DOI: 10.1111/j.1365-263X.2008.00941.x

Clinical, salivary, and bacterial markers for caries risk assessment in schoolchildren: a 4-year follow-up

LEONOR SÁNCHEZ-PÉREZ[1], JORDAN GOLUBOV[2], M. ESTHER IRIGOYEN-CAMACHO[1], PATRICIA ALFARO MOCTEZUMA[1] & ENRIQUE ACOSTA-GIO[3]

[1]Professor, Area for Research in Clinical Sciences, Health Care Department, Metropolitan Autonomous University (UAM), Xochimilco Unit, [2]Professor, Man and His Environment Department, Metropolitan Autonomous University (UAM), Xochimilco Unit, [3]Professor, Division of Postgraduate Studies and Research, School of Dentistry, National University of Mexico

International Journal of Paediatric Dentistry 2009; 19: 186–192

Background. In Mexico, there is a high prevalence of dental caries and large groups of children still show extensive untreated dental damage.
Aim. This study aims to evaluate, in a cohort of 6-year-old Mexican children, the relationship between caries increment at 4 years and the following caries risk markers: fissure morphology, caries experience, salivary flow rate, Snyder test results, and mutans and lactobacilli counts.
Design. To predict new caries lesions in 110 schoolchildren, clinical, salivary, and bacteriological caries risk markers were used, including fissure morphology, caries experience, salivary flow rate, Snyder test, and *Streptococcus mutans* and lactobacilli counts. To determine the validity of these markers, the baseline data were compared with the caries increment after 4 years.
Results. The risk model's capacity to predict caries was moderate (specificity 79.6% and sensitivity 78.6%). Caries experience ($P = 0.0001$), Snyder test ($P = 0.002$), and fissure morphology ($P = 0.024$) had the strongest association with caries increment. Salivary flow rate, lactobacilli, and *S. mutans* counts did not contribute significantly to the prediction of caries lesions in these children.
Conclusion. In addition to the initial caries experience, tooth morphology and Snyder test proved to be useful predictors for caries. These three risk markers may be particularly useful in targeting caries prevention efforts in developing countries.

Introduction

In the last 30 years, caries risk assessment has centred on the analysis of bacteriological, salivary, and clinical markers that can be used as risk predictors[1]. The most commonly used markers are mutans streptococci 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].

Risk assessment studies have shown that past caries experience has the highest association with caries increments[4]. Bacterial markers, particularly those employing rapid assays, tend to have low sensitivity and high specificity[5] and, therefore, have poor positive predictive values, probably induced by the choice of culture medium and type of sample (saliva versus plaque) used. On the other hand, mutans counts from plaque on TSY20B are correlated with caries and explain a significant 20.4% of variations in caries experience[6].

Models that include several markers have a better predictive power in caries risk assessment[3,7,8]. In a Mexican sample, lactobacilli counts and the Snyder's test (ST), which assess acid production by oral bacteria, could identify 95% of children in the lowest risk category and 84% of children in the high risk who developed caries[9].

To assess and optimize the use of the scarce resources for caries prevention 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 6-year-old Mexican children, the relationship between caries increment at 4 years and the following caries risk markers: fissure morphology, caries experience, salivary flow rate, Snyder test results, and mutans and lactobacilli counts.

Correspondence to:
Leonor Sánchez-Pérez, Calzada del Hueso 1100, Área de Investigación en Ciencias Clinicas, Edificio H 101, Colonia Villa Quietud, C.P. 04960, México D.F., Mexico.
E-mail: tlsperez@correo.xoc.uam.mx; leonor.sanchezperez@gmail.com

Materials and methods

Children

In 2001, we initiated a cohort study of 6-year-old schoolchildren from public (state funded) schools in southern Mexico City. All table salt available in Mexico City is fluoridated. In this area, the fluoride content in the water is < 0.02 p.p.m. The sample size was calculated based on caries increments recorded in other studies where caries increment was 97% in the high-risk group and 60% in the low-risk group[6]. The sample was calculated without continuity correction, with a $\alpha = 0.05$ power of 80%, using the software package Primer of Biostatistics (McGraw-Hill, NY, USA). A minimum of 60 children was required. A total of 135 children were registered in the surveyed schools, their parents were invited to participate. The parents of 110 children accepted to participate, allowing for dropouts. No data were collected from the 25 children whose parents declined to participate.

