with *Streptococcus mutans*[19].

Conclusion

The number of bacteria in saliva and the amount of plaque are directly related to the occurrence of caries. Particularly strong indicators are the number of pathogenic *mutans streptococci* present in plaque, saliva, and fissures on the occlusal surfaces of the teeth, which make each patient at high risk for the occurrence of caries. The data we obtained for the detection of *Streptococcus mutans* in saliva in children with permanent teeth in the experimental group, out of a total of 40 (56.30%) children, in 28 (39.40%) children Streptococcus mutans was isolated from the saliva and in 12 (16.90%) children *Streptococcus mutans* was not isolated. In the control group, out of a total of 31 (43.70%) children, in 12 (16.90%) children *Streptococcus mutans* was isolated from the saliva and in 19 (26.80%) children *Streptococcus mutans* was not isolated.In the presented distribution of data relating to the detection of *Streptococcus mutans* in saliva in children with permanent teeth, there is a statistically significant difference between the two groups ($p<0.01$).

Data relating to the detection of *Streptococcus sobrinus* in saliva in children with permanent teeth, in the experimental group of 40 (56.30%) children in total, in 30 (42.30%) children *Streptococcus sobrinus* was isolated from saliva and in 10 (14.10%) children *Streptococcus sobrinus* was not isolated from saliva. In the control group of 31 (43.70%) children in total, in 12 (16.90%) children *Streptococcus sobrinus* was isolated from saliva, however in 19 (26.80%) children it was not isolated from saliva. The distribution of data for the detection of *Streptococcus sobrinus* in saliva in children with permanent teeth indicates that there is a statistically significant difference between the two groups ($p<0.01$).

Data refer to the detection of *Streptococcus salivarius* in the saliva of children with permanent teeth. In the experimental group of a total of 40 (56.30%) children, in 35 (49.30%) children *Streptococcus salivarius* was isolated from the saliva and in 5 (7.00%) children *Streptococcus salivarius* was not isolated from the saliva. In the control group of a total of 31 (43.70%) children, in 10 (14.10%) children *Streptococcus salivarius* was isolated from the saliva and in 21 (29.60%) children *Streptococcus salivarius* was not isolated from the saliva.

The detection of *Streptococcus salivarius* in the saliva of children with permanent teeth indicated that there was a statistically significant difference between the two groups ($p<0.001$).

Further is the data regarding the detection of *Streptococcus mitis* in the saliva of children with permanent teeth. In the experimental group of 40 (56.30%) children in total, in 37 (52.90%) children *Streptococcus mitis* was isolated from the saliva and in 2 (2.90%) children *Streptococcus mitis* was not isolated. In the control group of 31 (43.70%) children in total, in 8 (11.40%) children *Streptococcus mitis* was isolated from the saliva and in 23 (32.90%) children *Streptococcus salivarius* was not isolated from the saliva.

In the control group of 31 (43.70%) children, *Streptococcus salivarius* was isolated from the saliva in 10 (14.10%) children and in 21 (29.60%) it was not isolated. Data relating to the detection of *Streptococcus salivarius* in the saliva of children with permanent teeth indicated that there is a statistically significant difference between the two groups ($p<0.001$).

The data relating to the detection of *Streptococcus mitis* in saliva in children with permanent teeth showed that in the experimental group of 40 (56.30%) children, in 37 (52.90%) children *Streptococcus mitis* was isolated from the saliva, in 2 (2.90%) children *Streptococcus mitis* was not isolated from the saliva. In the control group of 31 (43.70%) children, in 8 (11.40%) children *Streptococcus mitis* was isolated from the saliva and in 23 (32.90%) children *Streptococcus salivarius* was not isolated from the saliva.

The data relating to the detection of Streptococcus mitis in saliva in children with permanent teeth indicate a statistically significant difference between the two groups of subjects ($p <0.001$). When determining the significance of the contribution to the presence of dental caries of each bacterium, it was determined that *Streptococcus mitis* $p<0.01$ had the greatest influence, followed by *Streptococcus salivarius* $p>0.05$, while *Streptococcus mutans* $p>0.05$ and *Streptococcus sobrinus* $p>0.05$ had no predictive value for the presence of dental caries.

Children in whose saliva *Streptococcus mitis* was detected compared to children in whose saliva *Streptococcus mitis* was not detected, had a 29.14-fold significantly higher probability of presence of dental caries. Children in whose saliva *Streptococcus salivarius* was detected compared to children in whose saliva *Streptococcus salivarius* was not detected, had a 3.27-fold insignificantly higher probability of dental caries occurrence.

This study provides evidence that *Streptococcus mutans* may play a role in shaping the salivary microbial community. Our results highlight that future caries research should consider additional species as health/disease microbial mark-

ers, in conjunction with *Streptococcus mutans*, to improve diagnosis and caries management of the caries-discordant population.

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