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Salivary *Streptococcus mutans* level: value in caries prediction for 11-12-year-old children

X. Hong and D.Y. Hu

Department of Preventive Dentistry, West China College of Stomatology, Sichuan University, China.

Objectives: To analyze the associations of salivary *Streptococcus mutans* (SM) level with caries experience and incidence in children. ***Methods*:** Five hundred 11-12-year-old children participated in this study, one year later at follow-up 485 children remained. Scores of decayed, missing or filled (DMF) indices were recorded according to WHO criteria at baseline and at follow-up. Baseline salivary SM concentrations were determined with species-specific monoclonal antibodies. ***Results*:** Pearson's correlation coefficient for SM concentration and DMFS at baseline was 0.14 (P=0.002), for SM concentration and DMFS increment was 0.22 (P<0.001), for baseline DMFS and caries increment was 0.39. Salivary SM concentration was divided into three categories (scoring 1-3 respectively): 0.01-3, 3.01-25, and $>25\times10^4$/ml. Compared with children who were classified as category 1, children in category 2 were approximately twice as likely to develop new carious lesions (relative risk=1.83), whereas those in category 3 were about three times as likely (relative risk=2.67). However, caries prediction based on salivary SM levels provided negative predictive values (0.770~0.839) much higher than positive predictive values (0.341~0.436). ***Conclusions***: Salivary SM level associates positively with caries experience, caries incidence and increment. Caries prediction solely relied on salivary SM levels is not recommended. However, such test may help to identify those at risk children who have not been affected by caries yet.

Key words: Dental caries; risk assessment; Streptococcus mutans

Introduction

Oral diseases qualify as major public health problems owing to their high prevalence and incidence in all regions of the world, and as for all diseases, the greatest burden of oral diseases is on disadvantaged and socially marginalized populations (Petersen, 2003). Over the past few decades, a decline in caries prevalence has been observed in most industrialized countries as the result of a number of public health measures. However, dental caries still remains a major oral health problem as it affects 60–90% of school-aged children and the vast majority of adults (Petersen *et al*, 2005). Data from a study of representative samples of schoolchildren in the US reveal that 20% of the children have about 60% of all the caries. This well represents the skewed distribution of caries in all the high-income countries today that has led to much interest in targeted prevention and caries risk assessment (Stamm *et al*, 1991).

For years, great efforts have been made in searching for a method to predict patients' caries risk accurately. It has been advocated that a comprehensive caries assessment should consider many factors, such as past and current caries experience, diet, fluoride exposure, presence of cariogenic bacteria, salivary status, behavioral and physical factors and so on (Fontana and Zero, 2006). Among all the risk factors that have undergone various studies, *Streptococcus mutans* (*S.mutans,* SM) has traditionally captured much of researchers' attention. Given the unquestioned role of *S. mutans* in cariogenesis, it was hypothesized that level of *S. mutans* in saliva or plaque samples associates positively with caries experience and incidence thus could be used to predict future caries onset.

In most studies that intended to explore such associations (Alaluusua *et al*, 1989; Beighton *et al*, 1996; Chosack *et al*, 1988; Ishikawara *et al*, 2006; Kopycka-Kedzierawski and Billings, 2004; Seki *et al*, 2003; Teanpaisan *et al*, 2007; Zhang *et al*, 2007), techniques widely used for detection and quantification of *S. mutans* were either bacterial culture with selective media (such as MSB) or chairside cultural tests (such as Dentocult SM, Dentocult SM Strip mutans) which are commercially available. However, such culture-based quantification methods could only detect cultivable cells, which would result in inevitable underestimations (Gu *et al*, 2002; Shi *et al*, 1998). In addition, specificity for such tests is questionable as the selective media also allow growth of many bacteria other than *S. mutans* which could produce false-positive results. Moreover, bacterial growth and recovery rates for these methods do not agree with each other, so caution is indicated when comparing results between studies that employ different cultural methods for enumeration (Gu *et al*, 2002; Hildebrandt and Bretz, 2006).

