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Correlation of Level and Duration of *Streptococcus mutans* Infection with Incidence of Dental Caries

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The caries incidence at various levels of *Streptococcus mutans* infection was analyzed in a control group and a test group. In the control group, the incidence of caries and the duration of *S. mutans* infection were significantly correlated. In the test group, the *S. mutans* infection was suppressed by antimicrobial measures when the number of *S. mutans* exceeded 250×10^3 CFU per ml of saliva. The results illustrate that the level and duration of the *S. mutans* infection are strongly correlated to the incidence of caries. The findings support the concept of *S. mutans* as a key cariogenic microorganism and illustrate the value of antimicrobial treatment in the prevention of caries.

There is considerable evidence implicating *Streptococcus mutans* as a specific infectious agent in the initiation of dental caries (for a review, see reference 4). In this study, we examined the level and duration of *S. mutans* infection in relation to the development of caries in teenagers. At the same time, the effect of the reduction of the *S. mutans* infection was analyzed.

A total of 91 children, randomly assigned to a control group ($n = 47$) and a test group ($n = 44$), completed a clinical trial (6). The subjects were 13 to 14 years old at the start of the study and belonged to an elementary school in the city of Mölndal, Sweden, which has negligible fluoride (0.1 parts/10^6) in the community water supply. The children were followed up to 3 years with respect to *S. mutans* infection and the development of caries. The children in the control group followed the conventional annual prophylactic and restorative dental treatment program of the Swedish Public Dental Health Service. In addition, the children in the test group with an infection level of *S. mutans* above 250×10^3 CFU per ml of saliva were given chlorhexidine treatment at intervals to suppress the *S. mutans* population. Furthermore, the fissure sealing of all unfilled sound occlusal surfaces of premolars and molars was carried out in the test group at the start of the study. This was done to eliminate retention sites in which *S. mutans* colonizes. Written informed consent was obtained from the children's parents after information about the purpose, benefits, and risks of the study had been disseminated.

Paraffin-stimulated whole saliva samples were taken at intervals, and the number of *S. mutans* per ml of saliva was determined by the micro-method described by Westergren and Krasse (5). The salivary *S. mutans* level was determined every 6 months in the control group and every 4 months in the test group.

Dental caries were recorded after 1, 2, and 3 years, using criteria described by Gustafsson et al. (3). These criteria include both incipient and frank lesions.

The cumulative mean number of new carious lesions per child in the control group during the 3 years of the trial in relation to the level of *S. mutans* infection on the six sampling occasions was calculated. When the level of *S. mutans* infection was below the threshold value of 250×10^3 CFU per ml of saliva throughout the 3 years, the number of new lesions was 4.8. This figure increased to 6.8, 10.5, and 16.5 when the *S. mutans* infection level exceeded this value on 1 to 2, 3 to 4, and 5 to 6 sampling occasions, respectively (see Table 5). The correlation between the caries incidence and the duration of *S. mutans* infection during the 3-year observation period was statistically significant ($r = 0.53$, $P < 0.001$).

The effect of the infection level on caries activity for each of the 3 years of the trial was also examined. From the samples taken at the beginning, middle, and end of each year, the mean number of *S. mutans* per ml of saliva for each child was calculated. The incidence of caries in relation to this infection level for each separate year is shown in Table 1. The children with a mean value of $>10^6$ *S. mutans* per ml of saliva showed a considerably higher caries activity than did those with lower levels of *S. mutans* infection. It is evident from Table 1 that the children with the highest infection level run the highest caries risk.

TABLE 1. New carious lesions in the control group in relation to the salivary level of *S. mutans*

Yr	No. of new carious lesions (mean ± SD) per child at salivary level of *S. mutans* of:		
	$<250 \times 10^3$ (A)	$\geq 250 \times 10^3$–$<10^6$ (B)	$\geq 10^6$ (C)
1	2.4 ± 2.3 (21)[a]	2.6 ± 3.4 (14)[b]	8.1 ± 8.5 (12)[c]
2	1.7 ± 1.4 (26)	2.7 ± 3.0 (11)	5.1 ± 3.3 (10)[d]
3	2.2 ± 2.1 (28)	2.8 ± 3.4 (11)	6.3 ± 7.0 (8)[c]

[a] The number of children in each group is given in parentheses.

[b] The difference between B and C was significant at $P < 0.05$ (the Student t test).

