ORIGINAL ARTICLE

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Reproducibility of a new caries risk test under different oral conditions

Received: 6 December 2004 / Accepted: 16 March 2005 / Published online: 25 May 2005 © Springer-Verlag 2005

Abstract The aim of the present study was to evaluate the reproducibility of the caries risk test Clinpro Cario L-Pop (3 M Espe, D-Seefeld), which measures the lactic acid produced by different bacteria of the oral flora, under stable oral conditions. In a group of healthy volunteers (n=31), the test was carried out ten times during 2 weeks. During the test period, the subjects were requested not to change their oral hygiene habits to ensure stable oral conditions. To register possible alterations of oral conditions by medication, a questionnaire was used. In subjects who did not alter their oral conditions (n=20), the reproducibility was 82.0%, with especially low score variations in subjects with low lactate signal scores. Some subjects exhibited, mainly due to colds and their medication, alterations in their oral conditions. The reproducibility in this group was 60.0%. Both reproducibility values differ significantly. In conclusion, the new diagnostic device shows high reproducibility under stable oral conditions. The lower reproducibility under altered oral conditions gives evidence that the test might reflect changes in the oral microflora following preventive interventions and thus could be used to monitor the effect of such interventions.

Keywords Caries risk assessment · Lactic acid indicator · Reproducibility · Risk monitoring

Introduction

Dental caries is a multifactorial disease, which is characterized by acid-producing bacteria and frequent exposure to

U. Schiffner (⊠) · A. Torres-Quintero Section of Preventive Dentistry, Department of Restorative and Preventive Dentistry, School of Dental and Oral Medicine, University of Hamburg, Martinistr. 52, 20246 Hamburg, Germany e-mail: schiffner@uke.uni-hamburg.de Tel.: +49-40-428032276 Fax: +49-40-428034962 fermentable carbohydrates. If the unbalanced situation of the bacterial oral flora, carbohydrate-rich diet and some internal-modifying risk factors (e.g. behaviour) occurs repeatedly, hard tissue destruction will become obvious [3, 9, 20]. Even though the aetiology of dental caries as well as the preventive principles are known, the disease is widespread within the general population [2, 15, 20, 22, 24, 26, 29]. For this reason, dentistry focuses on measures to recognize the individual caries risk as early as possible.

Common concepts of caries risk assessment are based on early recognition of risk factors, long before there is a visible hard tissue defect. In particular, mutans-streptococci or lactobacilli were shown to be strongly correlated with caries aetiology [8, 14, 17]. Factors which explain this correlation are among others the ability to produce acids rapidly and to maintain their metabolism at low pH [7, 8, 16]. Based on the knowledge of this correlation, caries risk tests have been developed evaluating the presence and amount of microorganisms like Streptococcus mutans or *Lactobacilli* spp. in saliva. Jensen and Bratthall [11] developed a chair-side method which uses a selective broth with high sucrose concentration and bacitracin. Similar tests are available for simple evaluation of salivary lactobacilli counts [12, 13]. However, in studies investigating the caries prediction based on no more than one of these tests, it was shown that a correct caries risk assessment (sensitivity) did not exceed about 50-60% of the individual cases [5, 6, 19, 23, 25, 27].

A recently developed caries risk test (Clinpro Cario L-Pop, 3 M Espe, D-Seefeld; CCLP) does not determine specific bacteria in the oral cavity but their main metabolic product lactic acid. This biochemical device determines lactic acid produced by all bacteria of the oral microflora that are capable to produce lactate. Bacterial samples are taken from the tongue dorsum using a stick with a cotton swab which is impregnated with sucrose. The sucrose is metabolised to lactate by the microorganisms rapidly. By enzymatic and chemical transversion, a colour signal is generated. The reaction is based upon the enzymatic degradation of lactic acid to pyruvate by lactate dehydrogenase and coupled to a cascade of redox indicators which gives a blue colour signal within 2 min. The darkness of this colour signal is postulated to reflect the ability of the plaque to produce acids and thus to promote the caries process [10].

Until now, there is only spare information about the usefulness of the new device to measure the actual caries risk or to predict caries. One prerequisite to determine the caries risk under specific conditions or measures is that the test is reliable. To achieve reliability, the test has to prove both good validity and reproducibility. There is one short report concerning the test's reproducibility [10] which shows a reproducibility of 90.6%. Yet, there is only little information about the specific oral conditions of the tested subjects, and there is no report about the extent in which altered oral conditions will influence the reproducibility, in the sense that individual results stay stable over repeated measurements on different days.

For this reason, the aim of the present study was to evaluate the reproducibility of the CCLP test under known oral conditions during a short period of time.

Materials and methods

Thirty-two adults who had given informed consent participated voluntarily at the study. Inclusion criteria were healthy persons who had not taken any medication nor visited their dentist 2 weeks prior to the study. The participants were recruited from a catholic student hostel in Hamburg. During each 5 days of two consecutive weeks, the CCLP test was performed according to manufacturer's instructions. Special effort was made to ensure equal test conditions each day. For this reason, the examination was conducted at the participant's home each day at the same time. Five minutes prior to testing, the participants brushed their teeth with their own toothbrush and their own dentifrice.