None of the children in the study group had received antibiotics in the 3 weeks before bacteriological sampling. None of the participating children was under medication for central nervous system conditions, which could affect saliva secretion and bacteriologic results. Before sampling, informed consent was obtained in writing from all parents. This study was approved by the Research Commission at the Autonomous Metropolitan University.

Caries

The caries experience (CE) index was calculated as the sum of decayed, missing, and filled surfaces (dmfs, DMFS, and dmf + DMFS), using criteria recommended by the World Health Organization[10]. Radiographs were not obtained. One calibrated examiner (Kappa 0.92, $P < 0.01$) carried out all the clinical examinations under natural light. Two groups were formed: caries-free and ≥ 1 dmf + DMFS.

Fissure morphology

Based on the degree of penetration of the periodontal probe-tine in both first permanent lower molars, fissure morphology (FM) was scored as no penetration, minimal, and deep[3,8]. To avoid bias, the same clinical researcher assessed FM.

Caries increment

To determine caries increments, the dental examination was repeated at the fourth year 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 by surface subtracting the baseline dmfs or DMFS score from the last available dmfs or DMFS score, without considering reversals. The net increment was dichotomized as 0 surface newly affected (41% of the children, $n = 39$) versus 1 or more new surfaces affected (59% of the subjects, $n = 56$).

Sample collection

Plaque sampling. Two hours after the most recent meal, bacterial dental plaque was obtained from the right lower first molar's central fissures and pits using a short sterile hypodermic needle[11]. The needle was then immersed in 2 mL of thioglycolate broth with three sterile glass beads. Plaque samples were vortexed for 30 s and used undiluted for further analysis.

Stimulated saliva flow rate

After plaque collection, the children were instructed to chew a wax tablet and to dribble into sterile graduated plastic containers for 5 min. The saliva flow rate (SFR) could then be calculated in millilitre per minute (mL/min). Samples were transported in ice within 2 h of collection, vortexed for 30 s, and diluted 1 : 1000 in isotonic saline solution prior to inoculation. To analyse SFR, data were dichotomized as < 1 mL/min and ≥ 1 mL/min.

Bacteriological procedures

Mutans streptococci counts. Pairs of TSY20B[12] plates were inoculated with 100 μL of the undiluted plaque suspension or saliva diluted (10^{-3}). The seeded samples were dispersed with a sterile

glass rod and incubated in candle jars at 37 °C for 72 h. Colonies of mutans streptococci were morphologically identified, the colony forming units per millilitre (c.f.u./mL) were counted and transformed into an ordinal scale: $\leq 10^4$ and $\geq 10^5$.

Lactobacillus counts in saliva. Pairs of plates, containing Rogosa selective *Lactobacillus* agar (DIFCO, Detroit, MI, USA) were inoculated with 100 μL of the undiluted plaque suspension or diluted saliva, as described by Rogosa *et al.*[13] and incubated aerobically for 72 h at 37 °C. *Lactobacillus* sp. colonies were counted and transformed to c.f.u./mL in ordinal scale: $\leq 10^4$ and $\geq 10^5$.

Snyder test. To assess acid production by oral bacteria, 200 μL from each saliva sample were transferred to a test tube containing 8 mL sterile Bacto–Snyder test agar (DIFCO) maintained semisolid at 45 °C. The medium was then mixed uniformly with the inoculum and allowed to solidify and incubate in an upright position at 37 °C. Medium colour was observed every 24 h for 3 days, and colour change was classified from green to yellow into 'marked', 'moderate', 'low', and 'negative' as described by Snyder[14]. A non-inoculated medium tube served as a negative control. The data were transformed into an ordinal scale: 0 = negative and low acid production and 1 = a moderate and marked acid production.

Statistical analysis

To avoid bias, dropout's data were excluded from all statistical analyses. The clinical, salivary, and bacteriological markers were characterized with descriptive statistics. Bivariate analysis was carried out using caries increment (dichotomous) and each risk markers dichotomized at baseline (Pearson's χ^2 or Fisher exact test as required). Controlling for teeth present as a possible confounding variable, multiple logistic regression analyses were performed to compare each group of initial clinical, salivary, and bacterial variables versus caries increments, as well as caries increments versus the total set of risk markers including FM, CE, SFR, ST, mutans streptococci counts (MSC), and lactobacillus counts in saliva (LBC). Odds ratios (OR) and 95% confidence intervals were calculated. Receiver Operating Characterisitc (ROC) curves were constructed for caries increment at 4 years and the risk markers for the whole model, and for each marker as a single indicator. The area under the curve was summarized by index (A_z) while a perfect prediction is obtained at $A_z = 1$. Data were analysed using STATA/SE 8.0 (STATA Corporation, College Station, TX, USA). A critical value of $P < 0.05$ was considered statistically significant.

