Clinical Assessment of a New Caries Activity Test Using Dental Plaque Acidogenicity in Children under Three Years of Age

Hyo-Seol Lee*/ Eun-Song Lee**/ Si-Mook Kang ***/ Jae-Ho Lee ****/ Hyung-Jun Choi***** / Baek-Il Kim******

Objectives: *The aim of this study was to assess the validity of a new caries activity test that uses dental plaque acidogenicity in children with deciduous dentition.* ***Study design***: *Ninety-two children under the age of three years old underwent clinical examination using the dft index and examinations with two caries activity tests. Plaque samples for the new Cariview® test and the saliva sample for the conventional Dentocult SM® test were collected, incubated, and scored according to each manufacturers' instruction. The data were analysed using ANOVA and Spearman correlation analyses to evaluate the relationships between the test results and the caries experience.* ***Results***: *The mean dft index of all of the subjects was 4.73, and 17.4% of the subjects were caries-free. The levels of caries risk based on the new Cariview test score significantly increased with the caries experience ($p < 0.01$). The test results revealed a stronger correlation with caries indices (dft and dt index) than the conventional SM colony counting method ($r = 0.43$, $r = 0.39$, $p < 0.01$).* ***Conclusions***: *The new caries activity test to analyse the acidogenic potential of whole microorganisms from dental plaques can be used to evaluate caries risk in children with deciduous teeth.*

Key words *Acidogenicity, Caries activity test, Caries experience, Dental plaque*

INTRODUCTION

Caries risk assessment is essential for the control of dental caries and the prevention of their development [1,2]. It is well known that the use of caries activity tests is very helpful, and the development of a reliable and valid method is needed to more accurately assess caries risk [3]. Especially, the testing caries acitivity in children is important because caries in primary dentition have been strongly associated with the prevalence of caries in permanent dentition [4,5]. Among the various factors that are related to dental caries, the assessment of microbiological parameters is used to test for caries activity [6,7]. Based on previous studies, caries activity tests that use cariogenic bacteria have been considered to be a valid predictor of the caries prevalence, and several tests are commercially available and are applied in dental clinics [8-11].

One of the caries activity tests counts the colony-forming units of *Streptococcus mutans* (MS) or *Lactobacilli* (LB) using selective growth media that allow for the growth of the colonies of the specific cariogenic bacteria that contribute to the initiation and the progression of dental caries [6,12]. However, it has been reported that caries can occur in individuals without these specific bacteria, and other *non*-MS bacterial species can influence the development of caries [13,14]. According to the recent ecological plaque hypothesis, dental caries can be attributed to changes in the microbial composition within the dental biofilm rather than specific bacterial species [15,16]. Under normal conditions, dental plaque is composed of non-*mutans streptococci* (*non*-MS) and *Actinomyces.* When the acid production of low-pH *non*-MS and *Actinomyces* is maintained by changes in the oral environment, reduced plaque pH accelerates the growth

Hyo-Seol Lee, PhD, Department of pediatric dentistry, School of Dentistry, Kyung Hee University, Seoul, Republic of Korea.
Eun-Song Lee, PhD, Department of preventive dentistry & Public Oral Health, BK 21 PLUS Project, Yonsei University College of Dentistry, Seoul, Republic of Korea.
Both authors contributed equally to this paper
Si-Mook Kang, MS, Department of preventive dentistry & Public Oral Health, BK 21 PLUS Project, Yonsei University College of Dentistry, Seoul, Republic of Korea
Jae-Ho Lee, PhD, Department of pediatric dentistry, Yonsei University College of Dentistry, Seoul, Republic of Korea
Hyung-Jun Choi, Department of Pediatric Dentistry, Yonsei University College of Dentistry, Seoul, Republic of Korea
Baek-Il Kim, PhD, Professor and Chair, Department of Preventive Dentistry & Public Oral Health, Yonsei University College of Dentistry

Send all correspondence to
Baek-Il Kim
120-752, 50 Yonsei-ro, Seodaemun-Gu, Seoul, Republic of Korea
Phone: +82-2-2228-3070
Fax: +82-2-392-2926
E-mail: drkbi@yuhs.ac

of aciduric bacteria (such as MS and *Lactobacillus* (LB)). The imbalances in the resident microflora induce the demineralization of dental hard tissue and become carious lesions [15,17]. In other words, if we were able to measure the metabolic products of entire microorganisms in the plaque, it would be possible to assess caries activity and predict caries risk more accurately compared with focusing on the caries-related bacteria [18,19].

