High levels of salivary lactobacilli in Estonian schoolchildren

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Abstract. Aim *This was to assess oral salivary lactobacilli levels compared with oral health in a group of 12-year-old schoolchildren in Tartu, Estonia.* ***Methods*** *Whole saliva samples were collected and transferred to dip-slides (Dentocult LB) and incubated at 37°C for 3 days. Dental caries, dental plaque, and data concerning the general health, dental habits and eating patterns were recorded.* ***Results*** *Salivary lactobacilli were found in all children, with a quarter of them having high or very high lactobacilli counts. Caries prevalence of 75% and 2.6 DMFT were recorded. A positive correlation was found between the DMFS counts and the lactobacilli counts.* ***Conclusion*** *High levels of salivary lactobacilli were found in Estonian schoolchildren. Caries indicators of these children were slightly higher than in the same age group in Nordic countries.*

Keywords*: Children, Salivary lactobacilli, Dental caries.*

Introduction

Dental caries is an infectious disease that is related to acidogenic and aciduric microrganisms colonizing in the oral cavity. The demineralisation of teeth is caused by organic acid produced from the bacterial fermentation of dietary carbohydrates. The complexity of the bacterial community in dental plaque of humans has made it difficult to determine the single bacterial agent of caries; however, there is considerable evidence that mutans streptococci are involved in the initiation and lactobacilli in the progression of caries. They are able to rapidly metabolize carbohydrates into acid and to tolerate a low pH environment [Marsh and Martin, 1999].

On the other hand, as lactobacilli belong to a normal human microflora, they can be found also in caries-free people and their role in supporting human health has been shown repeatedly [Mikelsaar et al., 1998]. Large geographic variations in the composition of human microflora occur, for example lactobacilli of the intestinal tract are more frequently present and in higher numbers in populations of developing or Eastern European rather than in Western countries [Mikelsaar et al., 2002]. Less is known about geographic differences in oral lactobacilli, and no data for Estonian children are available. Accordingly, the present study was designed to compare salivary lactobacilli counts with oral health in a group of Estonian schoolchildren.

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Material and methods

The study was carried out in the eastern Estonian town of Tartu where the fluoride content in the drinking water is close to the optimal level (mean 0.7 ppm). 96 schoolchildren of grade 6 (54 girls and 42 boys) with mean age 12.2 years (range 11-14 years) were examined at a school dental unit. Before the examination all tooth surfaces were cleaned and dried. Dental caries was diagnosed using the criteria of WHO [1997] and incipient caries as "white spot" lesion on smooth surface and dental plaque [Silness and Löe, 1964] were recorded. General health, dental habits and eating patterns were recorded using questionnaires. Salivary lactobacilli were obtained by Dentocult LB (Orion Diagnostica, Espoo, Finland) method. Paraffin-stimulated whole saliva was collected and a 1 ml aliquot was transferred to a selective dip-slide. After 3 days of incubation at 37°C, the number of lactobacilli was estimated by comparing the slides with a density chart provided by the manufacturer. The results were grouped into four categories corresponding to low ($\leq 10^3$ CFU/ml), medium (10^4 CFU/ml), high (10^5

CFU/ml) and very high (>10^6 CFU/ml) count of lactobacilli. Mann-Whitney Rank Sum Test and Pearson Correlation were used for statistical analysis.

Results

All of the children carried lactobacilli in their saliva. 47% of the children had low, 28% medium, 18% high and 7% had very high counts of lactobacilli in their saliva. No statistically significant differences between boys and girls were recorded.

Clinical data of the children studied are given in Table 1. All the children considered themselves as healthy and no missing surfaces or teeth were recorded. A mild but positive correlation was found between DMFS counts and plaque scores (r=0.250, p=0.014). The frequency of use of non-sugary between-meal snacks showed an association with DMFT counts (r=0.245, p=0.016), and that of sweets consumption showed an association with the number of surfaces affected by incipient caries (r=0.208, p=0.042). A positive correlation was found between the DMFS counts and the lactobacilli counts (r=0.246, p=0.015). No statistically significant correlations were found between lactobacilli counts and other indicators.

Discussion

The present study showed that in this population of 12-year-old Estonian children all had lactobacilli in saliva with 25% of children having high or very high lactobacilli counts. This agrees with Kohler and Bjarnason [1987], who reported that 23% of the 12-year-old Icelandic children had more than 10^5 CFU per ml of saliva, however with much higher caries prevalence. The proportion of Finnish children with high count of lactobacilli was somewhat higher (38%) compared with the results of the present study [Raitio et al., 1996]. On the other hand, in Sweden there has been found a decreasing tendency in the number of oral lactobacilli during the last decade [Nylander et al., 2000]: 16% of children were free of lactobacilli in 1998, whereas only 12% of children had high or very high lactobacilli counts.

