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Research Article

Past Caries Experience and Salivary Levels of Streptococcus mutans in Caries Prediction; a 3-Year Follow-Up

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Abstract

Introduction: The prediction of caries risk has been of longstanding interest. Earlier prediction models usually involved the association of one variable with caries development. More recently multiple factors have been included in caries prediction.

Aims: The objectives of the present study were to evaluate, in a cohort of 13-year-old children, the relationship between caries increment of 3-years and the following caries risk factors: past caries experience and salivary *S. mutans* counts.

Settings and Design: Two-hundred four 13-year-old school children were selected randomly.

Methods and Materials: To predict new caries lesions in 204 schoolchildren, clinical and bacteriological caries risk factors were used, including caries experience and *S. mutans* counts.

Statistical analysis used: Spearman rank correlation coefficients for the categorized variables were calculated. Receiver Operating Characteristic (ROC) curves were constructed for caries increment at 3-years and the risk factors.

Results: The 3-year caries increment was positively correlated to the baseline DFS ($r = 0.355$, $p = 0.0001$) and salivary level of *S. mutans* ($r = 0.193$, $p = 0.003$). Among the tests, DFS was the most sensitive and specific. The sensitivity was 62% and the specificity 68% for the combination of *S. mutans* test and DFS and the positive predictive value was 64%.

Conclusion: In addition to the initial caries experience, salivary *S. mutans* proved to be a useful predictor of caries. These two risk factors may be particularly useful in targeting caries prevention efforts in developing countries.

Keywords: *Caries Experience; S. mutans; Caries Prediction*

Introduction

In the last 30 years, caries risk assessment has centered on the analysis of bacteriological, salivary, and clinical markers that can be used as risk predictors [1]. The most commonly used markers are *S. mutans* and lactobacilli counts, saliva flow rate or buffer effect, and clinical markers, including caries experience and fissure morphology, but the latter is a readily accessible indicator that has not been fully evaluated [2,3]. *S. mutans* has primarily been linked with the initial caries development and lactobacilli with the progression of the caries lesion [4,5]. Risk assessment studies have shown that past caries experience has the highest association with caries increments [6]. Bacterial markers, particularly those employing rapid assays, tend to have low sensitivity and high specificity [7] and, therefore, has poor positive predictive values probably induced by the choice of culture medium and type of sample (saliva versus plaque) used. Various other parameters to predict caries activity have also been used alone or in combination with microbiological tests. These include the number of incipient caries lesions and past caries experience, oral hygiene and dietary habits, buffer capacity of saliva, and rate of plaque formation [8]. For clinical purposes, however, the number of tests should be limited. The test or tests needed should be simple but yet accurate enough to predict future caries risk. There are several caries-related chair side salivary tests, which can be used under field conditions and in dental practice to select highly infected patients [9,10]. To assess and optimize the use of the scarce resources for caries preven-

tion available in developing countries, risk markers must be validated in local populations and under local conditions. The purpose of this investigation was to evaluate, in a cohort of 13-year-old children, the relationship between caries increment at 3 years and the following caries risk factors: caries experience and salivary *S. mutans* count.

Material and Methods

The present report is a 3-year longitudinal evaluation of caries-related findings in relation to caries increment of children in their teens. The caries examination was performed and the saliva samples were taken annually the total of four times.

Study cohort

The investigation was conducted in Davangere city, Karnataka state (India), between June 2008-June 2011. Thirteen-year-old schoolchildren in upper primary schools were included in the study and a two-stage random sampling technique was used. Upper primary schools were the primary sampling unit and individual children the unit of enquiry. Upper primary schools in Davangere city are divided into two geographical areas. Ten out of 67 private schools and 6 out of 39 government ones were randomly selected with probability proportional to the total number of schools within each area. The sample size was estimated to 182, using a design effect of two, a precision of 0.05 and a caries prevalence of 60% (based on pilot study). Approximately 11% was added to the sample size in order to oppose loss of sample. Within each of the 16 schools, 12 - 13 children were randomly selected from the total number of 13-year-olds present on the day of study. Parents of all children in the selected schools were approached and asked to give written consent. The final study population consisted of 204, 13-year-old schoolchildren. All the participants were residents of communities with low natural fluoride content (< 0.6 mg/l) in the drinking water. Ethical clearance was taken from the ethical committee of Government dental College, Bangalore City, India.