[c] The difference between A and C was significant at $P < 0.01$ (the Student t test).

[d] The difference between A and C was significant at $P < 0.001$ (the Student t test).

The length of time during which children had $>250 \times 10^3$ *S. mutans* per ml of saliva was calculated (Table 2). When high counts were found on 5 to 6 sampling occasions, 8 of 13 children showed a sequential of 36 months with high salivary counts. When a value of $>250 \times 10^3$ CFU was observed at 1 to 2 sampling occasions, only 2 of 13 children had high salivary counts for 6 months.

The infection level for the individual children in the control group remained fairly constant throughout the 3-year period, i.e., with few exceptions, children with high or low levels of infection at the start of the study remained at this level (6). This implies that conventional treatment and prevention had but little effect on the *S. mutans* infection. In the test group, however, the *S. mutans* infection in children with $>250 \times 10^3$ CFU per ml of saliva was reduced. To this end, a gel (pH 7.2) containing 1% chlorhexidine digluconate (ICI Ltd., Macclesfield, England), 2% methylcellulose, and flavoring agents was applied to the teeth in individually designed applicators 5 min/day for 14 days (1, 2). The antimicrobial effect was tested 2 to 3 days after completed gel treatment to determine whether a satisfactory drop in the *S. mutans* count had been obtained. The treatment was repeated every 4 months, if necessary. Some of the children received only one treatment during the whole 3-year period, whereas others needed seven to nine treatments. The level of *S. mutans* infection before and after the chlorhexidine treatment is shown in Table 3. The children were divided into three groups depending on the number of treatments received. The chlorhexidine treatment led to a considerable reduction of the salivary *S. mutans* count in all children. After some time, the number of *S. mutans* was again close to the threshold level; this time varied considerably (Table 4). The mean was 4.6 months for the children treated seven to nine times and 15.4 months for those treated one to three times. It is evident that the higher the infection level before the treatment, the sooner did the number of *S. mutans* return to more than 250×10^3 CFU/ml. The gradual reappearance of *S. mutans* is also illustrated in Fig. 1. Figure 1A shows the recovery of salivary *S. mutans* in highly infected subjects in another study (1). In that study, salivary samples were taken at intervals, and the data obtained during a 4-month period give the shape of the curve. Figure 1B shows a curve based on data from children in the present study treated with chlorhexidine seven to nine times. The shape of the curve was constructed after Fig. 1A. From Fig. 1 it can be calculated that the infection level was above 250×10^3 CFU for 27% of the 4-month period. The value 27% was used for the calculation of the time of infection at $>250 \times 10^3$ CFU in the test group in all of the cases where the *S. mutans* level after chlorhexidine treatment increased from $<100 \times 10^3$ to $>500 \times 10^3$ CFU after 4

TABLE 2. Distribution of children in the control group according to salivary levels of *S. mutans*

No. of sampling occasions with *S. mutans* $>250 \times 10^3$ CFU/ml	No. of children after mo of successive counts of *S. mutans* $>250 \times 10^3$ CFU/ml of:					
	6	12	18	24	30	36
1–2 (13)[a]	2					
3–4 (8)	1	7				
5–6 (13)				3	2	8

[a] The number of children is given in parentheses.

TABLE 3. Median number of *S. mutans* before and after chlorhexidine treatment

No. of children	No. of treatments	Salivary count (CFU) of *S. mutans* ($\times 10^3$) at:			
		Pre-treatment	2–3 days	4 mo	>4–36 mo
15	1–3	380	4	68	282
13	4–6	630	20	214	570
7	7–9	1,290	46	980	1,160

TABLE 4. Distribution of time intervals for samples of *S. mutans* with ≥250 × 10^3 CFU/ml of saliva after chlorhexidine treatment

No. of children	No. of CH[a] treatments per child (total)	No. of samples of *S. mutans* ≥250 × 10^3 CFU/ml of saliva at mo posttreatment of:					Mean interval for return of *S. mutans* to >250 × 10^3 CFU (mo)
		4	8	12	16	20–36	
15	1–3 (29)	7	5	7	1	9	15.4
13	4–6 (66)	36	18	8	4		6.7
7	7–9 (54)	47	6	1			4.6

[a] CH, Chlorhexidine.