Results

During the 4-year follow-up, 15 children dropped out from the study (14% dropout rate), mostly due to their parent's job mobility. Among these children, 67% ($n = 10$) were caries-free and 33% ($n = 5$) had caries (Fig. 1).

Of the 95 children who remained in the study, 46 (48%) were boys and 49 (52%) girls. Among them, only one had no risk factors (not exposed) at baseline, 54 (57%) brushed their teeth once at day, 33 (35%) twice at day,

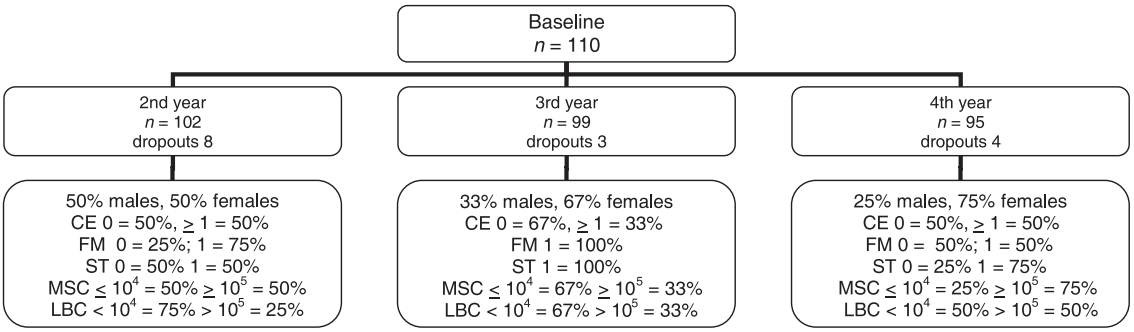

Fig. 1. Dropout diagram. CE = caries experience; FM = fissure morphology; ST = Snyder test; MSC = mutans streptococci counts; LBC = lactobacilli counts.

and 8 (8%) three times a day. No significant associations were found with the following confounding variables: gender, number of tooth brushing events per day, number of teeth present (permanent and primary), and caries increment (data not shown).

Of the 95 children, 40 (42%) were initially caries-free. After 4 years, 28 children (29%) remained without cavitated lesions and 12 developed caries. Initially, the caries prevalence at 6 years of age was 60%. At the end of the follow-up, the net caries increment was 3.7 new surfaces (Table 1). Due to exfoliation the overall CE decreased; the dmfs decreased from 5.5 to 4.6, while the DMFS increased from 0.1 to 0.6. Those 37 children with the highest caries increment contributed to 67% of the total caries increment. The distribution of caries net increment ranged from 1 decayed surface in 7% of the children to 30 surfaces in 1% of the children.

At baseline, FM (depth) scores were no penetration in 45 (47%) children, minimal in 40 (43%), and deep in 10 (11%). The SFR increased from 1.1 to 1.8 mL/min. Fifteen (16%) children had the same ST results as at baseline. Only 7 of 30 (23%) remained negative for acid production. Seventeen (18%) children had the same mutans counts as at baseline. Eleven children were initially mutans negative in plaque; among them only two remained negative. All the children had mutans streptococci in saliva. Lactobacilli were detected in the saliva from 49 (52%) of the children.

The initial CE, FM, and ST were the strongly associated with the caries increment at the fourth year ($P = 0.0001$, 0.011, and 0.0008, respectively), as shown in Table 2.

Logistical ordinal models were tested for each group of risk markers. Initial CE was the significant ($P < 0.0001$) variable in the model that included only the clinical variables. Initial acid production activity in saliva, as determined with ST, was the significant ($P < 0.0006$) variable in the salivary model. In the model

Table 1. Characteristics of the children at baseline and caries increment at 4-year follow-up.

