THE NUMBER OF *STREPTOCOCCUS MUTANS* AND *LACTOBACILLUS* AS WELL AS SALIVA BUFFER CAPACITY IN THE LUBLIN VOIVODESHIP RESIDENTS

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Abstract

Introduction. Metabolic processes of bacteria in the oral environment may contribute to pathological conditions in the oral cavity. *Streptococcus mutans* and *Lactobacillus* bacteria take part in carious process and may be the index of cariogenic.

Aim. The aim of the study was the assessment of the number of *S. mutans* and *Lactobacillus* cariogenic bacteria as well as saliva buffer capacity in the group of people aged 20-35 residing in Lublin Voivodeship. The analysis also included the number of cigarettes smoked daily in relation to the value of saliva buffer capacity.

Material and methods. The study was conducted in the group of 74 people (45 women, 29 men) aged from 20 to 35. The research material included mixed, non-stimulated and stimulated saliva collected between 9.30 and 11.30 a.m., from 1.5 to 2 hours after a meal. The evaluation of the number of *S. mutans* and *Lactobacillus* bacteria was conducted with the use of CRT bacteria test (Ivoclar Vivadent, Liechtenstein). Assessment of saliva buffer capacity was conducted with the use of CRT buffer test (Ivoclar Vivadent, Liechtenstein), assessment of cotinine concentration in the saliva using the Cotinine ELISA test (Calbiotech).

Results. No statistically significant differences in *S. mutans* number (χ^2=0.008, p>0.05), *Lactobacillus* number (χ^2=0.27, p>0.05) and saliva buffer capacity (χ^2=0.30, p<0.05) were stated in relation to place of residence of the studied. In people smoking up to 10 cigarettes daily, high saliva buffer capacity was stated more frequently in relation to people smoking more than 15 cigarettes daily (χ^2=5.85, p<0.05).

Conclusions. In the group of patients with a high number of cariogenic bacteria, oral hygiene instruction and dietary instruction should be carried out. It is recommended to conduct further multifaceted studies in a larger population that would assess risk factors of dental caries considering, among others, place of residence.

Keywords: saliva, *S. mutans*, *Lactobacillus*, place of residence.

Introduction

Metabolic processes of bacteria in the oral environment may contribute to pathological conditions in the oral cavity. Organic acids, including lactic acid, are produced by bacteria from glucose through Embden-Meyerhof-Parnas (EMP) pathway and initiate dental caries [1]. *Streptococcus mutans* (*SM*) bacteria are essential etiological factor of dental caries. The ability to live in low pH and adaptation to low pH affects

their cariogenicity. In people with poor oral hygiene and long-term unhealthy diet, *S. mutans* may be the dominant bacterium in dental plaque and cause caries [2]. A significant correlation was stated between the number of *SM* in the saliva and the presence of *SM* in dental plaque. *S. mutans* bacteria initiate the carious process, whereas *Lactobacillus* (*LB*) bacteria take part in the caries progression and may be the index of cariogenic diet which includes a lot of simple sugars. One of many factors protecting dental tissues from carious process is saliva buffer capacity which prevents the drop in salivary pH [3].

The measurement of saliva buffer capacity and the analysis of bacteria present in saliva are helpful in the assessment of the risk of caries development [4]. Dental caries is a multifactorial disease in which high number of *S. mutans* and *Lactobacillus* (*SM*≥10^5 CFU/ml saliva, number of colony-forming unit, *LB*≥10^5 CFU/ml saliva) reveal high risk of dental caries [5]. Cotinine is the marker of exposure to cigarette smoke assayed in various body fluids, among others, in saliva. The half-life of cotinine, the main metabolite of nicotine is about 17 hours, whereas, nicotine half-life is about 2 hours. On average, about 70-80% of nicotine is converted onto cotinine [6,7].

Aim

The aim of the study was the assessment of the number of *S. mutans* and *Lactobacillus* cariogenic bacteria, as well as saliva buffer capacity in the group of people aged 20-35 residing in Lublin Voivodeship. The analysis also included the number of cigarettes smoked daily in relation to the value of saliva buffer capacity.

Material and methods

The study was conducted in the group of 74 people (45 women, 29 men) aged from 20 to 35 who reported for dental treatment at the Chair and Department of Conservative Dentistry with Endodontics in Lublin. Cigarette smoking was declared by 34 people (26 residing in the city and 8 in the rural area). All participants of the study described their general health state as good. The research material included mixed, stimulated saliva collected between 9.30 and 11.30 a.m., from 1.5 to 2 hours after a meal. Saliva stimulated by chewing paraffin cube, was collected within 5 minutes by spitting it to disposable plastic cup. Immediately after saliva collection, a bacteriological test was performed and saliva buffer capacity was assessed.