In contrast, detection of *S. mutans* with species-specific monoclonal antibodies exhibits great specificity as well as sensitivity and is able to detect *S. mutans* in saliva samples accurately (Gu *et al*, 2002; Shi *et al*, 1998). Thus, it was the objective of the present study

Correspondence to: Dr. Xiao Hong, Department of Preventive Dentistry, West China College of Stomatology, Sichuan University, South Ren-min Road 3rd Section 14#, Chengdu, 610041, Sichuan Province, China. Email: hongxiao81@126.com

to determine the actual level of *S. mutans* in saliva in children, with the great accuracy offered by monoclonal antibodies, and correlate it with caries experience as well as caries incidence.

Materials and Methods

Ethical approval for the study was obtained from the Ethics Committee of Sichuan University. Five primary schools were randomly selected in Chengdu city. Participation in the study was voluntary and signed consents were obtained from parents prior to the examination.

Eligibility criteria were: overall healthy pupils who are
a) interested in and willing to participate in the study;
b) not undergoing orthodontic therapy or planning to receive orthodontic therapy in the following 12 months;
c) not currently taking, or have taken in the past 2 weeks, medicine especially antibiotics;
d) with no primary teeth remaining in the oral cavity.

Caries examination

Dental caries was examined twice, at baseline and one year later, by a single calibrated examiner (kappa value=0.91) with fiber-optic oral mirror. A standard dental probe was used to remove plaque or food debris and facilitate caries detection if necessary. Scores of decayed, missing or filled (DMF) indices were recorded according to the WHO criteria. No radiographs were taken.

Saliva samples collection and processing

Prior to dental examination, 0.5 ml of unstimulated whole saliva was obtained from each child by asking them to expectorate into sterile disposable plastic cups. The saliva samples were fixed with 1% formaldehyde immediately after collection and stored at room temperature for no more than one week before laboratory processing (Gu *et al*, 2002). Saliva samples were collected at least one hour after meal or consumption of sugar-containing soft drinks.

After being vortexed for 30 seconds, 10μl of each saliva sample was mixed with 10μl species-specific monoclonal antibodies against *S. mutans* (SWLA 1.2, by courtesy of Professor Shi Wenyuan at University of California, Los Angeles) and incubated at room temperature for 30 min, then mixed with 1μl fluorescein isothiocyanate (FITC) conjugated goat anti-mouse IgG antibody (Sigma, St. Louis, MO) and again incubated at room temperature for 30 min. Subsequently, the mixture was examined using fluorescent microscopy to determine the concentration of SM in saliva. Laboratory processing for saliva samples was performed by a single calibrated examiner. Approximately 10% of the total sample (every ten sample) was reexamined one week after the first examination to assess reproducibility.

Data analysis

Data collected were analyzed with SPSS 13.0 for Windows.

Caries prevalence at baseline and follow-up were compared using the Chi-square test. Caries experience at baseline and follow-up were compared with paired-samples t test. Simple correlation analyses were done to assess associations between salivary SM level and caries experience, between SM level and caries increment and between baseline caries experience and caries increment.

Salivary SM concentration was divided into three categories: 0.01-3, 3.01-25, and $>25\times10^4$/ml. Caries experience, incidence and increment between these categories were compared using Chi-square test and one-way analysis of variance (ANOVA). Differences in caries experience and increment between two adjacent categories were compared using Student-Newman-Keuls (SNK) test. Validity of SM estimation for caries incidence were analyzed by calculating sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and relative risk (RR). Reproducibility for SM examination was done by calculating intra-class correlation coefficient (ICC). The level of statistical significance was set at 0.05.

Results

At baseline, five hundred children aged 11 to 12 volunteered in this study. One year later, a total of 485 students (246 boys and 239 girls, 97.0% of the original sample) completed the study. Reasons for dropout were transfer to other schools and absence from school at the day of follow-up examination. Reproducibility for SM examination was 0.81.

