



THE PREVALENCE OF CARIOGENIC SALIVARY MICRO-ORGANISMS IN CHILDREN OF VARIOUS AGES

ENITA NAKAŠ^{1*}, AMILA ZUKANOVIĆ²

¹ Department of Orthodontics, Faculty of Stomatology,
University of Sarajevo Bolnička 4, 71 000 Sarajevo Bosnia and Herzegovina

² Department of Preventive and Pediatric Dentistry Faculty of Stomatology,
University of Sarajevo Bolnička 4a, 71 000 Sarajevo Bosnia and Herzegovina

* Corresponding author

ABSTRACT

The objective of the research was to obtain data on the values of salivary *Streptococcus mutans* and *Lactobacillus spp.* in 12 years old children and teenagers (13 – 18 years old children), and to compare the values of these cariogenic microorganisms in children of various ages, for the purpose of valuating the risk of caries. The sample comprised 32 persons divided into two groups. The first group included 17 children of the age of 12. The second group included 15 teenagers. The specimen of stimulated saliva, needed for the purpose of establishing the quantities of *Streptococcus mutans* and *Lactobacillus spp.*, was taken by the method of CRT[®]-bacteria, according to manufacturers instruction.

In the group of 12 years old children, the distribution of salivary *Lactobacillus spp.* was as follows: very low values 41,1%, low values 35,2%, high values 17,6%, very high values 5,8%, while in the group of teenagers, the results were the following: very low values 26,66%, low values 33,33%, high values 40%, very high values 0%. The levels of *Streptococcus mutans* were the following: 12 years old children had very low values in 41,7% cases, low values in 11,7% cases, high values in 41,7% cases, very high values in 5,8% cases, while the group of teenagers had very low values in 0%, low values in 33,3%, high values in 33,3%, and very high values in 33,3% cases.

The levels of *Streptococcus mutans* are higher in our teenage children than in younger children, while the differences in levels of *Lactobacillus spp.* among children of various ages do not exist. In terms of global distribution, the levels of *Streptococcus mutans* and *Lactobacillus spp.* are higher in our population than in other countries in both control groups.

KEY WORDS: *Streptococcus mutans*, *Lactobacillus*, children

INTRODUCTION

Mutans streptococci are a heterogeneous group of microorganisms divided into 6 species and 8 serotypes (1,2). They make normal micro flora in oral cavity. There exist several types of Streptococci, isolated from human dental plaque. The most common type is *Streptococcus mutans* (serotypes c, e and f), and *Streptococcus sobrinus* (serotypes d, g). They are connected with the development of caries in humans and animals (3, 4). *Streptococcus mutans* belongs to the group of Alfa hemolytic streptococci. Mutans streptococci are related to the occurrence of early demineralization, i.e., initial phases of the development of caries (5). In consequence of some of their vital characteristics, mutans streptococci are regarded potentially highly cariogenic: they are acidogenic (capable of producing acids that melt the tooth substance), aciduric (able to survive, grow and perform metabolic processes in the environment of very low pH), they have the capacity of creating extracellular polysaccharides, capability to transport sugar, capability to adhere to dental surfaces (6). Mutans streptococci infections are globally distributed. These microorganisms can be found in almost all humans in a population; however, in different persons, the levels of mutans streptococci vary from low to very high values. Their values in individuals are determined by the irregularities on the surface of tooth enamel, retention areas, as well as genetic and immuno-biological factors. Mutans streptococci are microorganisms that primarily colonize dental surfaces. In the concentration of 10^4 - 10^5 CFU/ml in saliva, *Streptococcus mutans* is able to colonise clean, smooth surface of a tooth. The presence of mutans streptococci on tooth surfaces increases the possibility that caries would develop thereon. Lactobacilli belong to the group of Gram-positive microorganisms. They are isolated as a part of normal human microflora. They are acidogenic and aciduric. As early as 1890, W.D. Miller proved the presence of lactobacilli in oral flora, and their capacity of producing acids. There are several kinds of lactobacilli, but the most common types found in oral flora are *Lactobacillus rhamnosus* and *Lactobacillus casei*. Lactobacilli alone have no important role in the initiation of caries (7). *Lactobacillus* is a secondary invasive bacteria connected with the progression of caries lesion. It is most commonly found in the dentine, in deep portions of carious lesion, in open cavities, but also in retention areas on teeth like, for example, poorly made edges of dental fillings. The saliva of persons that have active carious lesions, usually contains a large number of

