

SALIVARY MUTANS STREPTOCOCCI AND LACTOBACILLI AFTER SELF ARRESTING CARIES TREATMENT

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Abstract. Previous studies have shown that Self Arresting Caries Treatment (SACT) may be a useful technique to arrest active caries in Thai preschool children in remote areas. The objective of this study was to examine the effect of SACT on the levels of salivary mutans streptococci and lactobacilli in Thai children. Eighty-two subjects aged 4-6 years from primary school in a remote area of Thailand were recruited for this study. Each subject has at least two pairs of interproximal carious cavities between the first and second deciduous molars, and no history of severe pain. The subjects were divided into the control and SACT groups. The SACT technique was performed by removing food remnants and the surrounding overhang enamel of the carious cavity walls to enlarge the space between the carious cavities. The levels of mutans streptococci and lactobacilli in paraffin-stimulated whole saliva were determined at baseline, 2 and 4 months after treatment compared to those of the control group by the spatula method. The bacterial growth score was divided into three categories: 1 = 0-20 CFU, 2 = 21-100 CFU, and 3 \geq 100 CFU. At baseline, no differences in the mutans streptococci and lactobacilli scores were found between the SACT and control groups ($p > 0.05$). Lower salivary lactobacilli levels were found in the SACT group on follow-up at 2 and 4 months ($p < 0.05$), whereas the mutans streptococci level remained unchanged during these time periods. Our data confirms that the SACT technique can promote a self cleansing condition for interproximal lesions and can reduce the retentive sites in the oral cavity due to a reduction in salivary lactobacilli.

INTRODUCTION

Dental caries has been viewed as an infectious and transmissible disease which is strongly influenced by diet (Krasse, 1965). The current concept of dental caries centers on the fermentation of carbohydrates by cariogenic plaque bacteria, producing organic acids which act on the susceptible tooth (Keyes *et al*, 1963). The major groups of cariogenic bacteria are now considered to be mutans streptococci and lactobacilli. The presence of high numbers of mutans streptococci in the saliva is indicative of a high caries risk, while the presence of high numbers of lactobacilli is indicative of either high caries activity or a high sugar intake. The prevalence of mutans streptococci colonization is thought to increase with the age and number of

erupted teeth, from 6-10% 1 year-old to approximately 33% of 3 year-old children (Alaluusua and Renkonen, 1983; Mohan *et al*, 1998). Approximately 50% of preschool-age children harbor mutans streptococci (Twetman *et al*, 1994). Lactobacilli are highly dependent on the presence of retentive sites, and are generally less frequently detected in the saliva of young children (Van Houte, 1980).

The number of carious lesions, fillings and other retentive sites in the mouth, such as crowded arches and fixed orthodontic appliances, are thought to influence microbial colonization in a negative way. Self Arresting Caries Treatment (SACT) has been shown to be a useful technique in arresting active caries in Thai children in remote areas (Soo-Ampon, 2003). It is expected that any treatments which eliminate cavities and retentive sites can diminish the number of cariogenic microorganisms. The objective of this study was to examine the effect of SACT on the levels of salivary mutans streptococci and lactobacilli in Thai children residing in a remote area.

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MATERIALS AND METHODS

Subjects

Eighty-two children aged 3-5 years at a primary school in a remote area of Chachoengsao Province, Thailand were recruited for this study. Each subject has at least two pairs of proximal active carious lesions between the first and second deciduous molars and no history of severe pain. The subjects were divided into control and SACT groups.

Treatment

The SACT technique was performed in the SACT group by removing food remnants and the surrounding overhang enamel from the carious cavity walls to enlarge the space between the carious cavities. During the experimental period, children in the control group did not receive any treatment.

Microbiological procedures

The levels of mutans streptococci and lactobacilli were determined at baseline, 2 months and 4 months using the method described by Kohler and Bratthall (1979). The sampling was carried out on saliva, stimulated by chewing a piece of paraffin for 1 minute. A thin layer of saliva was collected by means of a wooden spatula rotated in the mouth of subject and withdrawn through the pursed lips of the child. The saliva sample was immediately transferred to Mitis-Salivarius agar by pressing both sides of the spatula against the agar surface. For lactobacilli, the same procedure was done and cultured on Rogosa agar. The plates were kept in plastic bags filled with expired air at 37°C until brought to the laboratory. Then, they were further incubated in a CO₂ incubator at 37°C for 48-72 hours. After incubation, the plates were examined under a microscope and the bacterial colonies resembled mutans streptococci and lactobacilli were counted. The bacterial growth score was divided into three categories: 1 = 0-20 CFU, 2 = 21-100 CFU, and 3 = > 100 CFU.

