The sensitivity and specificity of a colorimetric microbiological caries activity test (Cariostat) in preschool children

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Abstract

The purpose of this study was to test the ability of a colorimetric test, Cariostat® (Sankin, Tokyo, Japan) to identify preschool children with dental caries. Three- to 5-year-old children (N = 153) from seven preschool programs in Saskatoon, Canada, were examined for dental caries using a mirror and explorer. A caries severity index (csi) was calculated for each subject. In all subjects buccal plaque samples were obtained, incubated, and scored as per the manufacturer's instructions for the Cariostat test. In 98 of 153 children whole stimulated saliva was also collected and population counts of Streptoccocus mutans *(CFU/ml of saliva) were determined. The test group exhibited mean dft = 1.03, mean dfs = 1.53, and mean csi = 0.46 while 66.67% of subjects were caries free and had no restorations. The* S. mutans *count was found to be significantly correlated (P < 0.001) with dft, dfs, and csi. Significant differences (P < 0.001) were found between Cariostat groups with respect to dft, dfs, and csi. The sensitivity of the Cariostat test was found to be 98% while the specificity was found to be 14%. (Pediatr Dent 16:276–81, 1994)*

Introduction

Considerable time and effort has been spent on developing tests to identify individuals at risk for developing dental caries. Once identified, susceptible individuals could be placed on intensive programs to prevent dental caries from developing.

Even though dental caries is known to be a multifactorial disease process, the vast number of caries susceptibility tests developed so far are based on the microbiological aspect of dental caries. Hadley[1] developed a method for the selective growth of lactobacilli that allowed colonies of lactobacilli to be counted and caries activity to be estimated. Snyder[2] developed a simple colorimetric test to estimate the reactive numbers of lactobacilli in saliva. A similar colorimetric test of acid production in sucrose was developed by Rickles.[3] Alban[4] developed a colorimetric microbiological test similar to the Snyder test. Nevitt[5] and Hardwick[6] reported using aqueous methyl red as a possible indicator for identifying caries-susceptible teeth.

Recent microbiological tests have concentrated on estimating *Streptococcus mutans* levels in plaque or saliva. Some authors[7,8] have shown that *S. mutans* may play an important role in initiating the dental caries process. Lactobacilli, on the other hand, are considered to be important secondary invaders that contribute to the progression of dental caries.

Westergren and Krasse[9] developed a method for the quantitative estimation of *S. mutans* in stimulated saliva. In this method, serially diluted saliva was inoculated onto a selective growth medium for *S. mutans*. A simple dip-slide test based on a selective medium was devised by Jordan et al.[10] to detect and quantify *S. mutans* in stimulated saliva. As collecting stimulated saliva in young children can be difficult, Kohler and Bratthall[11] developed a practical method for estimating *S. mutans* levels in young children that used a wooden tongue depressor to collect saliva samples. In this method a saliva-contaminated wooden tongue depressor was pressed directly against a selective medium to inoculate the medium. Another semiquantitative technique, developed by Matsukubo et al.[12] used the ability of *S. mutans* to adhere to a glass surface in a sucrose-containing selective broth (mitis salivarius bacitracin broth) to determine the levels in saliva.

Numerous authors have reported positive associations between *S. mutans* levels in plaque and/or saliva and the prevalence of dental caries. This relationship has been found in adolescents[13–17] and preschool children.[18–22]

In 1975, a colorimetric microbiological caries activity test called Cariostat® (Sankin, Tokyo, Japan) was developed. This caries activity test uses a semisynthetic liquid containing sucrose, tryptose, a gram-negative bacteria growth inhibitor, and bromcresol green and bromcresol purple indicators. The test assesses acid production by cariogenic bacteria and thus indirectly assesses caries activity. The developer claims the Cariostat test is superior to the Snyder test especially in that its results are related more significantly to the increment of new carious lesions.[23]

The purpose of this study was to test the ability of the Cariostat test to identify Canadian preschool children with dental caries. Canadian preschool children have a lower caries prevalence than preschool children in Japan.[20,23,24] Since studies in Japan have shown the Cariostat test to be effective, this investigation was aimed at testing this correlation in a sample of preschool children with a lower caries prevalence than in Japan.

Methods and materials

Subjects and examinations

One hundred fifty-three preschool children (3–5 years old) from seven randomly selected preschool programs in Saskatoon, Canada, were examined for dental caries. The community water supply was fluoridated to the optimal level. Informed consent and a health history by questionnaire were obtained from the parents prior to conducting the clinical examinations. Children who had taken antibiotics within 30 days prior to the examination were excluded from the study to prevent any effects of the antibiotics on the oral microflora.