Lactobacilli spp.; however, lactobacilli as a part of normal flora can also be found in persons that have no caries (8). Infection with Lactobacilli has a global distribution. The levels of lactobacilli vary in different persons. The persons with a high level of *Lactobacilli spp.* have, or develop more carious lesions than those with lower levels of lactobacilli (9,10). The levels of *Lactobacillus spp.* and *Streptococcus mutans* in saliva indirectly point towards the risk that a new carious lesion would develop (11), so they are used as parameters in the assessment of caries risk. The objective of the research was to obtain data on the values of salivary *Streptococcus mutans* and *Lactobacillus spp.* in 12 years old children and teenagers (13 – 18 years old children), and to compare the values of these cariogenic microorganisms in children of various ages, for the purpose of assessing the risk of caries in these population groups.

MATERIALS AND METHODS

The sample comprised 32 persons divided into two groups. The first group included 17 children of the age of 12. The second group included 15 teenagers (13 – 18 years old children). The following criteria were applied in the selection of participants: - Patient did not use antibiotics 3 weeks before the testing. - No changes exist in the structure of tooth enamel. - No active carious lesion exists

Both groups of patients were the children coming from good-income families, who regularly maintain oral hygiene by brushing their teeth at least twice a day. All respondents had their teeth sanitized with dental fillings, without a single carious lesion. The levels of salivary *Streptococcus mutans* and *Lactobacillus spp.* were determined for each individual patient. The sample of stimulated saliva, needed for the purpose of establishing the quantities of *Streptococcus mutans* and *Lactobacillus spp.*, was taken by the method of CRT[®]-bacteria (Ivoclar-Vivadent[®] clinical), according to manufacturer instruction. Patients were given a piece of (factory-made) wax, which they chewed for 5 minutes in order to collect enough stimulated saliva (during wax chewing, the process of bacteria mobilization happens, from the plaque into saliva). While chewing, the respondents collected saliva into disposable plastic cups. After 5 minutes, disposable pipettes were used to take saliva and seed it on slides for *Streptococci mutans* and *Lactobacilli spp.* Firstly, after the protective coat was removed from sterile slides, 5 drops were neatly dripped on the slide (until its entire surface was cov-

ered in saliva), which was held under a slight angle, taking special care not to damage the surface of the slide with the sharp edge of pipette. Once the procedure was over, a tablet of NaHCO_3 was put in slide boxes, and then the boxes were closed and placed into incubator at the temperature of 37°C . 48 hours later, the results were read as indicated in the manufacturers scheme, according to the following scale (12):
 Class 1 = $<10^4$ CFU/ml saliva
 Class 2 = 10^4 - 10^5 CFU/ml saliva
 Class 3 = 10^5 - 10^6 CFU/ml saliva
 Class 4 = $>10^6$ CFU/ml saliva

The statistic software programme StatsDirect (StatsDirect version 2.6.1) was used in the analysis of the results. The prevalence of salivary *Streptococcus mutans* and *Lactobacillus spp.* in both groups of children was shown in percentages. T-test was applied in testing differences in the values of *Streptococcus mutans* and *Lactobacillus spp.* in respondents' groups. The value of $p < 0,05$ was taken as significant.

RESULTS

The results showed no differences in the levels of salivary *Lactobacillus spp.* between the group of 12-year old children and the group of teenagers ($p=0,4297$) (Table 1). A significant difference exists between the group of 12-year old children and the group of teenagers in the levels of salivary *Streptococcus mutans*, where the teenagers have significantly higher values of *Streptococcus mutans* in the saliva ($p= 0,0147$) (Table 2).

