Prevalence of Dental Caries in 4- to 5-Year-Old Children Partly Explained by Presence of Salivary Mutans Streptococci

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The correlation between dental caries and the number of oral mutans group streptococci (ms) present has been shown to be weak. The aim of this investigation was to study associations between caries experience (decayed, missing, and filled surfaces [dmfs]) and the number of ms in stimulated saliva, with emphasis on the level of disease and the confounding effect of regular intake of sweets, the presence of salivary lactobacilli, and oral hygiene. In some 2,700 4- to 5-year-old South African children of different ethnic origins, caries was diagnosed on the basis of World Health Organization criteria and saliva samples were analyzed for ms after cultivation on mitis salivarius-bacitracin agar and for lactobacilli by using the Dentocult kit. Oral hygiene was scored on the basis of the Greene and Vermillion simplified debris index, while data on intake of sweets were derived from extensive interviews. Pearson's coefficient of correlation was computed, and multiple regression analysis was performed to correct for confounding factors. The distribution of the children in the eight caries classes was strongly associated with the ms class ($P < 0.001$), with those in the lower ms classes generally having low dmfs scores and those in the higher ms classes having dmfs scores distributed over the whole range. The r value for the two variables was 0.25 for the total material; this was reduced to 0.18 by correction for confounding factors. The corresponding values for children with caries were 0.21 and 0.17, for those in the 1 to 6 dmfs interval they were 0.07 and 0.03, and for those in the 7 to 81 dmfs interval they were 0.16 and 0.14. The data imply that the explanatory values of ms, those for the lower caries interval not counted, ranged from 6 to 2%. The unexpected results for children with caries might be due to their distribution pattern. It is concluded that there is a need for reevaluation of ms as a risk factor in dental caries.

Mutans group streptococci have been said to be strongly associated with dental caries, particularly in the initial phase of development of the disease (9, 10, 15, 25). Results from studies on animals support such a theory (1, 5, 28), which is widely accepted by the scientific community. A large number of clinical studies have further reported positive associations between caries prevalence or incidence and the number of mutans group streptococci in stimulated saliva or dental plaque, both at group levels and in correlation studies with r values ranging from very low up to about 0.35. Sullivan et al. (21) reviewed this literature but questioned the clinical significance of bacterial counts at group levels. They also indicated that interpretation of results of statistical significance in correlation studies might have been exaggerated. In their own study, one group of Swedish children was examined yearly from ages 5 to 7 and another group was examined from ages 12 to 14. Coefficients of correlation were computed before and after correction for the confounding effects of oral hygiene and lactobacilli by subgroup analysis. The study showed the association between caries incidence and bacteria in saliva to be weak at the individual level. The variation of caries could, at most, be explained to only 6% by variation of the mutans group streptococcal count, a value that was considerably reduced after correction for the two confounders.

The numbers of subjects in the study groups of Sullivan et al. (21) were only 87 and 118, as a result of which some subgroups were quite small. In a recent study (7), we

therefore analyzed four large samples of 4- to 5-year-old South African children from three ethnic groups, about 700 in each, to see whether the figure of 6% could be improved. In simple linear correlations between caries prevalence and mutans group streptococcal counts, the explanatory values were between 3 and 11%. In multiple regression analyses that included five other potential etiological factors, the values were reduced, ranging from 2 to 6% as a result of interaction between the independent variables.

Convinced that the correlation between caries and mutans group streptococci is weak, even for large numbers of subjects, we felt that the next step, after pooling of all children, since little variation between the ethnic groups was found, would be to examine different groups with caries, i.e., after exclusion of caries-free individuals. It could be that the correlation between caries and mutans group streptococci is higher in such groups, particularly if the subjects are in a caries-active stage. Furthermore, since lactobacilli and oral hygiene were the only additional factors that had shown a statistically interesting association with mutans group streptococci (7), it could be of value to include these factors in the study of the different caries groups, as well as regular intake of sweets and snacks, which has also proved to be related to mutans group streptococci (23).

The purpose of the present study was thus to examine associations between caries prevalence and salivary mutans group streptococcal counts in preschool children, with particular emphasis on the level of disease and the confounding effects of regular intake of sweets, the presence of salivary lactobacilli, and oral hygiene, by using the same large data base as previously.

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MATERIALS AND METHODS

Subjects. In 1984, 2,728 South African preschool children, all living within areas with fluoride present at 0.2 to 0.33 ppm in the drinking water and aged 4 or 5 years at their last birthday, were examined for dental caries and a number of potentially related factors. The sample comprised rural black children (n = 671) living in the Gelukspan district of Bophuthatswana some 350 km west of Johannesburg; urban black children (n = 758) from Soweto, 16 km southwest of Johannesburg; urban Indian children (n = 588) from Lenasia, 35 km southeast of Johannesburg; and urban white children (n = 711) from Johannesburg. All descriptive data are available as a technical report by the South African Medical Research Council (3).