During the study period, the participants were asked to maintain their oral hygiene habits and to avoid all measures which could influence oral conditions. This was checked using a questionnaire.

At the beginning of the study, each subject filled out a questionnaire about oral hygiene measures, nutritional habits and smoking. To check if oral conditions were stable or had changed over the study period, each subject filled a short questionnaire (Table 1) about the last meal, last oral hygiene and the use of any medication prior to each single test. Additionally, subjects were asked if, in their opinion, there were any differences regarding their oral situation compared with the previous day.

The examination was performed by one examiner according to the manufacturer's instructions. Brand new samples of the caries risk test were used. A test swab was placed flatly on the middle of the tongue dorsum and turned back and forth with light pressure at least four times until it was well moistened with saliva. The test swab was then replaced in its blister package to allow the bacterial, enzymatic and chemical reactions to proceed. After 2 min, the test swab was removed and immediately compared with

Table 1	Short	questionnaire	to	determine	oral	conditions

When did you have your	Beforeh
last meal today?	
What kind was your meal?	Breakfast/lunch/snack
Do you smoke? When did you have your last cigarette?	No/yes, before h
What did you use to brush your teeth just before this test?	Own dentifrice/someone else's dentifrice/water
Apart from this last brushing, when did you have your last oral hygiene before?	Before h
What did you use?	Dentifrice/rinsing solution/others
Did you take any medication today or yesterday? Why and which medication?	No/yes,
Taken altogether, do you see any reason to assume that your oral conditions today are different from the previous days?	No/yes,

the colour fields of a nine-field reference colour chart under daylight. The subjects were not informed about their daily results to prevent a variation of their oral hygiene habits. During the course of the study, 10% of the colour signals were read by a second examiner independently from the main examiner in order to obtain an interpersonal calibration.

The results were compiled to an Excel datasheet and, for statistical evaluation, were transferred to the SPSS software (version 10.0.) Descriptive procedures (mean, median, quartiles and frequency) as well as comparing procedures (Mann–Whitney *U*-test) were used. To calculate the reproducibility of the test outcome, the frequency of reading the median score plus/minus one unit from the reference colour chart was determined.

Results

Thirty-two subjects started the study. As there was one drop-out, 31 participants finished it and were included in the statistical evaluation. This cohort consisted of 14 women and 17 men aged between 19 and 46 years, nine smokers and 22 non-smokers (Table 2).

Scoring of the test results by a second examiner revealed an accordance of 17 out of 31 sticks, while in ten cases, there was a deviation of ± 1 . In three cases, the disagreement was two scores.

Analysing the daily questionnaire, it became obvious that 11 subjects had modified their oral situation during the course of the study for different reasons. Some of them had taken analgetics, nose spray, tablets against sore throat, had changed their toothpaste or had received professional tooth cleaning. For this reason, a subgroup was formed which included subjects with altered oral conditions. Thus, sta-

 Table 2 Description of subjects with or without stable oral conditions

	All subjects	Group I (stable oral conditions)	Group II (altered oral conditions)
Number of subjects	31	20	11
Mean age (std. dev.)	29.9 (±6.4)	31.8 (±6.5)	26.6 (±4.9)
Median age	29	31	27
Male	17	11	6
Female	14	9	5
Non-smoker	22 (71.0%)	15 (75.0%)	7 (63.6%)
Smoker	9 (29.0%)	5 (25.0%)	4 (36.4)

tistical analysis separates between subjects who had not changed their oral situation (group I) and subjects with altered oral conditions (group II).

Table 2 also gives an overview about some descriptive variables in both groups. Both groups are similar with respect to their distribution by gender, age and smoking habits. Table 3 gives information on the mean CCLP score calculated from the individual's average score over ten examinations in both groups. The average score is 3.2 for the subjects in group I and 4.2 in group II. Both groups differ significantly (p<0.05, Mann-Whitney Utest). Table 4 shows results regarding the test reproducibility, i.e. the stability of the individuals' scores, where individual stability was measured as the percentage of scores ranging between his/her median score ± 1 unit in both groups. While there is a reproducibility of 82.0% (range 50–100%) in subjects with stable oral conditions, this value diminishes to 60.0% (range 30-80%) in subjects with altered conditions (p < 0.01, Mann–Whitney U-test).

Subjects of group I were split into subgroups according to their median score, and for these subgroups, the interquartile range (IQR) was determined (Table 5). It turned out that the difference between the 25% percentile and the 75% percentile is 1.2 in the group with the lowest median scores. In subjects with a median score of 3, the IQR is 2.2, whereas in the group with highest median scores, the IQR was 1.0.