In view of the caries process, caries activity tests have been developed for evaluating the acidogenic abilities of both *mutans streptococci* and the other aciduric bacteria in dental plaques [20,21]. For example, Cariostat® (Dentsply-sankin, Tokyo, Japan) was developed by Shimino *et al* in 1970 and is currently used in the clinic [22]. Based on previous studies, it has been confirmed that this method assessing the acidogenic potential of dental plaque is reliable for evaluating caries risk because it exhibits high levels of sensitivity and predictive power [23-25].

A new colorimetric caries activity test called Cariview® (All in One Bio, Seoul, South Korea) that was first developed in Korea evaluates the total amount of acid produced by all cultured microorganisms within dental plaque [26]. Using this test, we confirmed the level of caries risk by observing the colour changes that ranged from red at pH 3.0 to blue at pH 7.0 and could be quantified as on a 0–100 relative scale. Moreover, the sampling method is very simple; the plaque on teeth is scraped with a sterilized cotton swab. This method can be easily applied to uncooperative patients, such as infants, toddlers, and individuals with disabilities. However, to date, there is a lack of clinical studies that support the ability of the Cariview® test to assess caries risk in children.

Thus, the aim of this study was to assess the ability of this new caries activity test that uses the acidogenic abilities of all of the microorganisms in dental plaque in young children compared to the results of a conventional activity test. We hypothesized that this new caries activity test would have a significant correlation with the caries experiences and its level would be higher than the conventional test result.

MATERIAL AND METHOD

This cross-sectional study was performed according to protocols and procedures that were approved by the Yonsei University Institutional Review Board (2-2012-0038), and this study was ethically conducted according to the Declaration of Helsinki (World Medical Association). All subjects were children under the age of three who were admitted to the Yonsei University Dental College of Pediatric Dentistry from November 2012 to July 2013. The exclusion criteria were subjects who visited the dental hospital for dental trauma or orthodontic problems, had systemic diseases, or had taken antibiotics within the last three months. Prior to the initiation of the study, we explained the research purposes to the subjects and their parents, and written informed consent was obtained. The caries experiences were examined using the dft index of the primary teeth according to the World Health Organization criteria. The clinical examinations were conducted by two dentists, and the intra-examiner reliability k-value was 0.85.

In this study, caries activity tests were used to assess the counts of mutans streptococci in the saliva using the Dentocult SM® test (Orion Diagnostica, Espoo, Finland), and the acidogenic abilities of the dental plaques were assessed with the Cariview® test (Figure 1, All in One Bio, Seoul, Korea). All tests were performed out according to manufacturer's instructions. First, a sterile cotton swab from the test kit was rubbed across the buccal and labial cervical areas of the tooth surface, and this procedure was repeated 2-3 times. The cotton swab was then placed into the dedicated culture medium, and the medium with the plaque was incubated at 37°C for 48 h. Next, pH indicators were added to the cultured media to observe colour changes that were based on the media pH. The degrees of colour change were evaluated with an optic analyser (Figure 1(B)) and scored on a 0-100 point scale based on the medium pH; the scores ranged from 0 (blue, pH 7.0) to 100 (red, pH 3.0). The resultant scores were classified into three risk group levels according to the following arbitrary criteria: low risk, score 0-40 ($5.4 < pH \leq 7$ .0); moderate risk, score 41-70 ($4.4 < pH \leq 5.4$); and high risk, score 71-100 ($3.0 \leq pH \leq 4.4$).

The salivary SM levels were evaluated using a commercial Dentocult SM® kit. Bacitracin was added to the culture fluid 15 min before the test. Paraffin-stimulated saliva was collected for 1 min, and the test strip was placed into the collected saliva sample. The strip was cultured at 37°C for 48 h on the prepared culture media. Subsequently, the SM growth density was estimated using scores that ranged from 0 to 3 and were provided by the manufacturer's instructions (0: $<10^4$ CFUs/mL, 1: 10^4-10^5 CFUs/mL, 2: 10^5-10^6 CFUs/mL, and 3: $>10^6$ CFUs/mL).