Because of the young age of the subjects, those with caries were considered to be a caries-active group, although the data were from a prevalence study, particularly since caries was mostly untreated.

Dental caries. The children's teeth were examined with a mirror and a sharp probe on the basis of World Health Organization (26) diagnostic criteria after prior calibration of examiners to a 90% or greater modified percentage of reproducibility (18) by using extracted teeth mounted in plaster blocks (4). In the field, 10% of the sample was re-examined and it was found that the diagnostic reproducibility was maintained. The World Health Organization diagnostic criteria imply that only manifested carious lesions are registered, in practical terms, cavitation, to avoid the uncertain diagnosis of early precavitation stages of dental caries under field conditions. Caries was expressed as decayed, missing, and filled surfaces (dmfs).

Salivary mutans group streptococci. Each child chewed a 1-cm cube of sterile paraffin wax. The saliva secreted during the first minute was swallowed, and thereafter saliva was collected in a sterile jar. By means of a sterile disposable syringe, a 1-ml aliquot was removed in a portable laminar-flow cabinet and injected into a sterile tube with VMG II transport medium (16). After this, the specimens were placed in cool bags containing ice packs. The Lenasia, Soweto, and Johannesburg specimens were processed on the same evening, and the rural specimens were stored in a hotel cold room and processed within 2 to 3 days. The suspension was diluted in 10-fold steps in 0.02 M sodium phosphate buffer (pH 7.2) with 0.4% (wt/vol) NaCl. From suitable dilutions, cultivation was performed on mitis salivarius-bacitracin agar (6). Incubation at 37°C for 2 days was done in 95% nitrogen–5% carbon dioxide. CFU with morphology characteristic of mutans group streptococci were counted and expressed as numbers of CFU per milliliter of saliva.

Regular intake of sweets and snacks. Data on intake of sweets were derived from extensive interviews regarding nutrient intake and dietary habits (3). Parents or other adults accompanying individual children were asked about the children's recent weekly frequency of intake of sweets. Care was taken not to ask leading questions. The interviewers were skilled in this technique and able to speak the languages of those being interviewed. Sweets included chocolate, toffees, and candy, and snacks consisted of cake, biscuits, and ice cream.

Salivary lactobacilli. For lactobacillus scores, the prepacked Dentocult (Orion Diagnostica, Helsinki, Finland) was used (14). Dentocult is a dip slide method for evaluation of salivary lactobacillus counts designed for routine clinical use. The slide is covered with an agar substrate on both sides. Each slide was used for two children, one per side,
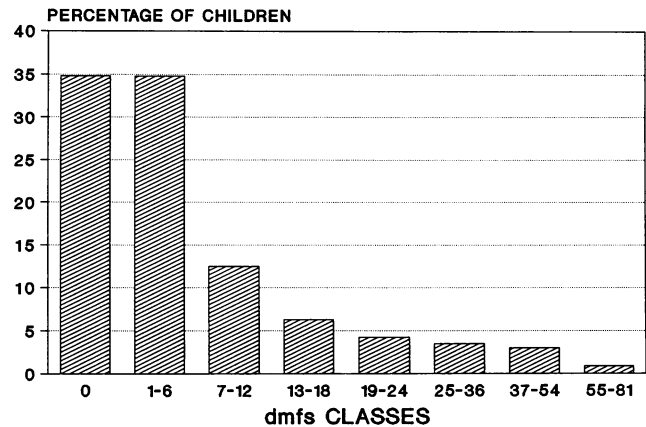

FIG. 1. Caries prevalence among 2,728 4- to 5-year-old children.

and cultured at room temperature (approximately 22°C) for 4 days. Lactobacillus scores were estimated from the comparison scale provided by the manufacturer. The scores (in microbes per milliliter of saliva) were as follows: 1, 1,000; 2, 10,000; 3, 100,000; 4, 1,000,000.

Oral hygiene. The simplified debris index (DI-S) of Greene and Vermillion (8) was used to determine the oral hygiene (amount of dental plaque) of all children. The following four scoring levels were used: 0, no soft deposits or extrinsic stain; 1, soft debris covering not more than one-third of the tooth surface or presence of extrinsic stain without debris regardless of the surface area covered; 2, soft debris covering more than one-third but not more than two-thirds of the surface; 3, soft debris covering more than two-thirds of the surface.