At the same time it is known that lactobacilli are common inhabitants of the oral cavity, although they

	Total (n=96)	Boys (n=42)	Girls (n=54)	P value
Prevalence of caries	75%	83%	69%	NS
Mean DMFS*	3.5	4.5	2.7	0.033
Mean DMFT	2.6	3.1	2.1	0.051
Mean DS	1.3	1.6	1.0	NS
Mean FS	2.2	2.8	1.7	NS
Mean DT	1.1	1.5	0.9	NS
Mean FT	1.5	1.8	1.2	NS
Prevalence of incipient caries	65%	62%	67%	NS
Mean plaque score	1.1	1.1	1.0	NS
Meal per day (2 / 3 / >3)	9 / 73 / 18%	12 / 64 / 24%	7 / 80 / 13%	NS
Sweets consumption**	5 / 41 / 43 / 11%	11 / 41 / 41 / 7%	0 / 41 / 44 / 15%	NS
Snacks consumption***	9 / 72 / 19%	7 / 67 / 26%	11 / 76 / 13%	NS
Toothbrushing frequency****	57 / 39 / 4%	40 / 50 / 10%	70 / 30 / 0%	0.006

*DMFS-decayed, missing and filled surfaces; DMFT-decayed, missing and filled teeth; DS-decayed surfaces; FS-filled surfaces; DT-decayed teeth; FT-filled teeth.
**never / 3-4 times a week / 1-3 times a day / more than 3 times a day;
***never / 1-2 per day / 3 or more per day
****twice or more than twice a day / once a day / some times a week or never

TABLE 1 - *Clinical data for dental caries, plaque scores and dietary data in population of 12-year-old Estonian schoolchildren.*

usually comprise less than 1% of the total cultivable microflora [Marsh and Martin, 1999]. Lactobacilli are not homogenous in terms of their cariogenic potential as certain species, like L. casei, L. fermentum and L. rhamnosus [Marchant et al., 2001] have been more frequently associated with caries lesions. Some lactobacilli strains have been found to be antagonistic against mutans streptococci, and they may even reduce the caries risk [Ahola et al., 2002]. Therefore, the presence of lactobacilli in saliva may not be directly related to caries risk if their species composition is unknown. In our study lactobacilli were also found in caries-free children, however, the counts were lower. Our study has some limitations, as species composition of the oral lactoflora was not determined.

Several studies have shown large geographic variations in human lactoflora [Mikelsaar et al., 2002]. When comparing intestinal lactobacilli in Estonian and Swedish children, it was shown that lactobacilli are more common in Estonia than in Sweden and with more diverse species composition [Mikelsaar et al., 2002], and there is a similar tendency for frequency of oral lactobacilli if comparing our data with those of Nylander et al. [2000]. The Estonian diet is still to a large extent based on locally produced foods and, in addition, with foodstuffs fermented by lactic acid bacteria, a possible source of lactobacilli [Mikelsaar et al., 2002].

The caries prevalence of 75% and 2.6 DMFT in our study group are somewhat higher as compared with the results of the Nordic countries, where 23% to 49% of 12-year-old children are caries-free and the mean caries prevalence is 1.2 to 2.5 DMFT [Von der Fehr, 1994; Nylander et al., 2000]. Treated dental caries (FS and FT) constituted most of the total caries picture in our study, which may reflect good access to dental care. Based on previous Estonian epidemiological studies, the average DMFT values for 12-year-old children were 4.6 in 1991 [Bjerner et al., 1992] and 4.1 in 1992 [Marthaler et al., 1996], while some recent data [Dragheim et al., 2000] have shown a declining tendency of dental caries in the same age group (1.8 DMFT in 1997). The oral health care system in Estonia is in transition, and in the past it has been mostly focused on curative care whereas community-based prevention and oral health promotion have not been systematically implemented [Dragheim et al., 2000]. In our study group with optimal fluoride concentration in the drinking water, but still a quite high prevalence of incipient caries, the reduction of consumption of sweets and snacks should be considered.

Conclusion

Salivary lactobacilli were found in all Estonian 12-year-old schoolchildren, with a quarter of them having high or very high counts. Caries indicators were slightly higher than in the same age group in Nordic countries.

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