TABLE 5. Caries incidence in relation to total *S. mutans* infection time ≥250 × 10^3 CFU/ml of saliva

Group	No. of children	No. of sampling occasions[a]	% of total time[b] *S. mutans* ≥250 × 10^3 CFU/ml of saliva	Total caries incidence (% reduction)[c]
Control	13	0	0	4.8 ± 2.7 (71)
	13	1 to 2 (1.4)	22.8 ± 11.6	6.8 ± 3.6 (59)
	8	3 to 4 (3.5)	64.1 ± 13.1	10.5 ± 11.2 (36)
	13	5 to 6 (5.7)	95.1 ± 8.0	16.5 ± 10.6 (0)
Test	9	0	0	2.4 ± 2.0 (85)
	15	1 to 3 (1.9)	8.6 ± 8.1	3.9 ± 3.3 (76)
	13	4 to 6 (5.1)	22.9 ± 7.4	5.0 ± 6.9 (70)
	7	7 to 9 (7.7)	36.9 ± 12.1	5.3 ± 4.3 (68)

[a] The mean is given in parentheses.
[b] Mean ± the standard deviation.
[c] Relative to the control group with the highest caries incidence.

months. In all other cases, the data were connected with straight lines, and the period above 250 × 10^3 CFU was calculated.

The percentage of time during which control and test subjects had *S. mutans* counts of >250 × 10^3 CFU/ml in relation to the caries incidence is shown in Table 5. In the control group, the children with *S. mutans* levels above this value in 5 to 6 samples, i.e., 95% of the total period, showed a caries incidence of 16.5 new lesions. A shorter time above the threshold value—either naturally in the control group or owing to antimicrobial measures in the test group—was accompanied by a correspondingly lower incidence of caries.

There was a difference in the caries activity of the zero control subgroup and that of the zero test subgroup which had not received chlorhexidine treatment (4.8 and 2.4, respectively). This difference was unexpected as the children came from the same population and had the same caries frequency at the start of the study. It is possible that the nontreated children in the test group became prevention conscious due to the chlorhexidine treatment of some of their classmates. Another explanation could be a reduced spread of virulent *S. mutans* within the class due to the repeated chlorhexidine treatment.

The low caries activity in the test group in spite of the fact that the children had an *S. mutans* infection level above 250 × 10^3 CFU four to nine times during the experimental period cannot be explained solely by the reduced duration of infection. Other important factors must be that the infection level was compara-

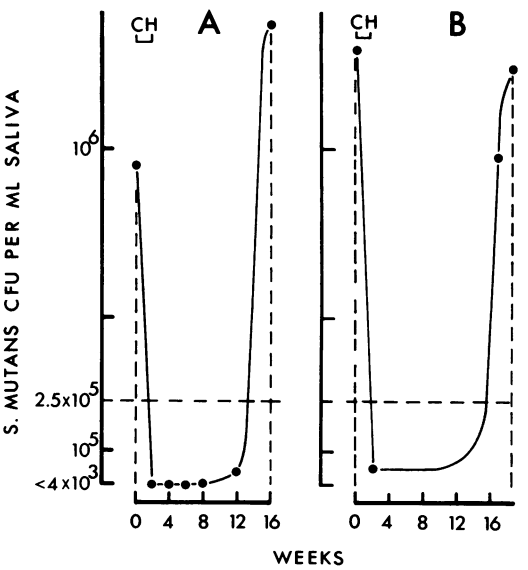

FIG. 1. Effect of 1% chlorhexidine gel treatment (CH) on salivary levels of *S. mutans* (median values). The shape of the curve was based on (A) data from five subjects highly infected with the organism (1) and (B) data from children treated seven to nine times during the 3 years of the trial.

tively low for a considerable time after the chlorhexidine treatment (Table 4) and that longer periods of high counts were not seen. In the control group, on the other hand, some children showed a sequential period of high *S. mutans* counts for up to 36 months (Table 2). This proved to be more destructive to the dentition than intermittent high counts.

In summary, the results clearly show that the level and duration of the *S. mutans* infection strongly correlated with the incidence of caries in Swedish teenagers. When a reduction in the *S. mutans* level was obtained by antimicrobial measures in highly infected children, the caries incidence was roughly the same as in children with a natural infection of the corresponding duration. The findings support the concept of *S. mutans* as a key microorganism in the development of caries. They also illustrate the value of controlled antimicrobial treatment for the prevention of caries.

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