The evaluation of the number of *SM* and *LB* bacteria was conducted with the use of CRT bacteria (Ivoclar Vivadent, Liechtenstein) according to the manufacturer's instructions. Concentration of *SM* and *LB* bacterial colonies was compared with the standard model (<10^5 CFU/ml low risk of caries and ≥10^5 CFU/ml high risk of caries for each microorganism).

Assessment of saliva buffer capacity was conducted with the use of CRT buffer (Ivoclar Vivadent, Liechtenstein) according to the manufacturer's instructions. After placing a drop of saliva on the marked place and after 5 minutes, the colour of test strip was assessed by comparing it with a standard model. Blue colour of the test strip indicated high salivary buffer capacity, the green one – moderate, yellow – low.

The survey data on the status of cigarette smoking (smoker/non-smoker) were verified with the use of Cotinine ELISA test (Calbiotech) according to the manufacturer's instructions. In order to assay cotinine concentration, non-stimulated saliva was collected between 9.30 and 11.30 a.m., from 1.5 to 2 hours after a meal, into Salivette tubes (Sarstedt, Germany), which were placed in the container with ice in the temperature of 4°C. Next, they were centrifuged for 12 minutes at 3000 rpm at the temperature of 4°C. In biochemical studies a supernatant was used which was stored at the temperature of -75°C until the assays were done. Statistical analysis was conducted with the use of χ^2 test. Statistically significant test values were those in which $p<0.05$.

Results

The studied group included 74 people, 60.81% women and 39.19% men. From among the investigated, 74.32% lived in the city, and 25.68% lived in rural area. Cigarette smoking was declared by 45.95% of the investigated, whereas 54.05% declared they had never smoked cigarettes. Among smokers, 50% reported smoking up to 10 cigarettes daily, whereas the remaining 50% smoked at least 15 cigarettes daily. Cotinine concentration in the smokers' saliva was from 29.931 ng/ml of the saliva to 924.528 ng/ma, while in non-smokers' saliva it was undeterminable. On the basis of determination of nicotine concentration in the saliva, there found 100% compatibility of declarations on the smoking status included in the survey studies with the results of biochemical assays (Table 1).

Among people residing in the city, high number of *SM* bacteria was stated in 32.73%, whereas low number in 62.27% of the studied. For people living in rural areas the values were 31.58% and 68.42% respectively. No statistically significant differences in *SM* number were stated in relation to place of residence of the studied ($\chi^2=0.008$, $p>0.05$) (Table 2).

High number of *LB* was stated in 43.64% of the studied living in the city and 36.84% in those in rural area. Low number was noted in 56.36% and 63.16% respectively. No significant differences were stated in the number of *LB* in relation to the place of residence ($\chi^2=0.27$, $p>0.05$) (Table 3).

High buffer capacity of the saliva was stated in 62.27% of the studied living in the city and in 73.68% from the rural area. Medium buffer capacity of the saliva was noted in 27.27% and 21.05% respectively, whereas low buffer capacity of the saliva in relation to the place of residence of the studied 5.45% and 5.26% respectively. No statistically significant differences were stated in the saliva buffer capacity in relation to the place of residence of the subjects ($\chi^2=0.30$, $p<0.05$) (Table 4).

In the group of people smoking up to 10 cigarettes a day, low buffer capacity of the saliva was not stated, medium buffer capacity was stated in 35.29% and high in 64.71%. In the group of people smoking 15 cigarettes a day or more, the values were 17.65%, 52.94%, 29.41% respectively. Statistically significant differences in saliva buffer capacity were stated in relation to the number of cigarettes smoked daily. In people smoking up to 10 cigarettes daily, high saliva buffer capacity was stated more frequently in relation to people smoking 15 cigarettes daily or more ($\chi^2=5.85$, $p<0.05$) (Table 5).

Table 1. The characteristics of the study group

Variable	N	%
Sex		
women	45	60.81
men	29	39.19
Place of residence		
city	55	74.32
country	19	25.68
Status of smoking		
yes	34	45.95
no	40	54.05
The number of cigarettes smoked per day		
up to 10	17	50
15 and more	17	50
The concentration of cotinine in the saliva		
smokers	29.931-924.528 ng/ml of the saliva	
non-smokers	non-detectable	
Declared compliance of smoking status with cotinine concentration	100%	

N – number of subjects
% – the percentage of subjects

Table 2. The number of *SM* bacteria(CFU/ml of saliva) in relation to the place of residence of the subjects

The number of *SM* bacteria	The place of residence			Test
	city	village	Total	
high	18	6	24	χ^2=0.008 (-); p>0.05
%	32.73%	31.58%		
low	37	13	50	
%	67.27%	68.42%		
Total	55	19	74	

The number of *SM* bacteria = high (≥105 CFU/ml),
The number of *LB* bacteria = high (≥105 CFU/ml),
The number of *SM* bacteria = low (<105 CFU/ml),
The number of *LB* bacteria = low (<105 CFU/ml),
(-) no differences p>0.05.