Analysis of data. All data were analyzed in an IBM 3083 J 24 computer by using Statistical Analysis System software (19). Simple correlation analyses between dmfs and mutans group streptococcal counts were performed for the total material and for three caries intervals (1 to 81, 1 to 6, and 7 to 81 dmfs). Pearson's coefficient of correlation was computed, and interdependence between the two variables was tested at class levels by using chi-square analysis. Multiple regression analyses were performed on all intervals to correct for the confounding effects of regular intake of sweets,
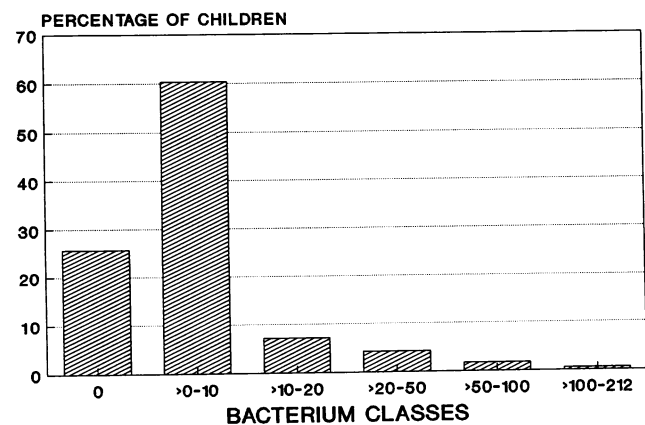

FIG. 2. Salivary mutans group streptococcal counts (10^4 CFU/ml) among 2,728 4- to 5-year-old children; 152 values were missing.

TABLE 1. Caries prevalence and salivary mutans group streptococcal counts in 2,728[a] 4- to 5-year-old children[b]

Bacterial count class (10^4 CFU/ml of saliva)	No. (%) of children in the following dmfs class:								Total (%)
	0	1–6	7–12	13–18	19–24	25–36	37–54	55–81	
0	326 (48.9)	217 (32.5)	61 (9.1)	25 (3.7)	12 (1.8)	8 (1.2)	14 (2.1)	4 (0.6)	667 (100)
>0–10	521 (33.5)	592 (38.0)	215 (13.8)	91 (5.9)	55 (3.5)	39 (2.5)	36 (2.3)	8 (0.5)	15 (100)
>10–20	23 (12.4)	59 (31.7)	26 (14.0)	21 (11.3)	24 (12.9)	15 (8.1)	12 (6.5)	6 (3.2)	186 (100)
>20–50	12 (11.0)	28 (25.7)	14 (12.8)	18 (16.5)	12 (11.0)	15 (13.8)	7 (6.4)	3 (2.8)	109 (100)
>50–100	3 (7.0)	14 (32.6)	8 (18.6)	5 (11.6)	2 (4.7)	4 (9.3)	5 (11.6)	2 (4.7)	43 (100)
>100–211	2 (14.3)	2 (14.3)	2 (14.3)	2 (14.3)	2 (14.3)	2 (14.3)	2 (14.3)	0 (0)	14 (100)
Total	887	912	326	162	107	83	76	23	2,576

[a] A total of 152 values were not included in this analysis.
[b] The chi-square test (df, 35) was used, $P < 0.001$.

presence of salivary lactobacilli, and oral hygiene. A 5% significance level was used for all statistical tests.

RESULTS

The distribution of caries is presented in Fig. 1, and that of mutans group streptococci is presented in Fig. 2. Both variables were typically skewed. About 70% of the children had ≤6 dmfs, and 60% had detectable mutans group streptococci at ≤10^5 CFU/ml of saliva. In Table 1, the data on the children are cross-tabulated with regard to the two variables. While the distribution in caries classes was extremely skewed for bacterium class 0, there was a uniform distribution for the highest bacterium class. Statistically, the interdependence between the two variables was highly significant ($P < 0.001$).

Table 2 shows the correlation between the two variables for different caries intervals before and after correction for confounding factors. The highest r value (0.25) was for the total material before correction, and the lowest (0.07) was for the 1 to 6 dmfs interval. The r values were all reduced by 13 to 57% after correction. All r values but one were statistically significant.

DISCUSSION

Estimation of the number of cariogenic bacteria in stimulated saliva has been used for prediction of dental caries for decades. It has been considered a reasonable measurement of the microbial load of the entire dentition, since saliva does not contain cariogenic bacteria per se, and a more practical measure in the clinical situation than sampling of bacterial plaque from tooth surfaces.

This study of preschool children confirms the finding in recent incidence studies of 5- to 7- and 12- to 14-year-olds (21) and of 15- to 18-year-olds (23) that only 6% of the variation of caries can be explained by the variation of

salivary mutans group streptococci in randomly selected materials. This value was further reduced to 3% after correction for the confounding effects of intake of sweets, presence of salivary lactobacilli, and oral hygiene, which also is in accordance with the above-mentioned studies.