Statistical Analyses

The caries indices of the caries risk groups that were classified according to the results of the Cariview tests and the Dentocult SM tests were assessed using one-way ANOVAs followed by Scheffe's post-hoc analysis to identify statistically significant differences between the groups. Spearman correlation coefficients were also calculated to analyse the associations between the caries activity test results and the caries indices including the dft and dt indices. All statistical analyses were performed using PASW Statistics 18.0 software (SPSS, IBM Corporation, Somers, NY 10589, USA), and the level of significance was set at $\alpha < 0.05$.

RESULTS

The demographic information of the 92 subjects under the age of three (41 males and 51 females) who participated in this study is presented in Table 1. The mean age was 2.36 years old, and the mean dft index was 4.73 ± 3.63. Among the examined children, 17.4% were caries-free, and there were no children who had required tooth extraction due to caries. There were no significant differences in the dft or mean Cariview scores of the girls and boys. The dft index increased with age, and this increase was not significantly different between the different age groups (Table 1).

According to the results of the caries activity tests of our patients, the three risk groups based on the Cariview scores exhibited relatively high distributions in the low- and middle-risk groups compared to the high-risk group. The score of 0 in the Dentocult SM test was most frequent and accounted for 30.4 % of all participants (Table 2).

Regarding the results of the Cariview tests, the dft and dt significantly increased with the caries risk ($p < 0.05$). The dft and dt indices of the high-risk group were approximately 2-fold greater than those of the low-risk group, and these differences were significant ($p < 0.01$, Table 2). The results of the Dentocult SM test revealed that the dft and dt indices did not increase proportionally with the risk group; the dt index was the lowest in the group with scores of 1 and highest in the group with scores of 3 (Table 2).

Figure 1. Cariview kit (A), Optical analysis apparatus involving a spectrometer (B)

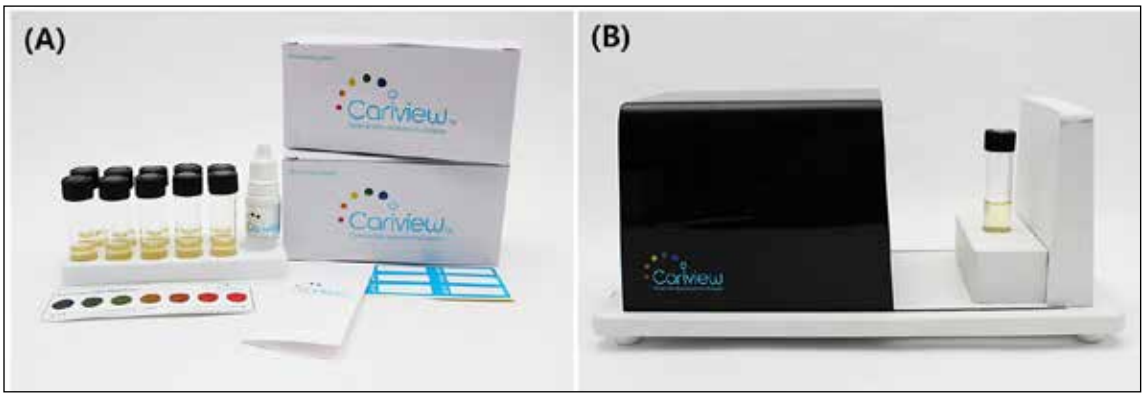

Figure 2. Cariview score and colour scale according to the resulting pH of the culture media.