The dental examinations were carried out in the preschool classrooms using a portable dental chair and fiberoptic dental light. A dental mirror and explorer were used to examine each patient for dental caries. No radiographs were exposed on the patients. All the children examined had only primary teeth present. One author (LK) examined all the children for dental caries. The examinations took place either in the morning or afternoon.

Caries criteria and scoring

Dental caries was recorded and scored using caries criteria similar to a system developed by Shimono et al.[23] Caries were scored as follows:

S = Sound
C1 = Obvious explorer catch, no soft walls or floor observed. Stained pits or fissures in enamel.
C2 = Obvious explorer catch with soft walls, softened floor or undermined enamel.
C3 = Caries exceeds C2 and involvement of the pulp exists. Fistula, abscess or hyperplastic pulpitis must be clinically present.
C4 = Crown is destroyed by the caries process, retained roots present clinically.

Intraexaminer reliability in using this caries criteria system was tested prior to the study using a group of preschool children from one author's (LK) clinical practice.

A caries severity index (csi) was calculated for each subject to obtain a representation of the severity of caries involvement present. Scores were obtained by assigning numerical values to the various caries criteria as follows: S = 0, C1 = 1, C2 = 2, C3 = 3, C4 = 4.

The csi (caries severity index) was then calculated for each subject using the following equation:

$$\text{csi} = \frac{\text{Sum of the caries scores for all surfaces}}{\text{Number of carious, filled, or extracted teeth}}$$

Higher csi values indicated a patient who had teeth with unrestored advanced caries.

Microbiological procedures

In all subjects (N = 153) buccal plaque samples were obtained and incubated as per the manufacturer's instructions for the Cariostat test. In this method, a sterile cotton swab was run across the buccal surfaces of all maxillary teeth present. The cotton swab was then placed into the ampule supplied by the manufacturer (2 ml of the culture medium). The samples were then incubated for 48 hr at 37°C. The test medium's color change was compared with four reference tubes provided. These standard reference tubes were colored and scored as follows: dark blue (pH 7) = Zero; Green (pH 5.5) = One; Yellow-green (pH 4.5) = Two; Bright yellow (pH 4) = Three.

These pH values corresponded to the point at which the color changes occur as per the manufacturer.

The results of all the Cariostat tests were scored by a laboratory technician. Prior to the study, intraexaminer reliability in scoring these results was tested using the preschool children in the caries examination reliability pretest.

In 98 children, samples of whole saliva were obtained for *S. mutans* count. Paraffin-stimulated whole saliva was collected in an ice-chilled glass container (100 ml), and the samples were transferred to the microbiology laboratory as soon as possible. Normally, all the microbiological processes were completed within 2 hr after saliva collection. To determine the number of *S. mutans*, the saliva was first agitated by a Vortex Mixer® (American Scientific Products; McGaw Park, IL) for 30 sec. The samples were then serially diluted in phosphate buffer. From each of the dilutions, 0.1 ml of the sample was placed on mitis-salivarius agar (Difco; Detroit, MI) containing sucrose and bacitracin,[25] in duplicate. The samples were spread on the MSB agar surface using an "L"-shaped glass rod. All the MSB plates were incubated under anaerobic conditions (10% CO_2, 10% H_2, and 80% N_2) at 37°C for 48 hr.

Results

Intraexaminer reliability using the caries criteria system was 0.97. Intraexaminer reliability in reading the Cariostat test results was 0.96.

Of the 153 children (71 girls and 82 boys) the mean dft = 1.03 ± 2.09, mean dfs = 1.53 ± 4.02 and mean csi = 0.46 ± 0.94. Of the children examined, 66.7% were caries free, had a dft and dfs equal to zero, and had no restored teeth or surfaces. In the caries active patients, the mean dft = 3.09 ± 2.56, mean dfs = 4.37 ± 5.45, and the mean csi = 1.52 ± 1.15.

There was no significant difference between the mean dft for girls (dft = 1.24 ± 2.38) and for boys (dft = 0.85 ± 1.79) using the Mann-Whitney U test (P = 0.66); between the mean dfs for girls (dft = 1.96 ± 4.52) and for boys (dfs = 1.16 ± 3.52) also using the Mann-Whitney U test (P = 0.63); or between the mean csi for girls (csi = 0.57 ± 1.21) and for boys (csi = 0.36 ± 0.60) (P = 0.65).