The remarkable result was that the correlation value for caries and mutans group streptococci was lower in the 1 to 81 than in the 0 to 81 dmfs interval and even lower in the 7 to 81 dmfs interval. It might be due to the more skewed distribution of subjects in dmfs classes for lower bacterium classes than for higher bacterium classes. This implies that low counts of mutans group streptococci are more indicative of low caries levels than high bacterial counts are of high levels, a conclusion that also can be derived from prediction studies. Almost invariably, specificity values are higher than sensitivity values (12, 17, 20, 22, 24). The explanatory value (r^2) of mutans group streptococci ranged from 2 to 3% after correction for confounding factors (excluding those children with dmfs scores of 6 or less).

High explanatory values for etiological factors are necessary for successful prediction, which in turn is a prerequisite for drawing up efficient individualized preventive programs. Unfortunately, all generally accepted factors in dental caries seem to show low explanatory values in observational studies compared with what is expected from many experimental investigations of humans, as well as from others using animal systems. Kingman (11) claimed that just one-third of the 3-year caries increment is accounted for by commonly acknowledged risk indicators in classical regression models. Prevalence studies report figures ranging from 12 to 25% (7, 23).

The above data are disappointing, particularly for mutans group streptococci, which have been regarded as a key factor in the occurrence of dental caries ever since the epoch-making work of Fitzgerald and Keyes (5) in 1960, which showed that rodents fed a sugary diet have to be infected with certain streptococci to develop caries. It is true

TABLE 2. Correlation between caries prevalence and salivary mutans group streptococcal counts in 2,778 4- to 5-year-old children[a]

dmfs interval	Value before correction					Value after correction				
	No. of children	Pearson's r	P	r^2	No. of values missing	No. of children	Pearson's r	P	r^2 (partial)	No. of values missing
0–81	2,552	0.25	0.0001	0.06	52	2,548	0.18	0.0001	0.03	180
1–81	1,689	0.21	0.001	0.04	91	1,675	0.17	0.0001	0.03	105
1–6	912	0.07	0.05	0.005	38	904	0.03	0.304	0.0009	46
7–81	777	0.16	0.001	0.03	53	766	0.14	0.0001	0.02	64

[a] Correction was for the confounding factors regular intake of sweets, presence of salivary lactobacilli, and oral hygiene.

TABLE 3. Interrelationship between mean mutans group streptococcal counts, caries prevalence, and weekly frequency of intake of sweets and sweetened drinks, food, and snacks in four groups of 4- to 5-year-old South African children[a]

Group	No. of children	Mean no. of MS[b] (10⁴ CFU/ml of saliva [SD])	Mean no. of dmfs (SD)	Mean weekly frequency of Sw[c] intake (SD)
Rural black	671	3.98 (14.06)	3.6 (7.1)	27.5 (11.0)
Urban black	758	7.11 (16.35)	6.8 (10.0)	35.8 (16.7)
Urban Indian	588	8.39 (18.23)	12.4 (14.9)	50.8 (20.3)
Urban white	711	2.74 (8.60)	5.9 (10.2)	52.9 (23.7)

[a] The data are from reference 3.
[b] MS, mutans group streptococci; some values are missing.
[c] Sw, sweets and sweetened drinks, food, and snacks; some values are missing.

that for humans, group mean values usually correspond to caries mean values and also to intake of sugar-containing products, which is demonstrated in Table 3, although high counts of bacteria have been found in younger teenagers with low caries prevalence (2). Consumption of sucrose has been said to facilitate oral implantation of mutans group streptococci in humans (13). At the individual level, however, Sundin and Granath (23) reached a coefficient of only 0.34 for the correlation between the number of salivary mutans group streptococci and the frequency of regular intake of sweets.

There are a few studies indicating that higher correlation figures might be obtained in selected groups. Thus, Zickert et al. (27) found an r value of 0.53 for the correlation between the 3-year caries incidence in a group of children initially aged 13 to 14 years and the duration of mutans group streptococcal colonization above a threshold value of 250,000 CFU/ml of saliva. Furthermore, Sundin and Granath (23) calculated an r value of 0.57 for the caries incidence in 22 teens from 15 to 18 years of age and the mean values of their respective salivary counts of mutans group streptococci estimated at the start and at the end of the period. All 22 children were characterized as having poor oral hygiene and a high regular intake of sweets, thereby constituting a high caries risk group. The generality of these results is, however, not clear.

The overall low predictive power of mutans group streptococci in randomly selected groups, accentuated in subjects with caries, speaks for the need for some kind of re-evaluation of this risk factor. First of all, the multifactoriality of the disease, including the potential action of several species of acidogenic and aciduric microorganisms, should be seriously considered. A unique specificity of mutans group streptococci has never been proved in observational studies. Another aspect is that the way we sample and estimate the bacteria might not be representative with regard to the role they play in the complicated ecology of dental plaque. Both aspects should challenge the scientific community.

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