DISCUSSION

The 2003 research by A. Zukanović about the prevalence of lactobacilli among 12-year old children in Sarajevo, conducted on a random sample of 109 children, showed that 38,5% children had very low values, 25,7% had low values, 23,9% high values, and 11,9% had very high values of salivary *Lactobacillus spp.* (13). The results of our research obtained similar results, where the level of salivary *Lactobacillus*

<i>Streptococcus mutans</i>	Group 1 – 12 years old children		Group 2 – teenagers	
	n	%	n	%
Class 1: very low values	7	41,17	0	0
Class 2: low values	2	11,76	5	33,33
Class 3: high values	7	41,17	5	33,33
Class 4: very high values	1	5,88	5	33,33

TABLE 1. The prevalence of *Lactobacillus spp.* in 12 years old children and teenagers

spp. has the same distribution among 12-year old children and teenagers, and 60% of children have low and very low values of *Lactobacillus spp.*. Comparing the results obtained in children from Sarajevo with those obtained in their Swedish age-fellows in the research by Hänsel-Petersson et al.(14), we see that 51,6% 12-year old children in Sweeden have very low values, 19,6 % low values, 20,4 % have high values and 8,4 % very high values of *Lactobacilli* in saliva. The study conducted among 12-year old children in Estonia shows that 47% children have very low values, 28% low, 18% high and 7% very high values of *Lactobacilli* (15). Comparing the values of *Lactobacilli* in saliva among Sarajevo children with the above researches, we see that our children have higher levels of lactobacilli in saliva, which indicates a higher risk of the development of caries. The results of the research by A. Zukanović of 2003 concerning the values of *Streptococcus mutans* in saliva among 109 12-year old children in Sarajevo, selected by the method of random sample, prove that most 12-year old children have high values of *Streptococcus mutans* in saliva. In 25,7% of children we have very low, in 24,8% low, in 34,9% high, and in 14,6% very high values of *Streptococcus mutans* (13). The following table displays the values of *Streptococcus mutans* in 12-year old children in several countries (Table 3): The results of our research show that the finding of the prevalence of salivary *Streptococcus mutans* in 12-year old children, whose dental status includes no active carious lesion, remains the same as that in their age-fellows selected by the method of random sample, where the dental status presented no criterion for participation in the study. In our research, twelve-year olds had no carious tooth, unlike the research of A. Zukanović, where the respondents had KEP-index 4,81, where caries component was 2,13, which means that each child in that participants' group had 2,13 carious teeth on average (13). Comparing the values of *Streptococcus mutans* found in our children of various ages, we see that teenagers have significantly higher values of salivary *Streptococcus mutans*. S. Bjarnason *et alia* (1993) (20) made a longitudinal study that followed the levels of *Streptococcus mutans*, *Lactobacillus spp.* and KEP index through a three-

<i>Lactobacillus</i>	Group 1 – 12 years old children		Group 2 – teenagers	
	n	%	n	%
Class 1: very low values	7	41,17	4	26,66
Class 2: low values	6	35,29	5	33,33
Class 3: high values	3	17,64	6	40,0
Class 4: very high values	1	5,88	0	0

TABLE 2. The prevalence of *Streptococcus mutans* in 12 years old children and teenagers

	Very low values of <i>Streptococcus mutans</i>	Low values of <i>Streptococcus mutans</i>	High values of <i>Streptococcus mutans</i>	Very high values of <i>Streptococcus mutans</i>
Bangkok Songpaisan Y. (16)	17 %	32%	13,3%	18%
Cambodia Bratthall D. (17)	7%	27%	14,1%	25%
Pakistan Maher R. (18)	22%	22%	31%	25%
China Shy Y. (19)	14%	28%	13,5%	23%
Sweden Hänsel Petersson G. (14)	39%	16,3%	23,5%	21,2%
Bosnia and Herzegovina Zukanović A. (13)	25,7%	24,8%	34,9%	14,6%

TABLE 3. The values of *Streptococcus mutans* in 12 years old children in different countries:

year period. Through their work we see that 41% of 18 years old participants have very high levels of *Streptococcus mutans* (10^5), while 21% of participants have high level of *Lactobacillus spp.* (10^5). The above authors found the increase in the levels of examined microorganisms, in comparison with 15-16 years old children. When we compare these details with ours, we see a higher level of *Streptococcus mutans* in the group of our teenagers, too; however, when we compare the levels, we perceive a lower level of *Streptococcus mutans*, but much higher level of *Lactobacillus spp.* in our population, which can be explained with a higher consumption of carbohydrates and higher number of meals than in the Swedish population. The work of Steckens-Blicks reveals correlation between a higher consumption of carbohydrates and the levels of *Streptococcus mutans* and *Lactobacillus spp.* (21). It has been proved that the number of *Lactobacillus spp.* in saliva depends on the type of food consumed during the day, and especially on the frequency of consumption of carbohydrates. Restricted consumption of carbohydrates brings to decline in the number of lactobacilli in saliva. Therefore, recent practices of monitoring the dietary habits in humans and active participation of patients in preventive programs include measuring of the levels of *Lactobacillus spp.*. Hence, the level of *Lactobacillus spp.* in saliva indirectly points to the risk of the development of new carious lesion (11). On the sample of 101 teenage participants, I. Zickert *et al.* (1982) found *Streptococcus mutans* in 89% participants, among them 21% had very high levels thereof (22). The same authors also give details on the level of *Lactobacillus spp.* the presence of *Lactobacillus spp.* was found in 79% participants, among them 11% had high values. In our study, 100% of participants had the presence of *Streptococcus mutans*, among them 33,33% very high levels thereof, which is almost twice as much as in Swedish teenagers. Similar are the data on *Lactobacillus spp.*, where the presence of lactobacilli was found in 100% of our teenagers, with 40% having high values thereof (22).

Similar results were publicized by B. Kohler *et al.* (1992) on the sample of 155 participants, *Streptococcus mutans* was isolated in 85% cases; among them, 17.7% had very high values (23). *Lactobacillus spp.* was found in 81% participants, where 14.3% had a high level thereof (23). Neither of the above authors gives explanation for the increased levels of *Streptococcus mutans* in children of older age. When it comes to the global distribution of salivary *Streptococcus mutans*, the research by authors El-Nadeef and Bratthall (24) is interesting. They compared the values of *Streptococcus mutans* in children of various areas, which had different levels of fluoride in water. The authors found higher levels of *Streptococcus mutans* in those areas where drinking water contained lower concentration of fluoride. Drinking water in the area of Sarajevo contains minimal quantities of fluoride (less than 0,1 ppm). This detail is another proof that the fluoridisation of drinking water in BiH remains a desirable preventive method for the improvement of oral health of the population because, among other effects, it brings to the reduction of the number of salivary *Streptococcus mutans*. Our study has shown that, in the control group of children, the levels of *Streptococcus mutans* and *Lactobacillus spp.* are higher in comparison with the global distribution of these cariogenic microorganisms. The detection of population groups, but also individuals, with the higher levels of *Lactobacillus spp.* and *Streptococcus mutans* effectively points to high-risk groups/individuals in terms of caries development. For persons and groups that bear a high risk of caries development, it will be necessary to design targeted programs of prevention based on the high-risk strategy, which will reduce caries risk to minimum. It should be noted that both the level of *Streptococcus mutans* and the level of *Lactobacillus spp.* present considerable risk-factors of caries, but whether and when caries would develop depends on many other factors as well, because caries is a complex infective condition of multi-causal etiology.

CONCLUSION

The level of *Streptococcus mutans* in our children is higher in teenagers than in the younger children, while the differences in the level of *Lactobacillus spp.* in children of various ages do not exist. In view of the global distribution, the levels of *Streptococcus mutans* and *Lactobacillus spp.* are higher in our population than in other countries in both age groups concerned. The above data should be taken into consideration in the creating and implementing preventive programs for the improvement of oral health at all levels (individually, within families, schools, communities and similar).