Caries

The caries experience was calculated as the sum of decayed, missing, and filled surfaces and teeth (DMFS and DMFT), using the criteria recommended by the World Health Organization. Radiographs were not obtained. One calibrated examiner (Kappa 0.96, $p < 0.05$) carried out all the clinical examinations under natural light. Two groups were formed: caries-free and with caries (DFS ≥ 1).

Caries increment

To determine caries increments, the dental examination was repeated annually for 3- years of the study by the same examiner. To avoid observer bias, the children were evaluated without access to the child's previous caries record. For each child, the caries increment was calculated from surface subtracting the baseline DFS score from the last available DFS score, without considering reversals. The net increment was dichotomized as 0 surface newly affected (63.07% of the children, n = 123) versus one or more new surfaces affected (36.92% of the subjects, n = 72).

Bacteriological procedures

Paraffin wax stimulated saliva was collected from each subject and microbiological assay commenced within 24 hours of saliva collection. The sample was transported to the laboratory immediately after collection using the thioglycolate broth and processed on the same day. The sample was vortexed (15 sec,) and diluted 1:1000 in an isotonic saline solution prior to inoculation. Using an inoculation loop (4mm inner diameter), One loop ($1/1000^{th}$ ml of sample) was streaked in duplicate on Mitis salivarius bacitracin agar (MSB) selective for *S. mutans*. The MSB agar plates were incubated anaerobically for 48 hours at 37°C. Following incubation, counts were made of colonies with morphological characteristic for *S. mutans* on the MSB agar using an electronic colony counter. The identity of the presumptive *S. mutans* isolates was established by means of a short set of biochemical tests like mannitol and sorbitol fermentation. Gram staining was also performed. Categories for *S. mutans* were as follows: not detected = score 0; low = score 1 ($<10^4$ CFU/ml); moderate = score 2 (10^4 -10^5 CFU/ml), and high = score 3 ($> 10^5$ CFU/ml).

Statistical analysis

To avoid bias, dropout's data were excluded from all statistical analyses. The clinical and bacteriological markers were characterized with descriptive statistics. Spearman rank correlation coefficients for the categorized variables were calculated for exploring the bivariate

associates. The capability of the tests to select caries risk patients was evaluated by the cross-tabulating the subjects according to their suspected caries risk on the basis of their salivary tests and/or baseline DFS score and the actual 3-year caries increment. The baseline microbial screening levels used were; *S. mutans*, score ≥ 105 CFU/ml and the DFS level selected was ≥ 1 DFS. Sensitivity and specificity, as well as the positive and negative predictive value of the tests were calculated from the cross-tabulations. The scale was based on the mean yearly caries increment of the study population. Receiver Operating Characteristic (ROC) curves were constructed for caries increment at 3-years and the risk factors (*S. mutans* and past caries experience). Analysis was performed using the Statistical Package for Social Science version 17 (SPSS INC Chicago link). All statistical tests were two-sided, and the significance level was set at $p < 0.05$.

Results

During the 3-year follow-up, 9 children dropped out of the study (4.41% dropout rate), mostly due to their parent's job mobility. Among these children, 55.55% (n = 5) were caries-free and 44.44% (n = 4) had caries. Of the 195 children who remained in the study, 97 (49.74%) were boys and 98 (50.26%) girls. Among them, 179 (91.79%) brushed their teeth once a day and 16 (8.21%) two times a day. No significant associations were found with the following confounding variables: gender, number of teeth brushing events per day, number of teeth present (permanent), and caries increment (data not shown).