Caries status of 485 children at baseline and at follow-up are summarized in Table 1. At baseline, 34.2% of children were affected by caries. The mean DMFT was 0.75 (SD=1.28), mean DMFS was 1.26 (SD=2.44). We found that 20% of the children had about 60% of all the carious teeth, and 80% of all the caries concentrated on about one third of the study population. One year later at follow-up, the proportion of the caries-affected children rose markedly to 45.4% (Chi-square test, p=0.001), with the corresponding caries experience increasingly

Table 1. Caries experience of 485 children at baseline and follow-up

	Prevalence	*DMFT (SD)*	*DMFS (SD)*
Baseline	34.2%	0.75(1.28)[a]	1.26(2.44)[b]
Follow-up	45.4%	1.04(1.69)[a]	1.67(2.83)[b]

Paired-samples t test: a: P<0.001; b: P<0.001

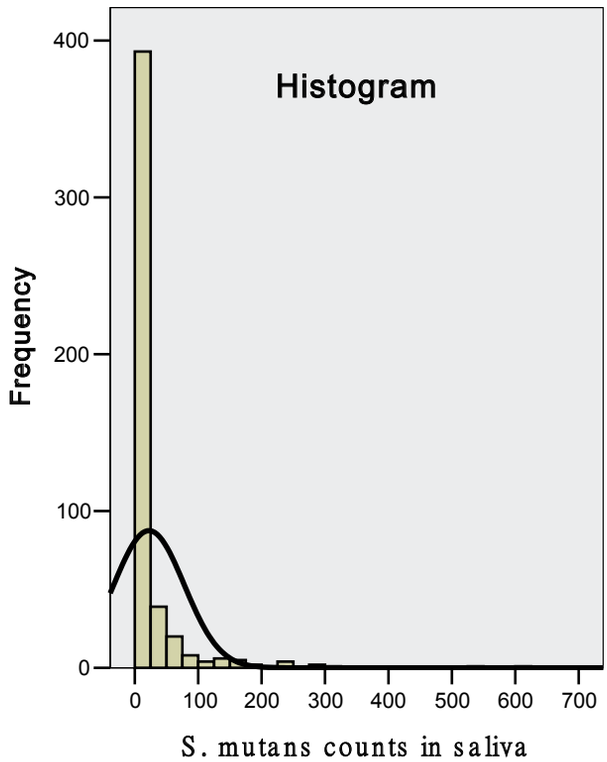

Figure1. *Distribution of salivary S. mutans concentration (×10⁴/ml) in 485 school children*

Table 2. Salivary SM category and its relation to baseline caries experience

SM Category	*Range (×10⁴/ml)*	*Number of children (%)*	*Caries prevalence (%)*	*DMFT (SD)*	*DMFS (SD)*
1	0.01-3	192(39.6)	43.2[a]	0.56(1.06)[b,d]	0.78(1.70)[c,f]
2	3.01-25	199(41.0)	49.7[a]	0.74(1.27)[b,d,e]	1.18(2.17)[c,f,g]
3	>25	94(19.4)	52.1[a]	1.17(1.60)[b,e]	2.42(3.93)[c,g]

Chi-square test: a: p=0.271
ANOVA: b: p=0.001; c: p<0.001
Student-Newman-Keuls test: d: p=0.031; e: p=0.002; f: p=0.004; g: p<0.001

Table 3. Salivary SM category in relation to caries incidence at follow-up

SM Category	*Caries incidence (%)*	*DMFT increment (SD)*	*DMFS increment (SD)*	*Relative risk (95% CI)*
1	16.2[a]	0.13(0.35)[c,e]	0.19(0.56)[d,g]	-
2	29.7[a,b]	0.35(0.58)[c,e,f]	0.50(0.89)[d,g,h]	1.83(1.10-3.05)
3	43.6[b]	0.45(0.69)[c,f]	0.65(1.11)[d,h]	2.69(1.65-4.39)