There was no significant difference between the csi for each of the different age groups of children (Table 1). However, there were significant differences between

the dft and dfs values for each of the different age groups of children.

The mean dft and dfs values for the 3- to 4-year-old age group were significantly less than the mean dft and dfs values for the 5- to 6-year-old age group ($P < 0.05$, Mann-Whitney U test). The mean dft and the mean dfs were not significantly different between the other combinations of age groups (Table 1).

The *S. mutans* count was significantly correlated with dft ($Z = 3.71$, $P < 0.001$), dfs ($Z = 3.78$, $P < 0.001$), and csi ($Z = 3.90$, $P < 0.0001$) (Spearman rank correlation analysis).

S. mutans counts were also grouped into four categories as follows: O = < 400 CFU/ml of saliva, A = 400–10^5 CFU/ml of saliva, B = 10^5–10^6 CFU/ml of saliva, and C > 10^6 CFU/ml of saliva. The distribution of these categories with respect to the presence or absence of dental caries then was examined (Table 2). This distribution showed that 10 children (10.2%) had very low *S. mutans* counts and had caries present. Conversely, two children (2.0%) had very high *S. mutans* counts (>10^6 CFU/ml of saliva) and had no caries present. The sensitivity of the *S. mutans* count for detecting dental caries was 64% while the specificity was 71%.

The relationship between the Cariostat results and the presence or absence of dental caries then was examined (Table 3). The sensitivity of the Cariostat test for detecting patients with dental caries was 98% while the specificity was 14%.

The mean dft and dfs were significantly different ($P < 0.001$, Kruskal-Wallis test) between the four Cariostat groups (Zero, One, Two, Three). Using the Mann-Whitney U test both the mean dft and dfs were significantly different between category Zero and Three ($P < 0.01$), between category One and Three ($P < 0.01$), and between category Two and Three ($P < 0.05$) (Table 4).

Similar to the dft and dfs values, the mean csi was significantly different ($P < 0.001$) between the four Cariostat groups. Using the Mann-Whitney U test the mean csi was significantly different between Cariostat category Zero and Three ($P < 0.001$), between category One and Three ($P < 0.01$), and between category Two and Three ($P < 0.05$, Table 4).

The mean *S. mutans* count was significantly different between the four Cariostat groups ($P < 0.01$, Kruskal-Wallis test). The distribution of Cariostat categories and *S. mutans* counts is shown in Table 5.

The mean *S. mutans* counts between the morning and afternoon patients were not significantly different ($P = 0.56$, Mann-Whitney U-test).

No significant difference ($P = 0.09$, Kruskal-Wallis test) was found between the mean *S. mutans* count for the three age groups.

Discussion

In this study, the caries rate was found to be quite low, (dft = 1.03 ± 2.09, dfs = 1.53 ± 4.02), and 66.7% of the children were found to be caries free and have a dft and dfs equal to zero. Similar results were reported in

Table 1. Relationship of age to dft, dfs, and csi

Age (Years)(N)	*Mean dft (± SD)**	*Mean dfs (± SD)**	*Mean csi (± SD)*
3–4 (32)	0.34 ± 0.83	0.44 ± 1.29	0.33 ± 0.80
4–5 (81)	0.88 1.80	1.30 3.43	0.51 1.11
5–6 (40)	1.90 ± 2.93	2.88 ± 5.88	0.45 ± 0.61

* Significant at $P < 0.05$; Kruskal-Wallis test.

dft: (3–4) vs. (5–6); significant at $P < 0.05$; Mann-Whitney U test.

dfs: (3–4) vs. (5–6); significant at $P < 0.05$; Mann-Whitney U test.

Table 2. Distribution of dental caries and Streptococcus mutans counts

	Group O	*Group A*	*Group B*	*Group C*	*Total*
Caries present	10 (16.67)	5 (27.78)	11 (68.75)	2 (50.00)	28 (28.50)
No caries	50 (83.33)	13 (72.22)	5 (31.25)	2 (50.00)	70 (71.50)
Totals	60 (100)	18 (100)	16 (100)	4 (100)	98 (100)

(O = < 400 CFU/ml of saliva, A = 400 – 10^5 CFU/ml of saliva,
B = 10^5 – 10^6 CFU/ml of saliva, C = > 10^6 CFU/ml of saliva).

Sensitivity = (18/28x100) = 64%.

Specificity = (50/70x100) = 71%.

( ) = percentage.