Markers	Mean (SD) Baseline	Range Fourth year
Caries indices		
dmfs	5.5 (7.9)	4.6 (6.6)
DMFS	0.1 (0.2)	0.6 (1.5)
Clinical markers		
Fissure morphology*	0.6 (0.5)	
Caries experience	5.6 (7.9)	5.0 (7.2)
Initial salivary markers		
Salivary flow rate	1.1 (2.2)	1.8 (0.6)
Snyder test**	1.7 (1.2)	1.4 (1.2)
Initial bacterial markers		
mutans counts in $saliva_{Log10}$	7.1 (0.6)	7.4 (0.4)
mutans counts in $plaque_{Log10}$	4.3 (1.9)	5.4 (2.3)
lactobacilli counts in $saliva_{Log10}$	2.9 (2.8)	7.6 (0.7)
lactobacilli counts in $plaque_{Log10}$	0.8 (1.6)	4.7 (2.8)
Caries increment		
In primary teeth		2.9 (3.9)
In permanent teeth		0.6 (1.5)
Total increment in fourth year		3.7 (5.0)

*Mean of morphology codes 0 (no penetration in the fissure), 1 (minimal penetration < 0.5 mm), 2 (extensive penetration > 0.5 mm); dmfs, mean of decay, missing, and filled primary surfaces; DMFS, mean of caries decay, missing and filled permanent surfaces; Caries experience = dmf + DMFS; **mean Snyder test acid production scores 0 = negative, 1 = low, 2 = moderate, and 3 = marked; SD, standard deviation.

Table 2. Bivariate analysis of initial clinical, salivary, and bacteriological risk markers versus 4-year caries increment.

	No caries increment		New caries increment			
Marker	*n*	%	*n*	%	χ^2 value	*P*
Fissure morphology						
0	25	63.4	14	45.7	4.674	0.011
1	20	28.0	36	72.0		
	45		50			
Caries experience						
0	28	71.8	11	28.2	40.585	0.0001
1	7	12.5	45	87.5		
	35		56			
Salivary flow rate						
0	14	35.9	25	64.1	0.464	0.4958
1	24	42.9	32	57.1		
	38		57			
Snyder test						
0	26	66.7	13	33.3	11.167	0.0008
1	17	29.1	39	70.9		
	43		52			
Mutans counts						
0	26	66.7	13	33.3	5.228	0.0222
1	24	43.9	32	57.1		
	50		45			
Lactobacillus counts						
0	32	54.3	27	45.8	11.184	0.008
1	7	19.5	29	80.6		
59		36				

P-value using χ^2 Pearson test.

Table 3. Logistic regression model for caries risk markers and caries increment after 4-year follow up.

Marker	Odds ratios	Standard error	$P>\|z\|$	95% Confidence interval	
Fissure morphology	19.10	24.9289	0.024	1.4807	246.5058
Caries experience (initial)	12.86	7.3388	0.0001	4.2033	39.3543
Snyder test	6.23	3.7178	0.002	1.9364	20.0640
Total teeth	0.70	0.1451	0.083	0.4640	1.0486

Total teeth: total primary and permanent teeth present at first examination. Fissure morphology: no penetration (0 mm), and minimal penetration (< 0.5 mm). Caries experience: children without cavitated caries lesions (0). Snyder test values negative and low (0).

constructed with bacteriological variables, both MSC and LBC were significant ($P < 0.05$) (data not shown).

In the whole model, where FM, CE, SFR, ST, MSC, and LBC markers are included, the significant variables were FM ($P = 0.0371$), CE ($P = 0.0034$), and ST ($P = 0.0086$).

After controlling for total teeth present at the first examination, a nominal logistic regression model was constructed using the dichotomized caries increment as the dependent variable. This model identified as significant (LR $\chi^2 = 40.7$, $P < 0.000$) initial independent variables FM (OR = 19.1), CE (OR = 12.9), and ST (OR = 6.23), while *Streptococcus mutans* counts, lactobacilli, and SFR did not come out as significant variables (Table 3).

With the nominal logistic model a ROC curve was constructed, with the three groups independent of markers versus the dichotomized variables. This model had relatively moderate sensitivity (78.6%) and specificity (79.6%) to discriminate between the children who developed caries after 4 years and those who did not (area under the ROC curve = 0.88) (Fig. 2). The individual predictors had lower ROC curve areas, the highest among them was CE (area under the curve = 0.79), followed by the ST (area = 0.67), SMC (area = 0.61), and fissure morphology (area = 0.57).

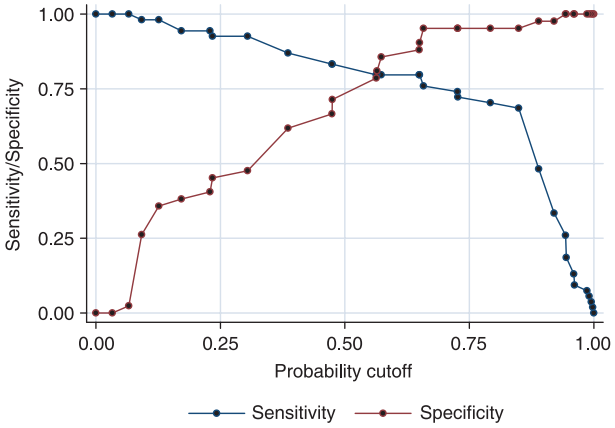

Fig. 2. ROC curve of combined prediction markers 4-year caries increment.

