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Discriminant ability for caries risk of modified colorimetric tests

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Abstract

Objetive: The aim of this study was to evaluate the relationship between the caries risk in children over a two-year period and their baseline caries status, salivary levels of mutans streptococci and lactobacilli, and results of the Alban test and modifications thereof using different substrates.

Study design: Ninety-five children aged 6-7 were examined in Granada (southern Spain) for dental caries at baseline and every six months. Stimulated saliva was sampled and inoculated in 7 colorimetric tests based on Snyder's medium with different sugars and polyalcohols. A mutans streptococci and lactobacilli count was performed (Dentocult SM strip® and Dentocult LB®). Caries risk proportions were contrasted against the potential predictor variables, i.e., basal caries history and salivary tests, by means of the Mantel Haenszel test for linear association, based on a chi-square distribution with 1 degree of freedom (df).

Results: Caries index, lactobacillus count and colorimetric tests showed significant, but limited, and non-different discriminant abilities. Increasing values of all predictor variables, except for Dentocult SM®, were related to increasing caries risk proportions.

Conclusion: Colorimetric test results and caries history showed similar correlation values as caries predictors.

Key words: *Alban test, mutans streptococci, lactobacilli, caries risk, schoolchildren, salivary tests.*

Introduction

There has been an overall decline of dental caries in developed countries, with the disease now concentrated among a small percentage of children (1). It is therefore increasingly important to identify caries-prone individuals.

Multiple variables have been used alone or in combination to predict an increase in caries. Prior caries history and salivary bacterial counts of lactobacilli (LB) or mutans streptococci (MS) have been correlated with caries increase (2). Various chairside techniques have been proposed for salivary bacterial counts in the clinical setting, including Dentocult SM strip (3) and Dentocult LB (4). Yet the cost of these techniques may impede their use in countries with weak economies.

Although MS are recognized as an important cariogenic plaque organism , Van Houte (5) proposed that a group

of "low-pH" microorganisms, including non-mutans streptococci, LB, *Bifidobacterium* strains and *Actinomyces*, may be involved in cariogenesis. Kleinberg (6) developed a mixed theory, including non-specific and specific hypothesis, to shed light on the microbial etiology of dental caries.

Before the development of quantitative tests, less costly colorimetric tests were used. The Alban test (7) determines the acidogenic capacity of the bacteria present in saliva, using glucose in a low pH medium that incorporates a pH indicator. However, although glucose and fructose have been found to be somewhat less cariogenic than sucrose (8), all three sugars are readily metabolized by many oral bacteria, including MS and LB. Other substrates, such as maltose (9), galactose (10), inverted sugar (11), and sorbitol (12), appear to be less cariogenic. The use of less cariogenic sugars and polyalcohols as the only fermentable substrate for salivary bacteria may exert a selective or discriminatory effect against bacteria that cannot metabolize them or can only do so with difficulty.

The aim of this study was to compare, in 6-7-yr-old children, the discriminant ability for caries risk over 2 years of different factors measured at baseline: caries status, salivary levels of MS and LB, the original Alban test and six modified Alban tests with different sugars and polyalcohols.

Materials and Methods

-*Study sample and data collection*

This study was conducted in Granada (Southern Spain). Five primary schools were randomly selected from all 21 elementary schools in the northern section of the city, with a middle or lower-middle socio-economic level. There was no school-based preventive (sealants, brushing, fluoride rinse or tablets, etc.) or restorative dental program, and none was implemented during the study period. The informed written consent of parents was received for 112 children (91.05%) out of the initially selected population of 123. The present results refer only to the 95 children who were followed up for 24 months. This sample size, according to MedCalc v.9.4.2.0 (MedCalc Sofware, Mariakerke, Belgium), allows one to detect a difference of 0.15 in paired comparisons of areas under the ROC curves (discriminant ability) of the considered variables (basal caries, MS, LB, and Alban tests), with a 0.05 alfa error, and 0.20 beta error; the value considered for priori non-parametric correlations between the two variables being compared was 0.60 (based on our previous experience with these variables), for both the caries risk and the non-caries risk children. The study was approved by the Ethics Committee of the School of Dentistry of the University of Granada.