Chi-square test: a: p=0.002; b: p=0.025
ANOVA: c: p<0.001; d: p<0.001
Student-Newman-Keuls test: e: p<0.001; f: p=0.512; g: <0.001; h: p=0.331

Table 4. Two screening criteria and resulting ratios

Screening criteria	*Children classified as at risk (%)*	*Sensitivity*	*Specificity*	*PPV*	*NPV*
SM category 1/23	60.4	0.763	0.455	0.341	0.839
SM category 12/3	19.4	0.313	0.850	0.436	0.770

significantly (paired-samples t test, p<0.001). During the interval, 27.0% of the children developed new carious lesions, the average increase of DMFT and DMFS was 0.29 (SD=0.56) and 0.41 (SD=0.80) respectively. Pearson's correlation coefficient for baseline caries experience and DMFS increment was 0.39 (p<0.001).

The number of *S. mutans* in collected saliva samples ranged from 1.0×10^2 to 6.0×10^6/ml. Figure 1 gives a profile of salivary SM level among the children. A skewed distribution pattern was observed. On average, the salivary SM concentration was 2.2×10^5/ml (SD=5.5×10^5/ml), however, the level at which 80% of children harbored *S. mutans* in saliva was below 2.4×10^5/ml. Pearson's correlation coefficient for salivary SM concentration and baseline DMFS was 0.14 (p=0.002), and for salivary SM concentration and DMFS increment was 0.22 (P<0.001).

Salivary SM concentration was divided into three categories: 0.01-3, 3.01-25, and >25×10^4/ml (see Table 2). Differences in baseline caries prevalence was not statistically significant among the three categories (Chi-square test p=0.271). However, as the category score rose, baseline DMFT/DMFS, caries incidence and increment increased as well, which was statistically significant except for caries increment between category 2 and 3 (Table 2 & 3). Compared with children who were classified as category 1, children in category 2 were approximately twice as likely to develop new carious lesions (relative risk=1.83), whereas those in category 3 were about three times as likely (relative risk=2.67, see Table 3).

When SM category 2 was used as the screening criterion, the resulting sensitivity and specificity for predicting new caries during the one year interval was 0.763 and 0.455 respectively (Table 4). When it comes to SM category 3, the specificity rose markedly to 0.850 which means 85.0% of children would be correctly diagnosed as at low caries risk. However, this was at the expense of sensitivity, by which only 31.3% of children would be correctly recognized as at high risk of caries attack.

Discussion

During the last thirty years, the industrialized nations have experienced a dramatic decline of caries prevalence. However, the decline did not occur to the population evenly, which results in a skewed distribution of caries that we observe today (Stamm *et al*, 1991). Findings of the present study well represent a profile of such skewed distribution pattern of dental caries. We found that 20% of the children had about 60% of all the carious teeth, and 80% of all the caries concentrated on about one third of the study population. If the children at high risk could be identified before lesions develop and receive targeted prevention programs, considerable benefit could be achieved.

In order to achieve a successful application of such high risk strategy, there are three basic prerequisites, one of which is to identify those at higher risk precisely (Hausen, 1997). For years, researchers have spent painstaking efforts in testing various methods trying to make the identification of high risk populations accurate.

As the principal role of *S. mutans* in caries initiation has been widely accepted, the associations between its level in saliva or plaque, caries prevalence and development have undergone extensive investigations. Most of the studies found positive correlations between *S. mutans* level and caries experience (Alaluusua *et al*, 1989; Beighton *et al*, 1996; Chosack *et al*, 1988; Ishikawara *et al*, 2006; Teanpaisan *et al*, 2007; Zhang *et al*, 2007), as well as caries development (Chosack *et al*, 1988; Kopycka-Kedzierawski and Billings, 2004; Seki *et al*, 2003; Teanpaisan *et al*, 2007). There were a few exceptions too (Ishikawara *et al*, 2006; Zhang *et al*, 2007). Taken into consideration the intrinsic drawbacks of the enumeration methodologies used that heavily relied on bacterial cultures, it could be possible that the real situation was to some extent veiled.