Of the 195 children, 122 (62.562%) were initially caries-free. After 3 years, 73 children (37.43%) remained caries free and 40 developed caries. Initially, the caries prevalence at 13 years of age was 37.44% (n = 73, DFS = 1.72 ± 2.99). At the end of the follow-up, the net caries increment was 0.60 ± 0.94. Those 12 children with the highest caries increment contributed to 58% of the total caries increment.

Table 1 shows caries increment in children at different follow-up interval, the distribution of caries net increment ranged from one decayed surface in 20% of the children to four surfaces in 2.05% of the children at the end of 3rd-year-followup.

Follow-up period	Number of individuals with caries increment				
	Zero increment	One surface increment	Two surface increment	Three surface increment	Four surface increment
First - year-follow-up	146 (74.9)	32 (16.4)	14 (7.2)	3 (1.5)	0
Second-year-follow-up	140 (71.8)	35 (17.9)	16 (8.2)	3 (1.5)	1 (0.5)
Third-year-follow-up	124 (63.6)	39 (20.0)	21 (10.8)	7 (3.59)	4 (2.05)

Table 1: *Distribution of caries increment in children at first, second, and third year follow-up (n = 195). Values in parenthesis represent percentage.*

At baseline, *S. mutans* was not detected in 1.03% of the children; the level was low in 32.82% of the samples, moderate in 52.31% and high in 13.85% of the samples. 15% of the children maintained the same *S. mutans* score level throughout the study and 31.03% were within 2 scores.

The caries increment was positively correlated with the salivary microbial level and the baseline DFS (Table 2). The strongest associations were found between caries increment and the baseline DFS values (r = 0.355). The sensitivity and specificity of individual tests calculated from cross classifications of the subjects according to their predicted and actual 3-year caries increment is shown in table 3. The baseline DFS count was most sensitive (sensitivity = 58%), and it was highly specific in predicting future caries development (specificity = 78%). The positive predictive value for *S. mutans* test was 42%. The value of a combined test was higher than that of DFS alone.

Parameters	3-year-caries increment	p value
Baseline DFS	0.355	< 0.001.
Salivary *S. mutans*	0.193	0.003

Table 2: *Correlation coefficients between three-year caries increment, baseline salivary and clinical parameters.*

Screening criteria	SN	SP	PPV	NPV	AUC	SE
S. mutans score ≥ 10^5 CFU/ml	38	72	42	72	0.57	0.10
DFS alone (≥1)	58	78	58	85	0.66	0.11
DFS ≥ 1and *s. mutans* score ≥ 10^5 CFU/ml	62	68	64	75	0.65	0.11

Table 3: *Sensitivity (SN), Specificity (SP), Positive (PPV) and Negative (NPV) prediction values, area under the ROC curves (AUC), and Standard error (SE) of tests based on salivary microbial level and baseline DFS value.*

ROC curve shown in figure 1 indicates, curve area was highest for past caries experience (area = 0.66), followed by combined test (area = 0.65), and *S. mutans* (area = 0.57).