The birth date and gender of the children were recorded, along with their socio-economic status (13). Caries was scored at baseline and 6 monthly following 1988 WHO criteria (14) by a principal and a secondary examiner to assess agreement.

-*Microbiological procedure*

Stimulated mixed saliva samples were obtained from all children at baseline. Saliva was stimulated by chewing a piece of sterile paraffin wax (approximately 1g) and swallowing for 2 min. The saliva was then collected during a 5-min period in a sterile universal bottle, which was rapidly transported to the laboratory for processing within 30 min of its collection.

We used two commercial tests, Dentocult SM strip® (3) and Dentocult LB® (4) (Vivacare/Vivadent, Liechtenstein), and seven colorimetric tests prepared in our laboratory based on the same Snyder agar base medium, of which 5 cc was dispensed in 100x16 mm tubes and sterilized at 120ºC. While maintaining the tubes at 50º C in a water bath, a filtration-sterilized solution of glucose, sucrose, maltose, mannose, galactose, sucrose + sorbitol or glucose + fructose substrates was added to the tubes in sufficient quantity for the molarity of the medium to meet that of the Alban test. All media were refrigerated at 4º C for a maximum of one week, and removed from the refrigerator 15 min before their use, allowing inoculation at room temperature.

The Dentocult SM strip® method and Dentocult LB® test were performed according to the manufacturers' instructions. The other tests were inoculated with stimulated saliva samples. For the Alban test and its modifications, a calibrated pipette was used to place 0.2 ml of saliva on the surface of the medium. All tests were incubated at 37ºC, and test readings were made at 48 hours for Dentocult SM strip® and at 96 hours for Dentocult LB® using the scale provided by the manufacturers, and at 72 hours for the Alban test and its modifications, using a linear scale from 0 to 4 according to the depth of color change from the original green to yellow. Results refer only to those at 48 hours for MS, at 96 hours for LB, and at 72 hours for the colorimetric tests. All test readings were made by the same examiner, who was blind in relation to the composition of the colorimetric tests, and 25% of the tests were randomly selected for a second reading by the principle examiner and a reading by a second independent examiner to determine the intra- and inter-observer agreement.

-*Statistical procedure*

Reliability was analysed using the Kappa test for the caries findings and the intra-class correlation coefficient (ICC) for the salivary test results, assessed according to the Landis and Koch scale (15).

For the discriminant ability analysis, caries risk was defined as developing at least one new caries (in permanent or deciduous dentition) during the 2-year period, detected in any one of the biannual visits. Caries risk proportions were contrasted against the potential pre-

dictor variables, i.e., basal caries history and salivary tests, by means of the Mantel Haenszel test for linear association, based on a chi-square distribution with 1 degree of freedom (df) (16). The areas under the ROC curves were calculated —together with their standard errors, for the basal caries history and all the salivary tests— using the Wilcoxon test (17), and were compared by pairs according to the method proposed by Hanley and McNeil (18).

Results

Out of the 112 children originally included in the study, 16 (15.17%) were lost in the follow-up. There were no significant differences in the baseline variables analyzed between these children and those who completed the study (results not shown).

At baseline, the mean age of the study population was 6.71 years (range, 6-7 years); 53.7% were male, and the socio-economic distribution was as follows: 1.2% high, 8.3% high-middle, 16.7% middle, 61.9% low-middle and 11.9% low. The mean (SD) of decayed and filled deciduous teeth and surfaces were 2.21(2.9) and 4.04(6.72). For only permanent first molars the mean (SD) of decayed and filled teeth and surfaces were 0.47(0.99) and 0.58 (1.28), respectively.

The inter-observer agreement results for caries scoring were 0.796 at baseline, 0.809 at 6 months, 0.968 at 18 months, and 0.968 at 24 months. The intra-observer agreement results were 0.984 at baseline and 0.991 at 12 months.

The inter- and intra-observer agreement for the saliva tests measured using the ICC varied from 0.73 to 1.00, considered adequate according to the Landis and Koch (15) scale.