 Table 3
 Average CCLP scores after ten examinations for subjects with or without stable oral conditions

	Group I (stable oral conditions)	Group II (altered oral conditions)	
Mean CCLP score (std. dev.)	3.2* (±1.3)	4.2* (±1.1)	
Median	3	4	
Minimum	1.7	2.2	
Maximum	6.7	6.1	

*p<0.05

189

Ta	ble 4	Re	produc	ibility	v of the med	ian CCLP	score in sul	bjec	ts with
or	witho	ut	stable	oral	conditions	(average	frequency	of	scores
agi	reeing	wit	th the r	nedia	$n \pm 1$ unit)	. –			

	Group I (stable oral conditions)	Group II (altered oral conditions)
Minimum frequency (%)	50	30
Maximum frequency (%)	100	80
Mean frequency (%)	82*	60*

*p<0.01

Table 5Average interquartilerange (IQR) in relation to thescore median in subjects withstable oral conditions	Score median	Number	IQR
	1 or 2 3	11 6 3	1.2 2.2
	All scores	20	1.5

Discussion

In an era of considerable caries decline for about 80% of the children and adolescents but increasing polarization of a group with high caries experience, it is mandatory to give this group special care. However, to have optimal results, this group of about 20% of the younger individuals has to be selected early before carious lesions occur. Specific chair-side bacterial tests reflecting the number of S. mutans or lactobacilli do not predict caries development to a satisfying extent. This may be related to the fact that also non-mutans-streptococci form a significant proportion of the aciduric microflora in dental plaque, exhibit acid tolerance and are able to contribute to the hard tissue demineralisation [1, 8, 18, 21, 28]. The new caries risk test CCLP is a biochemical device, which measures the lactic acid produced by all bacteria which are capable to do so in the presence of sucrose. Thus, the test avoids one of the shortcomings of the specific bacterial tests.

The inter-examiner reproducibility of the test to prove exactly the same score was fair (Kappa=0.45). However, most discrepancies were restricted to ± 1 scoring unit, which reflects the difficulty to distinguish between two similar colour fields by different persons. Considering the predominantly minor discrepancies, it seems, under practical aspects, justified to count a deviation of ± 1 unit as less relevant. When only discrepancies of more than one score are included, inter-examiner reproducibility is excellent (Kappa=0.84). Based on this consideration, only disagreements of more than ± 1 unit were taken into account in the study. In the same way, Häberlein et al. [10] handled a deviation of ± 1 as clinically not important.

The aim of the present study was to evaluate the reproducibility of the CCLP during a period of 2 weeks under stable oral conditions. For this purpose, healthy subjects were included in the study who should not exhibit alterations of their oral parameters under the study period. However, at the end of the study, 11 subjects had some

medication or events which could influence the oral flora. From a methodological point of view, this shows that examining oral alterations using a questionnaire was suitable. As these subjects obviously could not follow the study protocol, they are excluded from the main analysis, but the respective results are compared and discussed.

In subjects with stable oral conditions, the CCLP test had a reproducibility of 82%. This means a high degree of reproducibility in the sense of stability with respect to the median scores, although this reproducibility is less if compared with the study of Häberlein et al. [10], who found a reproducibility of 90.6%. In some of the subjects in group I, single test scores were strikingly different from the median. One reason for this phenomenon could be a change of habits, which was not revealed by the questionnaires. As the stronger deviations from the median were mainly observed at the first test following a weekend, it can be speculated that a special diet at weekends caused these differences, as plaque formed under a special diet regime like consuming toffee and sugar lumps is more tenacious and less readily removed from the tooth surface [4].

Due to the range of reproducibility from 50 to 100% in group I, a more detailed analysis was carried out. It becomes obvious that in subjects with low median scores (score 1 or 2), the deviation from the median is low. Given symmetry around the median, the interquartile range (IQR) of 1.2 for these subjects means that 50% of all test results are within the median ± 0.6 . A similar low IQR was obtained for the group of persons with scores of at least 4, but this group is formed by three persons only. In the group of persons with a median score of 3, the IQR was 2.2. For these subjects, 50% of the obtained values are in the range of the median ± 1 , and the remaining 50% are deviating more than 1 score from the median. However, it has to be considered that these observations are based on a few cases only.

Transferred to clinical aspects, these results indicate that there is good reproducibility, especially for low CCLP scores.

The results for subjects who did not follow the study protocol but had alterations in their oral conditions (group II) differ significantly from group I subjects. They exhibit a higher median CCLP score, which might be due to the medication itself or to the reasons for the different medications. The subjects in group II also showed a significantly lower reproducibility rate of 60.0%. Under clinical aspects, as this figure is associated by altered oral conditions, this means that the test might be able to reflect changes of oral conditions sensibly and thus be a promising tool to monitor procedures which are aimed to improve oral hygiene.

Conclusion

In conclusion, the study shows a reproducibility of the CCLP test of 82.0% in subjects with stable oral conditions. Especially subjects with low test scores exhibit minor

deviations of repeated tests from this score. In situations with altered oral conditions, the reproducibility of the test is significantly lower. This indicates that the test could be a useful tool to monitor preventive measures.

Acknowledgements The study was conducted with financial support of 3M Espe, D-Seefeld. The authors are indebted to Michael Bubenheim (Institute for Medical Biometry and Epidemiology, University Hospital Hamburg-Eppendorf) for statistical assistance.

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