REFERENCES

- (1) Perch B., Kjems E., Ravn T. Biochemical and serological properties of *Streptococcus mutans* from various human and animal sources. *Acta Pathol. Microbiol. Scand. Sect B* 1974; 82:357-370
- (2) Beighton D., Russel R.R.B., Hayday H. The isolation and characterisation of *Streptococcus mutans* serotype h from dental plaque of monkeys (*Macaca fascicularis*). *J. Gen. Microbiol.* 198;124:271-279
- (3) Hamada S., Masuda N., Oshima T., Sobrie S., Kotani S. Epidemiological survey of *Streptococcus mutans* among Japanese children: identification and serological typing of the isolated strains. *Jap. J. Microbiol.* 1976; 20:33-44
- (4) Thomson L.A., Little W.A., Bowen W.H., Sierra L.I., Aguirrer M., Gillespie G. Prevalence of *Streptococcus mutans* serotypes, actinomyces and other bacteria in the plaque of children. *J. Dent. Res.* 1980; 59:1581-1589
- (5) Loesche W.J. Role of *Streptococci mutans* in human dental decay. *Microbiol. Rev.* 1986; 50:353-380
- (6) Gibbons R.J., Cohen L., Hay D.I. Strains of *Streptococcus mutans* and *Streptococcus sobrinus* attach to different pellicle receptors. *Infect. Immun.* 1986; 52:555-561
- (7) Crossner C.G. Variation in human oral lactobacilli following a change in sugar intake. *Scand. J. Dent. Res.* 1984; 92:204-210
- (8) Mikelsaar M., Mandar R., Sepp E. Lactic acid microflora in the human microbial ecosystem and its development. In: Salmien S., Von Wright A. editors. *Lactic acid bacteria: microbiology and functional aspects.* New York: Marcel Dekker; 1998. pp.279-342
- (9) Radford J.R., Balayne H.M., Nugent Z., Beighton D., et al. Caries associated microorganisms in infants from different socioeconomic backgrounds in Scotland. *J. Dent.* 2000; 28(5):307-312
- (10) Campus G., Lumbau A., Bachisio S.L. Caries experience and streptococci and lactobacilli salivary levels in 6-8 year old Sardinians. *Int. J. Paediatr. Dent.* 2000; 10(4): 306-312
- (11) Crossner C.G. Salivary lactobacilli counts in the prediction of caries activity. *Community Dent. Oral. Epidemiol.* 1981; 9:182-190
- (12) Montalto M., Vastola M., Marigo L., Covino M., Graziosetto R., Curigliano V., Santoro L., Cuoco L., Manna R., Gasbarrini G. Probiotic treatment increases salivary counts of lactobacilli: a double-blind, randomized, controlled study. *Digestion.* 2004;69(1):53-56.
- (13) Zukanović A. Efficacy of "Cariogram" model in evaluation of caries risk-factors in 12-years old children (Master Thesis), (in Bosnian). Sarajevo: University of Sarajevo, 2005: 47.
- (14) Hänsel Petersson G., Fure S., Twetman D., Bratthall D. Comparing caries risk factors and caries risk profiles in children and elderly. Assessing Caries risk. *Swedish Dent. J. Supplement* 2003; 158:1-23.
- (15) Koll-Klais P., Mändar R., Leibur E., Kjaeldgaard M. High levels of salivary lactobacilli in Estonian schoolchildren. *Eur. J. Paediatric Dent.* 2004; 5(2):107-109
- (16) Songpaisan Y., Serinirach R., Kuvatanasuchati J., Bratthall D. *Mutans streptococci* in a Thai population: Relation to caries and changes in prevalence after application of fissure sealants. *Caries Research* 1994; 28:161-169
- (17) Bratthall D., Khim S.P., Durward C.S. Dental caries and prevalence of mutans streptococci in a group of Cambodian children. *Community Dent. Oral Epidemiol* 1993; 21:174 -175
- (18) Maher R., Khan A., Rahimtoola S., Bratthall D. Prevalence of mutans streptococci and dental caries in Pakistani children. *JPMA J. Pak. Med. Assoc.* 1992; 41:213-215
- (19) Shy Y., Barmes D., Bratthall D., Leclercq M.H. WHO pathfinder caries survey in Beijing extended with data for prevalence of mutans streptococci. *Int. Dent. J.* 1992; 42:31-36
- (20) Bjarnason S., Kohler B. A longitudinal study of dental caries and cariogenic microflora in a group of young adults from Goteborg. *Swed. Dent. J.* 1993;17(5):191-199
- (21) Stecksén-Blicks C. Lactobacilli and *Streptococcus mutans* in saliva, diet and caries increment in 8- and 13-year-old children. *Scand. J. Dent. Res.* 1987;95(1):18-26
- (22) Zickert I., Emilson C.G., Krasse B. *Streptococcus mutans*, lactobacilli and dental health in 13-14-year-old Swedish children. *Community Dent. Oral Epidemiol.* 1982;10(2):77-81
- (23) Kohler B., Bjarnason S. Mutans streptococci, lactobacilli and caries prevalence in 15 to 16-year olds in Goteborg. Part II. *Swed. Dent J.* 1992;16(6):253-259
- (24) El Nadeef M.A., Bratthall D. Intraindividual variations in counts of mutans streptococci measured by "Strip mutans" method. *Scand. J. Dent Res* 1991; 99:8-12