Table 3. The number of *LB* bacteria (CFU/ml of saliva) in relation to the place of residence of the subjects

The number of *LB* bacteria	The place of residence			Test
	city	country	Total	
high	24	7	31	
%	43.64%	36.84%		
low	31	12	43	χ^2=0.27 (-); p>0.05
%	56.36%	63.16%		
Total	55	19	74	

Table 4. Buffer capacity of the saliva in relation to the place of residence of the subjects

Buffer capacity of the saliva	The place of residence			Test
	city	country	Total	
low	3	1	4	
%	5.45%	5.26%		
high	37	14	51	
%	67.27%	73.68%		χ^2=0.30 (-); p>0.05
medium	15	4	19	
%	27.27%	21.05%		
Total	55	19	74	

Table 5. Buffer capacity of the saliva in relation to the number of cigarettes smoked per day

Buffer capacity of the saliva	The number of cigarettes smoked per day			Test
	<10	≥15	Total	
low	0	3	3	
%	0.00%	17.65%		
high	11	5	16	
%	64.71%	29.41%		χ^2=5.85 (*); p<0.05
medium	6	9	15	
%	35.29%	52.94%		
Total	17	17	34	

(*) difference on the level of p<0.05

Discussion

Dental caries is a disease with a complex etiology. Individual risk of caries may be assessed, among others, with the use of computer programme Cariogram, after typing

information on: DMF index, dental plaque (Silness-Löe Plaque Index), CRT bacteria and CRT buffer (Ivoclar Vivadent AG, Liechtenstein), measurement of the quantity of the stimulated saliva as well as the analysis of survey questionnaire filled by participants of the study (systemic diseases, drugs taken, oral hygiene, dietary habits) [8].

The assessment of the number of *SM* and *LB* is helpful in selecting people with large number of cariogenic bacteria and monitoring the changes in the oral hygiene and dietary habits. Lifestyle and profession may influence dietary habits e.g. people working in the evening may tend to consume sweet snacks [9].

Results of the studies conducted by us revealed high number of *SM* in 32.73% people living in the city and 31.58% living in rural areas. High number of *LB* was more frequently noted in people living in the city (43.64%) in relation to those living in the country (36.84%).

Our earlier studies revealed that the number of *SM* and *LB* in the saliva is not related to the status of smoking, number of cigarettes smoked and duration of smoking. Smokers less frequently had their dental appointments to check their oral health every six months in relation to non-smokers. Living in the city or in rural area did not influence part of dental follow-up studies among people aged 20-54 [10,11].

Instructions on proper oral hygiene and dietary guidelines given to the patient raise pro-health awareness and may result in modification of habits concerning oral hygiene as well as dietary habits that will contribute to lowering of the number of cariogenic bacteria in the saliva. Cigarette smoking is also a modifiable habit; therefore, minimal anti-nicotine intervention should be conducted in each smoking patient.

S. mutans are of importance not only in the development of carious process in dental hard tissues but also periodontal and systemic diseases. Durandset *et al.* [12] stated positive correlation between the number of *SM* and *Fusobacterium nucleatum*, and a positive correlation, although not statistically *SM* and *Aggregatibacter actinomycetemcomitans. F. nucleatum* and *A. actinomycetemcomitans* are pathogens of periodontal diseases. Streptococci are dominant species present in the oral cavity and in upper respiratory tract. They can enter the bloodstream as a consequence of invasive procedures in the oral cavity e.g. tooth extraction or dental procedures in the oral cavity, during mastication, tooth brushing and using dental floss. Elimination of these bacteria from the blood stream takes place with the aid of the immune system and takes a few seconds, however, in rare cases interaction with platelets can occur which is significant in the pathogenesis of the infectious endocarditis [13].

S. mutans strains were isolated from the samples collected from the infected heart valve and the oral cavity in patients with bacterial endocarditis [14].

Conclusions

In people smoking up to 10 cigarettes daily, high saliva buffer capacity was stated more frequently in relation to people smoking 15 cigarettes daily or more.

It is recommended to conduct further multifaceted studies in a larger population that would assess risk factors of dental caries considering, among others, place of residence.

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