Table 3. Distribution of dental caries and Cariostat categories

	Category Zero	*Category One*	*Category Two*	*Category Three*	*Totals*
Caries present	1 (6.25)	31 (36.05)	15 (31.91)	4 (100)	51 (33.33)
No caries	15 (93.75)	55 (63.95)	32 (68.09)	0 (0.00)	102 (66.67)
Totals	16 (100)	86 (100)	47 (100)	4 (100)	153 (100)

Sensitivity = (50/51x100) = 98%.

Specificity = (15/102x100) = 14%.

( ) = percentage.

Table 4. Relationship between Cariostat groups and caries indices

Cariostat Group	*Mean dft*	*SD*	*P-value*
Zero	0.31	1.25	
One	0.86	1.67	0.0009*
Two	1.19	2.42	
Three	5.75	3.30	
Cariostat Group	*Mean dfs*	*SD*	*P-value*
Zero	0.38	1.50	
One	1.12	2.78	0.0009*
Two	2.21	5.79	
Three	7.00	4.24	
Cariostat Group	*Mean csi*	*SD*	*P-value*
Zero	0.04	0.15	
One	0.42	0.81	0.0007*
Two	0.57	1.23	
Three	1.59	0.32	

* Significant at $P < 0.001$; Kruskal-Wallis test.

The mean dft, mean dfs, and csi are significantly different.

($P < 0.05$)(Mann-Whitney U-test) in the following combiniations of Cariostat groups: Zero vs. Three; One vs. Three; Two vs. Three.

Table 5. Distribution of S. mutans counts and Cariostat categories

Category	*Group O*	*Group A*	*Group B*	*Group C*	*Total*
Zero	9	0	0	0	9
One	42	8	7	1	58
Two	8	10	8	3	29
Three	1	0	1	0	2
Totals	60	18	16	4	98

(O = < 400 CFU/ml of saliva, A = 400 – 10^5 CFU/ml of saliva, B = 10^5 – 10^6 CFU/ml of saliva, C = > 10^6 CFU/ml of saliva).

Swedish preschool children.[21] Another study of Canadian preschool children found 37.8% of examined children to be caries free,[20] while in a group of Icelandic preschool children, 45.8% were caries free.[18]

There was no significant difference found between females and males with respect to the caries indices (dft, dfs, and csi). This is contrary to results of an Australian study of preschool children that found males to have a higher caries rate than females.[26]

The caries rate was significantly related to age. A significantly higher caries rate was found in the 5-year-old children than the 3-year-old children, which could be expected since the longer a tooth has been erupted and exposed to the oral environment, the more likely it will become carious.

In this study no significant difference was found between the *S. mutans* counts for patients examined in the morning and patients examined in the afternoon. Togelius et al.[27] also found no statistical difference in *S. mutans* counts between morning and afternoon subjects. However, other studies[28–30] have shown *S. mutans* counts to related to the time of testing.

S. mutans counts were found not to be significantly different with respect to the age of the patient. A study by Catalanotto et al.[31] found that as the number of deciduous teeth increased in young children the prevalence of *S. mutans* also increased. The greatest frequency occurred in patients with a complete deciduous dentition and contacts between the molar teeth. All the children examined in this study had a full complement of deciduous teeth so any age-related differences seen in other studies due to the eruption of deciduous teeth would not be seen in these patients.

The *S. mutans* count was significantly related to dfs, dft, and csi. These results are similar to numerous other studies that have found a relationship between the prevalence of caries and the number of *S. mutans* present in saliva or plaque.[13–15, 17–21] A definite association between a *S. mutans* concentration of more than 10^6 CFU/ml in saliva and a high dmft has been found previously in preschool children.[19] This same study also found a caries severity score to be significantly higher in children with a *S. mutans* level of more than 10^6 CFU/ml of saliva.

In this study significant differences were found between the Cariostat categories with respect to dft, dfs, and csi. There were significant differences between the dft, dfs, and csi for the first three categories (Zero, One, Two) and for category Three. These results may be interpreted to mean that patients who score in category Three have significantly higher caries rates than patients who score in the other categories. As the color change to yellow occurs at a pH of 4.0 (category three), this may mean that these patient harbor extremely high numbers of cariogenic bacteria.

Radiographs were not exposed during this investigation. As a result the reported dft, dfs and csi were probably lower than the real values. This also could have also affected the relationship between the microbiological tests and the dft, dfs, and csi. Patients with undetected interproximal radiographic caries may account for some of the cases in which patients had positive Cariostat results or high *S. mutans* counts and at the same time did not have clinical caries.

