Comparison of Two Chair-Side Tests for Enumeration of Mutans streptococci in Saliva

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Abstract

Aim: To compare the prevalence and levels of salivary Mutans Streptococci (MS) assessed with two commercial chair-side methods based on culture growth or monoclonal antibodies, respectively.

Material and methods: The study group consisted of a convenience sample of 89 adults, 23-72 years of age, referred to a maxillofacial hospital clinic with a caries history. Stimulated whole saliva samples were collected and the number of MS was assessed with the Dentocult-SM Strip Mutans (DSM) and the Saliva-Check Mutans (SCM). The outcome was compared with conventional anaerobic laboratory cultivation on selective MSB agar.

Results: The prevalence of $\geq 5\times10^5$ CFU was 21%, 62%, and 73% with the SCM, DSM and MSB-cultivation, respectively. The correlation between DSM and SCM tests was r=0.49 ($p<0.05$) but the agreement on the high levels was fair. In comparison with the MSB agar, DSM provided acceptable levels of sensitivity and specificity while the SCM displayed a poor sensitivity but a perfect specificity. Both chair-side methods were significantly related to the prevalence of active root caries lesions ($p<0.05$).

Conclusions: The two chair-side salivary tests were significantly correlated and due their high specificity, they may be sensitive enough for screening purposes and for patient-centered promotion of oral health.

Key Words: Caries, Risk assessment, Root caries, Streptococci

Introduction

High counts of salivary mutans streptococci are supposed to be a biomarker of a cariogenic environment and therefore, enumeration is incorporated into comprehensive Caries Risk Assessment (CRA) models [1,2]. The objective of CRA is to target primary or secondary preventive measures to those with the highest need and to monitor the outcome of the provided treatment. Several culture-based chair-sides tests are available for the clinician but the 2-4 day cultivation time is many times perceived as an obstacle for use. In recent years, a rapid immunological method based on monoclonal antibodies specific for *S. mutans* has been developed [3] but this advance seem to have gained relatively little attention in research. One study in preschool children concluded that the immunoassay accurately and rapidly detected *S. mutans* abundance in saliva from preschool children [4] and another paper has adopted the new method into a computer-based CRA model in adults [5]. To our knowledge, there is no head-to-head comparison available regarding simple chair-side methods for screening purposes in adults. The aim of the present study was therefore to compare the prevalence and levels of salivary mutans streptococci with two commercial chair-side methods, the Dentocult-SM Strip Mutans and the Saliva-Check Mutans. The null hypothesis was that the prevalence of salivary mutants streptococci assessed with the two methods would not differ from conventional cultivation on selective agar.

Material and Methods

The study group consisted of 89 adult patients of both sexes (41 males, 48 females), 23-72 years of age, referred to the maxillofacial unit at the Halland Hospital, Halmstad, Sweden for caries risk assessment and for planning of secondary and tertiary preventive treatment. The mean age was 51.2 years. The patients were consecutively included after informed consent and no formal inclusion criteria were employed. However, subjects with any mucosal disease or antibiotic therapy within one month prior to clinical examination and sample collection were excluded. The study had cross-sectional design and was approved by the Halland Hospital ethical committee.

Clinical examination and saliva sampling

The clinical examination, saliva collection and chair-side testing were conducted at one single occasion. All patients were examined by one single examiner using visual and tactile methods. Caries was registered according to the WHO criteria [6] and expressed as DMFT and DMFS, respectively. Presence of untreated root caries was scored on surface level according to Peterson et al. [7] as "active" (soft or leathery) or "inactive" (hard). After a thorough mouth rinse with water, paraffin stimulated whole saliva samples were collected in graded tubes during five minutes at any time between 9am and 4pm and immediately processed as described below. No restrictions concerning foods, drink, oral hygiene procedures or smoking prior to the sampling were applied.