Discussion

An association was observed between CE, FM, and ST that can be useful for caries risk assessment. In this, as in previous studies[3,15–17], initial CE proved to be the most valuable tool to identify children at high caries risk, and together with FM and ST had an increased prediction power.

Net caries increment was used as our dependent variable because others have demonstrated this variable to be a good outcome measurement[16]. In this study, 37 children with the highest caries increment contributed 67% of the total caries increment. Several reports regarding caries risk assessment using a variety of markers show sensitivities from 55 to 87% and specificities from 70% to 90%[1,18]. The markers used in the present study showed a moderate sensitivity (78.6%) and specificity (79.6%). Caries prevalence in the studied group was higher than that observed in several developed countries[4,16,17,19–22] and even in some developing countries[23]. In addition, the caries increment was approximately four new surfaces affected.

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 CE, FM, and ST as risk markers, and this information may be used to plan for the delivery of preventive services.

ST results have been used to assess caries-inducing conditions. Our observations suggest

an association between caries increments and ST as an indicator of the growth of all aciduric bacteria since only 6% of variations in lactobacilli counts were explained by the ST. Other oral bacteria such as streptococci, staphylococci, and *Candida* can grow and produce acid in the Snyder agar[24].

All the children had mutans streptococci in saliva. The observed prevalence was similar to that previously reported in Mexican children[6] and higher than that reported in other population groups[20,22]. In this investigation on 6-year-old children, the initial mutans counts were less related to caries prevalence than reported by others[3,20,22]. We only found a moderate association between caries active surfaces and mutans streptococci[18,25], probably caused by the small number of permanent teeth present in the children of this age group.

The ROC curve for the CE, FM, and ST demonstrates the advantage in using a model that includes a combination of predictor variables versus a single marker. The values of specificity and sensitivity of each marker was lower than those of the whole model and higher than those reported[1,18,26–28]. This study is planned as a 6-year follow-up, the duration of the elementary school program in Mexico, to assess the value of the selected risk markers during the first stages of the permanent dentition, to provide information on the natural history of caries in these schoolchildren, and to provide evidence on their oral health care needs.

Batchelor and Sheiham pointed out the limitations of the 'high-risk' approach for the prevention of dental caries[29]. Nevertheless, in most developing nations caries is still a growing public health problem, and in industrialized nations, where caries is no longer pandemic, groups of children remain at risk.

In Mexico, salt fluoridation is the chosen population-based prevention strategy implemented by health authorities. This intervention has reduced caries prevalence from 5.5 to 1.94 DMFT since 1990[30]. All table salt available in Mexico City is fluoridated. Unfortunately, however, this population-based prevention is far from achieving caries eradication. Risk-based prevention may help target the scant human and material resources at the group with the highest incidence, as approximately two-thirds of the surveyed children had caries, and half of them contributed to 67% of all new caries lesions.

The results of this investigation provide the basis to develop an affordable system for caries risk assessment applicable at the individual level. It is expected that caries-prone children be identified and selected for prevention, and children at low risk could be followed up. The diagnostic value of these markers is under evaluation in other groups of children.

What this paper adds

- In this study, fissure depth and Snyder test helped to identify children with increased caries susceptibility.
- Together with initial caries experience, fissure depth and Snyder test provide a caries prediction model that may be affordable and accessible when other resources are limited.
- When the caries prevalence is high, streptococci and lactobacilli counts do not necessarily show caries predictive value.

Why this paper is important to paediatric dentists

- For allocating and optimizing the use of scarce resources available for prevention, caries risk assessment is required to identify and target patients at greater risk of developing caries.
- In developing countries, paediatric dentists must be aware that in addition to the child's initial caries experience, the lower first molar's fissure depth and the Snyder test provide valuable caries predictors.
- In caries prediction, it is important to apply more than one risk marker, because the individual predictors have lower ROC areas and show less capacity to predict risk.

Acknowledgements

The authors acknowledge the support provided at the 'Espartaco' and 'Porfirio Parra' schools, as well as the willingness of parents to provide consent for the participation of their children in this study. This research was funded by grant 3450402 from Universidad Autonóma Metropolitan Xohimilco.

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