Increasing values of all predictor variables, except for Dentocult SM®, were related to increasing caries risk proportions (Table 1). All the variables showed significant discriminant ability for caries risk, with 95%-CI or their areas under the ROC curve above the null value of 0.50. The discriminant ability of the predictor variables remained between 0.640 and 0.744, with no paired significant differences between them (Table 2).

Discussion

The objective of this study was to compare the discriminant ability for caries risk of colorimetric salivary tests, basal caries status and salivary levels of MS and LB. As the efficacy of the tests can be influenced by a history of caries and other etiological and protective factors, we emphasize that our study population could be considered at medium or high risk of caries: their baseline caries values were higher than the Spanish mean for their age, with many untreated cavity lesions (decayed/decayed+filled teeth=0.0995); and their drinking water was not fluoridated.

We used the WHO caries criteria, which diagnose lesions in dentine and provide for high validity and reliability. The interpretation of the colorimetric tests may be less reliable than chairside techniques for MS and LB counts, although we obtained good inter- and intra-observer agreement using previously trained and calibrated examiners.

Interestingly, unlike other authors (19) we found increasing values for the Dentocult SM® test were unrelated to increasing caries risk proportions at 24-months, although differences in subject age, disease criteria, and methodology hamper comparisons among studies. It is not unusual, however, to find in the literature studies where MS counts show no real utility in the prediction of caries risk (20), even among schoolchildren (21).

Our results have various possible explanations. The test we used to determine saliva levels was the Dentocult SM® strip, whose composition is based on the selective medium MSB. Growth of non-MS has been described in this medium (22), and there is an underestimation of MS (23). The problems posed by MSB medium may be greater in chairside techniques. Despite the convenience of their use, they have been reported to underestimate Streptococcus mutans, and they are not effective for isolating colonies of less than 103 (24). Using the Dentocult SM strip®, we found that some colonies did not remain adhered to the plastic spatula, which could lead to an underestimation of the counts despite the good inter- and intra-observer agreement. MS counts present further drawbacks. Although the presence of MS is probably necessary or important for the development of caries lesions, high MS levels may not necessarily produce the disease, due to clonal variations with different levels of cariogenicity or the presence of protective factors. This may explain why some authors obtained higher negative than positive predictive values (25).

In contrast, increasing values of LB count, dft index and all of the colorimetric tests were related to increasing caries risk proportions. The association of LB count and caries risk has been observed previously (19, 20). The results of the dft index were predictable, since most studies have confirmed that a prior history of caries has a stronger correlation with caries increase as compared with salivary bacterial counts (26).

Colorimetric tests measure the potential of salivary microorganisms to produce a lowering of pH in a medium whose initial pH is low (4.8–5.1). As mentioned above, besides MS, numerous low-pH, non-MS organisms are involved in cariogenesis (5), including oral streptococci species capable of acidogenesis at a low pH (<4.4). Alongside these, other types of low-pH organisms exist in plaque, such as LB and even *Bifidobacterium* strains and *Actinomyces*. The colorimetric tests would reflect the capacity of producing acid at low pH of all the bacteria with that ability, and not of one specific bacteria.

Table 1. Association of studied variables at baseline with caries* risk in 24 months.

Variable Value	No. of children in interval	Caries risk proportions	p-value‡
dft†			0.013
0	43	0.744	
1	10	0.700	
2	11	0.818	
≥3	31	0.968	
Alban glucose (72 hours)			0.003
0	23	0.652	
1	30	0.767	
2	23	0.957	
3	15	0.933	
4	4	1.000	
Alban sucrose+sorbitol (72 hours)			0.005
0	24	0.625	
1	34	0.853	
2	25	0.880	
3	9	1.000	
4	3	1.000	
Alban glucose+fructose (72 hours)			0.005
0	24	0.667	
1	31	0.774	
2	21	0.952	
3	16	0.938	
4	3	1.000	
Alban mannose (72 hours)			0.002
0	24	0.625	
1	39	0.821	
2	20	0.950	
3	10	1.000	
4	2	1.000	
Alban sucrose (72 hours)			0.007
0	26	0.654	
1	32	0.844	
2	23	0.870	
3	12	1.000	
4	2	1.000	
Alban maltose (72 hours)			0.002
0	22	0.636	
1	29	0.793	
2	24	0.875	
3	16	1.000	
4	4	1.000	
Alban galactose (72 hours)			0.010
0	24	0.667	
1	33	0.818	
2	27	0.889	
3	9	1.000	
4	2	0.800	
Dentocult SM strip (48 hours)			0.466
0	45	1.000	
1	11	0.818	
2	27	0.815	
3	12	0.917	
Dentocult LB (96 hours)			0.043
0	58	0.759	
1	13	0.846	
2	8	1.000	
3	16	0.938	