Mutans streptococci enumeration

The levels of salivary mutans streptococci were assessed by two chair-side techniques; i) Dentocult-SM Strip mutans (DSM, Orion Diagnostica, Esbo, Finland) and ii) Saliva-Check Mutans (SCM, GC, Tokyo, Japan). As "gold standard", laboratory cultivation on selective MSB-agar [8] under anaerobic conditions for 48h in 37°C was conducted using serial dilutions in 10-fold steps in 0.05% phosphate buffer. Both chair-side methods were processed according to the instructions of the manufacturer. The plastic DSM strips were rotated 10 times directly on the tongue, withdrawn through lightly closed lips and then cultivated in a vial of selective culture broth for 48 h. The growth was scored in

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four levels (0-3) based on the density on the strip, as shown in *Table 2*. For the SCM, 250 µl of the saliva was treated with 50 µl tris-NaOH for 30 seconds and then mixed with 100 µl tris-acetate before added to the test device. The results was categorized as positive or negative based on a red line appearing after 15 minutes, indicating levels of *S. mutans* exceeding $5x10^5$ Colony Forming Units (CFU) per ml saliva. With the laboratory technique, colonies were identified based on morphology and the numbers of CFU were counted in a stereo microscope with 5-10 times magnification.

Statistical methods

All data were processed with the IBM-SPSS software (version 20.0; Chicago, Ill. USA). For comparison of proportions, chi-square tests with Yates' correction for continuity were applied (two-tailed p-values). For two-way classifications of the methods, chi-square tests were used. Sensitivity, specificity and Youden's index (sensitivity + specificity -1) was calculated against the gold standard. P-values <0.05 were considered statistically significant.

Results

The mean (SD) DMFT and DMFS values were 25.1 (6.8) and 60.5 (28.8) respectively, but the number of untreated caries lesions (the D-component) was low with a mean value of 1.1 DS (1.6). The prevalence of untreated root caries lesions considered as active was 7%. Both chair-side tests underestimated the presence of salivary mutans streptococci when compared with the conventional laboratory cultivation. The prevalence of $5x10^5$ CFU was 21%, 62%, and 73% with the Saliva-Check mutans, Dentocult SM Strip mutans, and conventional laboratory cultivation, respectively. The value for Saliva-Check mutans differed significantly from the culture based procedures ($p<0.05$). The sensitivity and specificity of the chair-side methods when compared with the laboratory evaluation is presented in *Table 1*. The DSM test displayed a reasonable high combination of sensitivity and specificity with a Youden's index of 0.68, indicating a highly useful performance of the test. On the other hand, the SCM test had a poor sensitivity but reached a perfect specificity. The proportion of correctly classified subjects (true positive and true negative) was higher with the DSM test (82%) than the SCM test (48%). The relationship between the two chair-side tests is shown in *Table 2*. The overall correlation was acceptable ($r=0.49$, $p<0.05$) but the agreement on the high levels was fair. The odds for a false negative test was 6.0 (95%CI 3.1-11.6) when a positive outcome for SCM was compared with class 2+3 of the DSM test. There was no statistically significant relationship between the salivary mutans streptococci levels, assessed by the two chair-side tests, and the total caries experience expressed as DMFS (data not shown). The mean number of root caries lesions judged as active displayed however a positive relationship ($p<0.05$) with the salivary MS counts as enumerated by both chair-side tests (*Table 3*).

Discussion

In the modern era of early detection and non-invasive caries management, there is a need for rapid and accurate biomarkers for caries activity and future caries risk. The present study was undertaken to evaluate the use of a novel chair-side test and compare it with established methods. The study group consisted of a convenience sample of adults referred to a

Table 1. *Sensitivity and specificity for the two chair-side test for salivary mutans streptococci enumeration compared with laboratory cultivation on selective MSB-agar.*

	Sensitivity	Specificity	Youden's index
Dentocult SM[a]	0.80	0.88	0.68
Saliva-Check mutans[b]	0.29	1.0	0.29

[a]positive test: class 2+3 = $\geq 10^5$ CFU
[b]positive test: detection level >$5x10^5$ C

Table 2. *Relationship between two chair-side tests in the detection of S. mutans in saliva.*

	Dentocult SM Strip mutans class[a]				
	0	1	2	3	sum
Saliva-Check Mutans					
Negative test	22	12	20	16	70
Positive test	0	0	3	16	19
Sum	22	12	23	32	89

[a]Class 0= <10^4 CFU; Class 1 = $\geq 10^4$-<10^5 CFU; Class 2 = $\geq 10^5$-<10^6 CFU; Class 3= >10^6 CFU