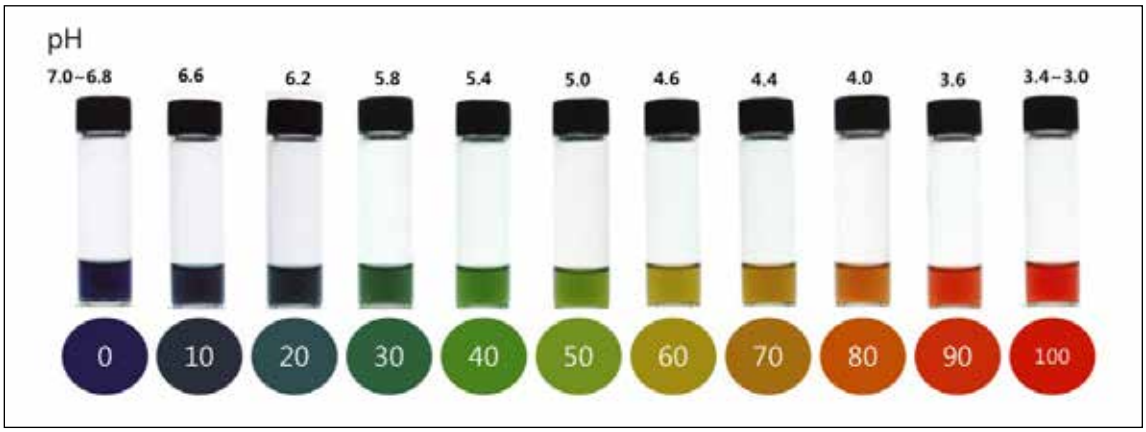

Table 1. Distribution and mean dft values and Cariview score according to the demographic characteristics

		N (%)	Mean ± S.D	
			dft	Cariview score
Total		**92(100)**	**4.73 ± 3.63**	**51.46 ± 18.66**
Sex	Male	41(44.6)	4.72 ± 0.55	50.24 ± 3.06
	Female	51(55.4)	4.89 ± 0.53	52.22 ± 2.72
Age	1	11(12.0)	3.00 ± 0.84	46.06 ± 4.69
	2	37(40.2)	4.46 ± 0.54	48.40 ± 2.91
	3	44(47.8)	5.39 ± 0.60	55.37 ± 2.97

No significant differences between the groups were observed based on two-sample t-tests and ANOVA at α = 0.05.

Table 2. Mean caries indices according to the score groups based on the results of the two caries activity tests.

Caries activity test	Score groups	N (%)	Mean ± S.D.			
			dft	*p*-value	dt	*p*-value
Cariview	Low (0-40)	37(40.2)	3.27 ± 0.57 [a]	0.001	3.03 ± 0.56 [a]	0.002
	Middle (41-70)	40(43.5)	5.13 ± 0.46 [a,b]		4.50 ± 0.48 [a,b]	
	High (71-100)	15(16.3)	7.27 ± 1.09 [b]		6.80 ± 1.14 [b]	
Dentocult SM	0	28(30.4)	3.61 ± 0.50 [a]	0.007	3.57 ± 0.50 [a,b]	0.014
	1	15(16.3)	3.60 ± 0.70 [a]		2.53 ± 0.55 [a]	
	2	22(23.9)	5.73 ± 0.83 [a,b]		5.36 ± 0.86 [a,b]	
	3	18(19.6)	6.61 ± 0.88 [b]		5.78 ± 0.96 [b]	

Within the same column, the different letters denote significant differences between the groups by Scheffe's post hoc analyses at α = 0.05.

Table 3. Correlation coefficients between the caries activity tests and the caries indices

Caries activity tests		dft index	dt index
Cariview	*r*	0.43**	0.39**
	p-value	< 0.0001	< 0.0001
Dentocult SM	r	0.34**	0.25*
	p-value	0.001	0.022

r indicates the Spearman correlation coefficient; *$p < 0.05$, **$p < 0.01$.

Regarding the correlation coefficient, the caries activity tests were significantly correlated with the dft and dt indices (Table 3). The Cariview results were more strongly correlated with caries experience ($r = 0.43$, $p < 0.01$, and $r = 0.39$, $p < 0.01$) than the Dentocult SM ($r = 0.34$, $p < 0.01$, and $r = 0.25$, $p < 0.05$, Table 3). Moreover, a significantly moderate correlation was identified between the results of the Cariview and the Dentocult SM tests ($r = 0.34$, $p < 0.01$).

DISCUSSION

According to Caufield *et al* , the "window of infectivity", i.e., 19 to 31 months, is an important time for caries-inducing bacteria, and most children are infected during this period [27]. If caries-inducing bacteria are identified in children before they turn three years old, the children are highly predisposed to the occurrence of caries in the future [4,28]. Because caries prevalence in the deciduous teeth has been shown to be a predictor of the development of future caries, it is important and crucial to evaluate caries activities in children below the age of three [29].