Statistical analysis

The microbial levels in the SACT and control groups were compared at baseline with the non-parametric Mann-Whitney test. At 2 and 4 months, bacterial scores were analyzed com-

pared to baseline both in the control and SACT groups using the Friedman and multiple comparison tests. The level of statistical significance was set at $p < 0.05$.

RESULTS

At baseline, no differences in mutans streptococci and lactobacilli scores were found between the SACT and control groups. The mean score for mutans streptococci in the control group was 2.40, and in the SACT group was 2.31; the mean score for lactobacilli in the control group was 1.52, and in the SACT group was 1.82. A significant reduction of salivary lactobacilli was demonstrated in the SACT group at 2 and 4 months ($p < 0.05$) (Table 1). After 4 months, approximately 18% of children in the control group had a high lactobacilli score (score 3), whereas no child with the same level was found in the SACT group. In contrast to lactobacilli, the mutans streptococci levels in the SACT group at 2 and 4 months were similar to those observed at baseline (Table 2). However, the mutans streptococci score in the control group had increased significantly at 4 months ($p < 0.05$).

DISCUSSION

This study investigated the effects of SACT on the levels of caries associated microorganisms in the saliva of preschool children residing in a remote area of Thailand. At baseline, the prevalences of salivary mutans streptococci and lactobacilli in general were rather high (the mean mutans streptococci score was 2.40 in control group and 2.31 in the SACT group; the mean lactobacilli score was 1.52 in the control group and 1.82 in the SACT group) (Tables 1 and 2). Thus, the oral cavity environment in this group of children was very conducive to caries development. Caries prevention was urgently needed but a large-scale curative treatment was not feasible due to the remote living area. SACT, a primary treatment measure, was performed to control the initial carious lesions and prevent the progression to more advanced lesions. In the course of this study it was found that the lactobacilli score dropped significantly immediately after SACT and the effect lasted for at least 4

Table 1
The levels of salivary lactobacilli in the SACT and control groups.

	SACT			Control		
	Baseline	2 months	4 months	Baseline	2 months	4 months
Mean, score	1.82	1.28	1.03	1.52	1.80	1.40
Score %						
1	40.47	79.48	96.87	47.36	44.44	77.77
2	35.71	2.82	3.12	23.68	30.55	3.70
3	23.81	7.69	0.00	28.94	25.55	18.51

Table 2
The levels of salivary mutans streptococci in the SACT and control groups.

	SACT			Control		
	Baseline	2 months	4 months	Baseline	2 months	4 months
Mean, score	2.31	2.04	1.90	2.40	2.25	3.00
Score %						
1	14.28	20.51	12.50	15.79	16.66	3.70
2	26.19	38.46	25.00	23.68	41.66	11.11
3	59.52	41.02	62.50	60.52	41.66	85.18

months. This is in agreement with a previous observation that the lactobacilli count was reduced following the restoration of the cavities (Shklair *et al*, 1956) or the arresting of caries progression (Bjorndal and Larsen, 2000). Lactobacilli were found to be the predominant microorganisms in carious dentin and involved in the progression of the lesion. Demineralization caused by these kinds of bacteria is due to their ability to produce large amounts of acid, particularly lactic acid, and their aciduric property (Van Houte, 1980). The prevalence of lactobacilli increases with advanced carious lesions (Nyvad, 1993). A substantial reduction in the frequency and proportions of carious lesions reflects a change toward a non-acidic environment, which favors the arrest of carious lesions and demineralization of tooth tissues (Bjorndal *et al*, 2000). In contrast to lactobacilli, mutans streptococci has been demonstrated to be associated with the initiation of carious lesions (Nyvad, 1993). They are considered cariogenic organisms because they can synthesize water-insoluble glucans from sucrose to mediate their

irreversible adhesion and colonization of the teeth (Tanzer *et al*, 1984). In addition, they can produce lactic acid, which is potent in driving tooth demineralization (Johnson *et al*, 1980). Our results show that the levels of mutans streptococci in the saliva of children were not decreased 2 and 4 months after SACT. This is similar to a study by Twetman *et al* (1999) which demonstrated that lactobacilli levels diminished more dramatically than mutans streptococci levels after primary restorative treatment. They reported that approximately 50% of the children still exhibited high counts of mutans streptococci at 1 and 6 month follow-up visits. It is interesting to note that the mutans streptococci score in the control group of our study increased significantly in comparison to the baseline, reflecting the severity of the disease in this child population in remote areas of Thailand.

In conclusion, the results from this study show that SACT can promote a self cleansing condition and it is effective in reducing retentive sites in the oral cavity due to a decrease in lactobacilli levels.

ACKNOWLEDGEMENTS

This work was supported by a Mahidol University grant (2001) for the projects entitled: Research package for prevention and control of oral diseases of children in the underserved area, and risk factors for dental and gingival problems at the Thai-Myanmar border.

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