*: When any new caries in permanent or deciduous teeth is detected during 2 years with 6-month examinations.
†: Decayed and filled deciduous teeth. ‡: Mantel-Hanszel test for linear association χ^2. For the dft variable it was calculated with the original values (not collapsing dft≥3).

Table 2. Discriminant ability of studied variables* at baseline with caries risk† in 24-months. Areas under the ROC curves.

Variable	Area±se§,θ	95%-CI¥
dft‡	0.674±0.062	0.552-0.796
Alban glucose (72 hours)	0.728±0.063	0.605-0.851
Alban sucrose+sorbitol (72 hours)	0.714±0.066	0.585-0.843
Alban glucose+fructose (72 hours)	0.716±0.064	0.591-0.841
Alban mannose (72 hours)	0.744±0.059	0.628-0.860
Alban sucrose (72 hours)	0.706±0.065	0.579-0.833
Alban maltose (72 hours)	0.735±0.059	0.619-0.851
Alban galactose (72 hours)	0.694±0.066	0.565-0.823
Dentocult LB (96 hours)	0.640±0.067	0.509-0.771

*: Dentocult SM strip has been excluded due to lack of linear tendency (see Table 1).
†: When any new caries in permanent or deciduous teeth is detected during 2 years with 6-month examinations.
‡: Decayed and filled deciduous teeth, with the last category collapsed (dft≥3) (See Table 1).
§: Areas and standard error calculated according to Hanley & McNeil [*].
θ: No paired comparison was statistically significant according to the method of Hanley & McNeil [*].
¥: 95% Confidence Intervals. All are significantly higher than 0.500.

All those predictor variables that showed association with caries risk had areas under the ROC curve, indicating significant discriminant ability for caries risk —all confidence intervals are above 0.5, and there are no significant differences between them. Yet our finding of areas in the range of 0.640 and 0.744 (Table 2) points to a limited discriminant ability that should be interpreted as "fair", and may reside in limitations of the tests themselves; for instance, they do not provide information about other cariogenic characteristics such as the ability to synthesize intra- and extracellular storage polysaccharides (27). Furthermore, caries is a multifactorial disease, influenced by fluoride, diet and other factors.

The fact that variables predicting a risk of caries share information among themselves may explain the lack of differences seen among the discriminant ability of the tests studied here. MS and low-pH bacteria in general are a consequence of diet, and in the presence of a weak host these two variables are the main factors that determine caries lesion development. The children in our study had untreated open caries, which can act as a reservoir for cariogenic microorganisms (28). Therefore, the colorimetric tests may reflect to some extent both the microbiological risk and the baseline caries situation.

Although we had surmised that the different substrates of the colorimetric tests might exert a selective or discriminatory effect against bacteria that cannot metabolize them, these tests revealed no differences among them despite the different levels of cariogenicity of the substrates. Glucose and fructose are somewhat less cariogenic than sucrose (8), as are maltose (9), galactose (10), and inverted sugar (11). The sorbitol-sucrose combination even reduces acid production from sucrose and from dental plaque in vivo (29). A great number of bacteria form part of the biofilm of bacterial plaque, some of them largely unknown (30) and currently unculturable.

Caries prediction remains a complex scientific problem that is far from resolved. It can be concluded from the present study that results of the Dentocult LB® test, dft index and colorimetric tests showed significant, but limited, and non-different discriminant abilities in differentiating between children developing or not developing new caries in the period of two years. One advantage of the colorimetric tests, however, is their low cost compared with Dentocult SM® and LB®, enabling their utilization in countries with weak economies. Greater knowledge of the etiology of caries would further the development of new forms of assessing the presence of protective factors, and of predicting future caries in particular groups and regions.

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