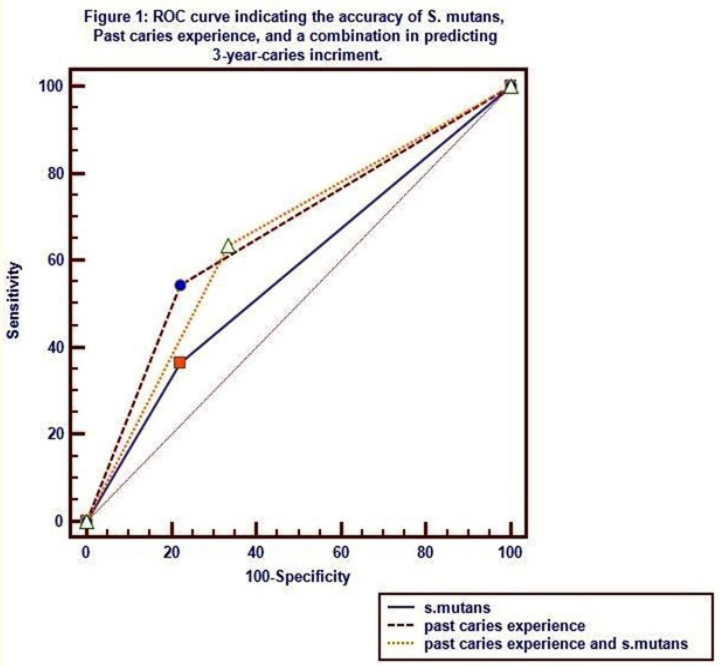

Figure 1: *ROC curves indicating the accuracy of S. mutans, Past caries experience, and a combination in predicting 3-year-caries increment.*

Discussion

The search for a practical method for detecting caries risk has spanned many decades and has included a wide range of potential risk factors. Given the multifactorial nature of the disease, prediction models have included detailed social, psychological, behavioral, dietary, and familial histories [11]. Other models have focused primarily on those risk factors which are individual specific, such as oral hygiene, current caries experience, salivary analysis, gingival indices, fissure morphology, and plaque and salivary microbiology.

Characteristic of the children of the present study was very low mean caries experience at baseline and a low mean caries increment during the 3-year study period. Polarization of caries experience and caries increment was also evident. Whereas 37.43% of the children had no new lesions in 3 years, the highest increment was four lesions. With the declining caries prevalence and incidence figures, child populations like this have become more common, and we considered it important for practical reasons to study validity of various caries screening methods for such child groups.

The tests analyzed in detail were baseline past caries experience and the salivary *S. mutans* level. As the intention was to limit the number of tests to a practical level, the salivary flow rate and buffering capacity were not included in evaluation of sensitivity and specificity since their association with caries increment was found to be low. An association was observed between caries experience and *S. mutans* that can be useful for caries risk assessment.

The sensitivity values of both clinical and microbial tests were low. Of the individual tests, the baseline DFS count was more sensitive and more specific than the microbial test. It was highly specific in predicting future caries development. Our present sensitivity value for

DFS as screening criterion were on the same level, but with a specificity value of a higher level than in another study of children of the same age with much higher average DFS and caries increment figures [12]. The difference in caries prevalence between studied populations might explain the variation in the sensitivity-specificity pattern. This pattern is also affected when distributions of the test attributes differ in shapes among studied populations. The sensitivity was improved, when two screening tests, one microbial combined with the baseline caries score, were used in parallel, but the specificity was impaired. This is a common phenomenon when using parallel tests [13]. This is because some persons at risk are detected by one test but not the other, resulting in the detection of a higher proportion of individuals at risk by the combination of the tests. Also, each test usually contributes independently to the combined total of false positives, thus reducing the specificity. The general fact is that when a disease in a population is at a low level as was the caries in the present study, the positive predictive value is decreased. Since the value is dependent on the prevalence of caries, it is difficult to compare the results reported from other studies. The positive predictive values of the microbial test of the present study are however in accordance with the results obtained from child groups with approximately similar caries activity [13-15]. The present study showed that the positive predictive value of DFS was higher than that of the microbial test. The value increased when DFS was used in combination with the microbial test. The increase was, however, slight and of little practical value.

Conclusion

In conclusion, there was a significant positive correlation between caries increment and salivary *S. mutans* levels and DFS index value. However, individuals at risk for caries were difficult to detect in the present low caries activity population. The efficiency of a combination of DFS and the microbial test, however, was better than that of various alternatives alone in selecting individuals at risk. Depending on the resources allotted for caries prevention, a combination of DFS count and *S. mutans* test (moderate sensitivity, high specificity, moderate positive predictive value) can be considered. Developing countries need strategies to optimize the application of the scarce resources available for caries prevention. These results suggest the possibility of identifying children who are at high risk for caries using caries experience and salivary microbial count as risk markers, and this information may be used to plan for the delivery of preventive services.

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