Camling and Emilson[32] investigated the Cariostat test by inoculating pure samples of *S. mutans* and lactobacilli into the test medium. Patients' plaque and saliva samples also were tested with the Cariostat test. Samples

with very low or very high numbers of cariogenic bacteria were found in all score groups. The authors concluded that the Cariostat test did not satisfactorily differentiate samples with low or high numbers of microorganisms associated with dental caries. Even with these criticisms there were positive correlations found between the degree of color change in tubes inoculated with saliva or plaque and the numbers of mutans streptococci and lactobacilli in saliva.

In this study *S. mutans* counts were significantly related to the Cariostat categories. As in the previously mentioned study,[32] very high or very low numbers of cariogenic bacteria were found in categories One and Two. No subjects in category Zero had *S. mutans* in their saliva (Table 5). In this study only *S. mutans* levels in saliva were determined. Other cariogenic bacteria (lactobacilli) may have been present in high enough numbers to result in a color change greater than would be expected solely due to *S. mutans* levels. Schroder and Ewardsson[21] found that higher predictive values were obtained when presence/absence of lactobacilli and *S. mutans* were combined as predictors of caries.

The *S. mutans* count was found to have a high sensitivity and specificity while the Cariostat was found to have a very high sensitivity but a low specificity for the detection of dental caries. Sensitivity is defined as the ability of a test to detect the presence of a disease in patients with the disease, while specificity is the ability of a test to detect the absence of a disease in patients without the disease. The Cariostat — by virtue of its simplicity and high degree of sensitivity — could be used to screen patients for dental caries. More intense preventive programs could then be established for these patients to prevent future dental caries from developing.

However, the high specificity of the Cariostat test can mean that a high number of false positive readings occurred. Some of the false positive results for the Cariostat test and *S. mutans* count could be explained by the presence of interproximal caries that was not diagnosed clinically. In both cases the number of false positive results would be reduced if radiographs were exposed to detect interproximal caries. Also in false positive patients, more intensive preventive programs may be pursued that are not truly necessary, depleting available resources.

The *S. mutans* count and the Cariostat test have been shown to be significantly statistically related to the presence of dental caries. However, the clinical significance of both tests has to be addressed. Both tests are time consuming, relatively expensive, and can have ambiguous results for certain individuals. A more effective method of screening patients for caries susceptibility may continue to be visual examination. The best known predictor of future caries susceptibility continues to be past caries history.

The Cariostat test has problems similar to other caries activity tests. It relies on one aspect of the caries process — production of acid by acidogenic bacteria — to quantify caries activity. As with the other caries activity test, it appears to have group correlation to caries prevalence. Such tests may be useful to identify susceptible subgroups within a population and to educate these patients to prevent future carious lesions. Stolpe[33] states that education and demonstration probably describe the real value of caries tests developed to date.

Further prospective studies using the Cariostat test could be done to investigate its ability to screen populations of children for caries susceptibility and observe the identified high-risk individuals for the subsequent development of dental caries.

Conclusions

In this study the following conclusions can be drawn:

1. The *S. mutans* count was found to be significantly related to the dft, dfs, and csi of the study group.
2. Significant differences were found between the four Cariostat groups with respect to dft, dfs, and csi.
3. The *S. mutans* count was found to be significantly related to the results of the Cariostat test.
4. Examination time and subject age were not significantly related to the *S. mutans* count.

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From the Archives

An Anglo reaction to a 19th century continental recycling proposal

The practical tendencies of the age have been rather unpleasantly illustrated by a paragraph which appeared in a late number of the *Gazette Médicale de Lyon*. Some of the more speculative of our modern utilitarianists propose to convert our dead friends and relatives to useful purposes. Why, they say, should such a vast quantity of organic matter as that which now fills our graveyards be allowed to go to waste? Coal is being exhausted, and, since the human carcase is capable of supplying a gas of good illuminating power, why should it not be employed to this end? ... By a process of combustion in retorts, a corpse of ordinary dimensions may be made to yield 25 cubic metres of illuminating gas, which, at a cost of 25 centimes per cubic metre, would give a value of about 8 francs for a deceased friend of about medium size. Truly, one hardly knows whether to smile at such a suggestion for its absurdity, or to reject it for its loathsomeness. It is certainly the offspring of that filthy and growing materialism which is developing itself amongst our continental neighbors.

in *Lancet*, 1867