In contrast, species-specific monoclonal antibodies exhibit great sensitivity as well as specificity and can be used to provide accurate quantification of SM in saliva samples (Gu *et al*, 2002; Shi *et al*, 1998). Such characteristics offer good opportunity for caries risk assessment (Shi *et al*, 1998). The present study evaluated the associations of salivary SM level with caries experience and incidence in children with one year follow-up. We found the correlation between SM level and baseline caries experience or incidence was, although statistically significant, modest (not exceeding 0.2 and 0.3 respectively). In a previous study, Beighton *et al* (1996) assessed 328 children aged 12, *S. mutans* level was determined by spreading stimulated saliva samples to mitis-salivarius agar supplemented with bacitracin and sucrose. A somewhat stronger correlation between SM level and caries experience (0.228-0.278) was found. However, due to the different enumeration methodologies used, direct comparison between results obtained from the present study and other investigations was deemed inappropriate.

The three SM categories corresponded well with baseline caries experience, caries incidence as well as caries increments. However, screenings solely based on SM categories offer higher negative predictive values than positive predictive values. This means a low SM category accurately predicts low caries risk, but people with higher SM categories do not necessarily develop new caries.

Unfortunately, such frustrations (NPV usually exceeds PPV in caries prediction) for dental researchers struggling to make accurate targeting with a variety of variables, including those comprehensive prediction models, were not uncommon. Therefore, difficulties are not unexpected.

Here one might ask how the three SM categories were decided? In the literature, there are already a lot of studies which analyzed the association between SM level in saliva and plaque with caries development. Unfortunately for us, few of them employed monoclonal antibodies to make SM quantification.

It is suggested that risk group whose size exceeds 30% of the population is unworkable (Hausen, 1997). Considering the huge population size in China, it is decided that the highest SM category comprise about 20% of the total sample. After extensive explorations on data we gathered, the three SM categories exhibited the highest correlation with baseline caries experience and caries increment. Thus it was decided that the three categories and the resulting ratios be presented as above. However, these cut-off points which resulted from cal-

culations are strongly recommended to be subjected to further investigations.

With the widespread use of topical fluorides around the world, not only is the caries prevalence declining nowadays, but also is caries progressing much slower than the past. In the sense, the relatively short follow-up of 12 months might not be enough to produce a significant DMFT/DMFS increment. Therefore, it could be possible that some of the children classified into higher SM categories might manifest new carious lesions beyond the observation span, in contrast to those in lower SM categories. This might account for the low correlation coefficients, PPV and RR achieved in this study to some extent. An extended follow-up period of more than two years could offer a better chance for caries evaluation.

On the other hand, however, even within the same observation span, the correlation between baseline caries experience and caries incidence was relatively stronger (nearing 0.4). Furthermore, if simple regression analysis was to be carried out by using baseline caries experience as the explanatory variable and the caries increment as the dependent variable, an adjusted R^2 value (the explained variance) of 0.153 would be achieved. Entering the salivary SM category into this model would result in a marginal, although statistically significant, additional adjusted R^2 value of 0.031. This was in good accordance with the literature where it was concluded that past caries experience served as the most powerful caries predictor (Fontana and Zero, 2006; Hausen, 1997; Stamm *et al*, 1991).

Because caries is a multifactorial disease, no single test can take into consideration all the causative factors and thus be solely relied on to predict an individual's susceptibility to caries accurately. Bacteriological tests are not exceptions (Fontana and Zero, 2006; Hausen, 1997; Stamm *et al*, 1991; Reich *et al*, 1999). Based on findings and within the limitations of the present study, caries prediction solely based on salivary SM examinations is not recommended.

What was interesting is that the three SM categories did not differentiate baseline caries prevalence, as each category was nearly equally comprised of caries-free and caries-affected children. This finding indicated that prediction based on these SM categories was not affected by baseline caries prevalence; thus could be used to aid caries risk assessment when the baseline caries experience was absent. In other words, these categories may help to identify those at risk children who have not yet been affected by caries.

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