This study confirmed that the Cariview could be a reliable and valid method for screening the progress of caries and predicting caries risk in children. In this study, the mean dft and dt of the teeth increased with the Cariview score groups, and a significant difference was observed between the groups (Table 2). It can be assumed that children with higher Cariview scores have significantly higher caries rates than children in the low-risk groups with lower scores. Based on the results, the Cariview score can identify susceptible subgroups within a population and distinguish the caries activity and caries risk of the individual. Moreover, the results of the Cariview test were significantly correlated with past caries experience ($r = 0.43$, $p < 0.0001$), which is known to be the most reliable and strongest single predictor of future caries risk in patients [9]. These findings suggest that the Cariview score could be used as an indicator of the caries risk of children.

According to the results of the Dentocult SM, the caries indices did not increase linearly with the test scores; the mean value of the score 1 group was lower than that of the score 0 group (Table 2). Although the patients who scored 0 did not have any SM colonies according to the Dentocult system, they might have had current caries and past caries experiences, which supports the notion that cariogenic bacteria other than mutans streptococci are responsible for caries activity and caries development [24]. Therefore, the test for mutans streptococci is not sufficient for clinically identifying patients with high caries risk or evaluating the level of caries risk. Moreover, the correlation coefficients of the SM test with the dt and dft indices were relatively low compared with that of the Cariview test (Table 3). In previous studies, the reported correlation coefficients between dental caries and the Dentocult SM range from 0.18 to 0.25 [8,14]. Granath *et al* explained that the correlation between the Dentocult SM and dental caries experience is low because the test identifies only *Streptococcus mutans* species, and this author emphasized that it is necessary to consider the ecological plaque hypothesis to more accurately assess the risk [14]. In view of the fact that the Cariview test can evaluate the acidogenic abilities of both mutans streptococci and other aciduric bacteria in the plaque, the Cariview test might be a more valid and reliable method for evaluating caries activity and predicting caries risk. However, to assess the potential of this test to predict future caries risk, it will be necessary to conduct a longitudinal study with numerous subjects in various age groups.

Given that dental caries are known to be a multifactorial disease process, it should be remembered that the test based solely on biological aspect of dental caries might have some limitations to assess caries risk. This study confirmed that the correlations of the test results with the caries indices were not very high, and these results correspond with previous studies that have reported correlation coefficients in the ranged of 0.20 to 0.30 [8,10,21]. Furthermore, the test results might have been affected by the presence of interproximal caries that were difficult to detect and diagnose clinically [24]. Moreover, the caries indices used in this study do not reflect the actual states of caries (i.e., active/arrested, incipient/moderate/advanced, and types of restoration) that might have contributed to the weak correlations with the caries activity test results.

In clinical application, the Cariview test has several advantages over the conventional test method. Because children with deciduous teeth can be noncompliant, a simple and easy method for evaluating caries activity in clinical situations was needed. Regarding the Cariview test, it is easy to quickly collect plaque samples a using cotton swab. In contrast, the Dentocult SM is difficult to use with uncooperative children due to the requirements of the placement of a bacitracin tablet 15 minutes prior to the test and one minute of paraffin wax chewing to stimulate the production of saliva. Therefore, we confirmed that this new test can be used to more easily examine the caries risk of uncooperative patients, including young children, individuals with behavioural control problems and those with special needs because it requires only minimal time for plaque sampling and no special equipment.

Additionally, the results of this test enable parents to instinctively understand the degree of caries risk by visualizing the results in a colour spectrum and quantifying them on a 0-100 point scale. This test will make it possible to measure slight changes in the degree of an individual's caries susceptibility by providing changes in a numerial score that reflecting the acidogenic potential of the entire microorganisms of the plaque.

CONCLUSION

This new caries activity test using acidogenic potential of dental plaque exhibited moderate corrlations with the caries experiences and caries status of children under the age of three. Therefore, the Cariview test could be a clinically useful and simple method for assessing the caries risk of children. Further longitudinal studies are needed to confirm the predictive value of this new test.

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