Table 3. *Number of surfaces with untreated active (soft and leathery) root caries lesions in adults (n=89) in relation to the levels of salivary mutans streptococci, assessed by two chair-side tests.*

Test result	n	Mean (SD)	P
Dentocult SM strip mutans			
Score (0+1)	34	0.15 (0.2)	
Score (2+3)	55	0.29 (0.4)	<0.05
Saliva-Check Mutans			
Negative test	70	0.20 (0.3)	
Positive test	19	0.44 (0.5)	<0.05

specialist clinic for assessment of future caries risk and for planning of individually targeted care. All were regularly attending private or public dental care but experienced a caries burden above average for their age group The patients exhibited a wide range of general medical conditions with frequent medication and 19% displayed signs of impaired stimulated saliva secretion (<1.0 ml/min). Thus, the present study group was likely not representative for a general adult population.

The main finding was that both chair-side methods underscored the *S. mutans* levels in comparison with the laboratory "gold standard". The method based on monoclonal antibodies displayed the lowest prevalence of positive tests (high *S. mutans* counts), 21% of all samples and for this technique, the null hypothesis was rejected. This figure was in concert with a previous report [5] and likely explained by the highly specific anti-*S. mutans* detector antibody on which the method is based [9]. The DSM corresponded better with the laboratory method which was expected since both methods are based on cultivation using the MSB medium, allowing growth of a greater variety of streptococci stains. Nevertheless, the correlation between the two chair-side methods was good and in agreement with findings of Matsumoto et al. [3] and Gao et al. [4]. The low sensitivity of the SCM method indicated that the test by far detected all patients with high *S. mutans* counts but on the other hand, no false positive tests were displayed. A high specificity may be an advantage in the screening for "less serious" non-fatal diseases, such as slowly progressing dental caries, where a slight under-treatment is preferred before a restorative over-therapy. Therefore, the new method offers advantages for the clinician as a "negative" screening tool. It is rapid and easy to use, even outside the dental office, and requires no incubator or special storage, thereby reducing labor and time. The advantage of the DSM-test is that no saliva sampling is required which is helpful when dealing with preschool children, mentally disabled patients and dry mouth elderly. Consequently, the informed clinician has to trade the higher accuracy of the test DSM test against the convenience and speed of the SCM-test, but bear in mind the both tests may underestimate the true bacterial counts.

Our observation that both chair-side tests were associated with untreated active root caries was interesting but may have occurred by chance, considering the low prevalence of such lesions in the study group. Systematic reviews have concluded that high salivary mutans streptococci counts constitute a strong risk factor for early childhood caries [10,11] albeit not strong enough for use as a single predictor [2]. The relationship of *S. mutans* to root caries in adults seems however less evident [12]. Out of eight studies on the association between *S. mutans* and root caries incidence that were identified in the review by Ritter and co-workers [12] only one was able to find a significant correlation [13]. Although the etiology and risk factors for root caries is basically the same as for crown caries, except for the higher organic component of dentin, it is important to keep in mind that *S. mutans* is considered a biomarker of a cariogenic environment rather than a direct causative microorganism for the disease.

Our present findings do not rule out the possibility that chair-side mutans streptococci estimations may have a role in the caries risk assessment of adults. The visual test results can be of didactic help in patient information and as a base for conducting motivational interviews. In any case, a comprehensive caries risk assessment should not rely on single variables but on multifactorial algorithm-based models [2,14]. Notably, one previous study has shown that the predictive ability of one such model, the Cariogram, was significantly impaired when the mutans streptococci enumeration was omitted [15]. The predictive value of the any tests or models for root caries must however be validated in prospective trials. Thus, further studies on the performance of the novel chair-side test seem justified.

Conclusions

In conclusion, the two chair-side salivary tests evaluated in this study correlated with each other but did not accurately detect the levels of salivary mutans streptococci in caries-active adults. Due to a high specificity, they may however be sensitive enough for screening purposes and for patient-centered education and promotion of oral health.

Author's Contributions

Both authors were involved in the study design. ST carried out data collection and LT performed the laboratory work. Both authors interpreted data and wrote the manuscript.

Statement of Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper. The Saliva-Check Mutans test kits were obtained free of charge from the local GC supplier.

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