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Original Article

Monitoring Caries Risks before the Window of Infection and Later Caries Increment: A Caries Prediction Study on Rapid Detection of *Streptococcus mutans* Using Monoclonal Antibodies

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Abstract

A new semi-quantitative enumeration system has been developed for the detection of *Streptococcus mutans* in saliva. Using two kinds of species-specific monoclonal antibodies, this system can quickly detect salivary *S. mutans* within 30 min and classify the results into three levels. The aim of this study was to evaluate the potential of this test kit in determining risk for the development of caries. Saliva samples collected during a compulsory dental examination from 56 children aged 18-months were tested. The children were classified into 3 groups according to the level of salivary *S. mutans* determined. After 18 months, 36 of the children underwent a second examination to investigate whether there was a correlation between salivary *S. mutans* level at the first examination and subsequent increment in caries. The results showed a good correlation between the two. This indicates that salivary *S. mutans* level before the window of infection is an indicator of caries risk and can be quickly detected using this test kit. This rapid test should be particularly useful in assessing risk of future caries in very young children.

Key words: *Streptococcus mutans*—Species-specific monoclonal antibody—Saliva—Caries increment—18-months-old children

Introduction

Dental caries is an infectious disease caused mainly by *Streptococcus mutans*, *Streptococcus sobrinus* and other oral microbes in human[13)].

Many studies have shown a correlation between caries prevalence and the number of *S. mutans* in the oral cavity[1,5,6,11)]. A positive relationship was found between caries increment and mutans streptococci and Lactobacilli parameters in the saliva of 18- and 19-year-old Swedish young persons[14)], in 12- to 14-year-old Scottish children and adolescents[11)], and in children and adolescents aged 12- and

13-years[2,9,10].

The risk factors for caries have been well studied, and some are now well established in children[12]. The presence of caries in the primary dentition has also been confirmed as a predictive indicator of caries in the permanent teeth[7].

In most of these studies, however, the age of the population group investigated has been over 3 years. According to the concept of the window of infection, children are infected with mutans streptococci between approximately 19 months and 31 months of age[3,4]. The purpose of this study was to investigate the effect of the number of salivary *S. mutans* before the window of infection on subsequent caries increment.

Recently, a new salivary *S. mutans* detection system (SALIVA-CHECK MUTANS®; GC, Tokyo, Japan) has been developed which can determine salivary *S. mutans* levels within 30 min[8]. That investigation revealed that this test showed a good correlation with MSB agar plate-based cultivation, suggesting the potential of this new system for the screening of patients with high salivary *S. mutans* levels in only a short period of time. Another aim of this study was to evaluate the potential of this test kit in assessing risk of caries before the window of infection.

Materials and Methods

1. Dental examination

Fifty-six children (31 boys and 25 girls) aged 18 months participated in this study. Saliva samples were collected using a swab and caries prevalence determined. A second dental examination was performed at the age of 3 years by the same dentist. All participants were receiving legal health examinations in Kamogawa City, Chiba Prefecture.

2. Test procedure

Two hundred fifty μl collected saliva sample was placed into a tube, treated vigorously with 50 μl Tris-NaOH solution for 30 sec, and then mixed with 100 μl Tris-citrate buffer to neutralize the pH. Two hundred μl treated saliva was loaded onto the test device (SALIVA-CHECK MUTANS®). If a red line appeared on the test line of the test device after 15 min, it was scored as level 3. If a line did not appear, an enhancing reagent (Silver Enhancer: KPL, Maryland, U.S.A.) was added. After another 15 min, it was scored as level 2 if a line was observed, or level 1 if no line was visible.

3. Statistical analysis

A one-way ANOVA was used to determine differences in average values. The data were analyzed using JUSE StatWorks (version 3.5). A level of less than 5% was considered to show statistical significance.

4. Ethical considerations

Code numbers were used to identify examination charts and the results for *S. mutans* levels in order to protect the anonymity of the participants. All personal information was protected and treated confidentially and ethical considerations taken into account. On implementation, an explanation was given in writing or verbally to the parents or guardians of the participants with regard to the purpose and method of the survey, protection of personal information, freedom of consent, and withdrawal. Informed consent was obtained from all the parents or guardians of the participants and the local authorities concerned.

Results

Figure 1 shows the mean caries prevalence for decayed and filled deciduous teeth (df-t) at each *S. mutans* level at baseline according to the results of the test kit. Thirty-eight children were at level 1 (68%), 13 were at level 2 (23%), and 5 were at level 3 (9%). The number of df-t in level 1, 2, and 3 was 0.1, 0, and 1.2, respectively. The differences in these values between level 1 and 3, and 2 and 3 were statistically significant (one-way ANOVA; $p<0.01$).

Figure 2 shows the correlation between the baseline *S. mutans* level and the caries

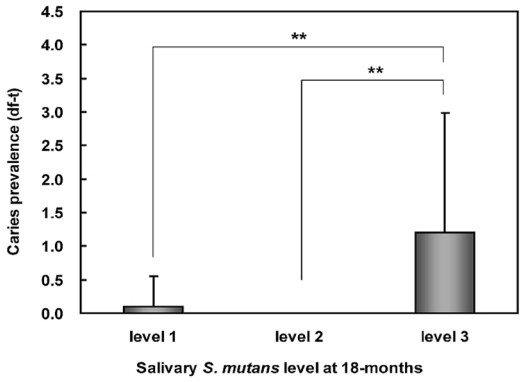

Fig. 1 Salivary *S. mutans* level and caries prevalence at 18-months (baseline)
**: one-way ANOVA; $p<0.01$

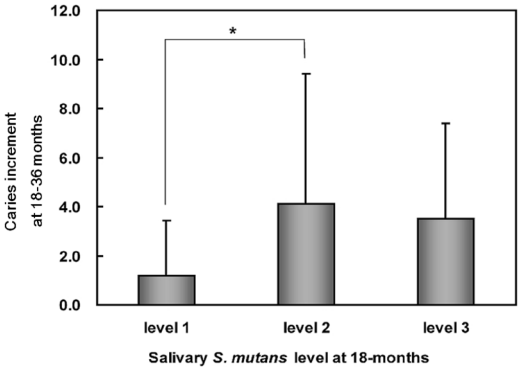

Fig. 2 Correlation between baseline *S. mutans* level and caries increment over next 18-months
*: one-way ANOVA; $p<0.05$

Table 1 Comparison of caries status at each salivary *S. mutans* level

	Caries prevalence at 18-months[1]		Caries prevalence at 36-months in caries-free children at 18-months[2]		
	with caries	caries-free	with caries	caries-free	drop-out
Level 1	2	36	6	15	15
Level 2	0	13	6	4	3
Level 3	2	3	2	1	0

[1]: Number of children with or without caries at 18-months at each level according to SALIVA-CHECK MUTANS®
[2]: Change in caries status at 36-months and number of drop-outs among children free of caries at baseline

increment at 18–36 months. The number of participants reduced to 36 due to some being withdrawn from the study. The drop off rate was 36%, yielding a participant number of 22 at level 1, 10 at level 2, and 4 at level 3. The caries increment value was 1.2, 4.1 and 3.5, respectively. A statistically significant difference was observed between level 1 and 2 (one-way ANOVA; $p<0.05$). Table 1 shows the number of children who had been free of caries at baseline who subsequently developed new caries during the study period. At baseline, 2 children were caries-positive and 36 were caries-free at level 1. Fifteen of these 36 children dropped out, 6 had new caries, and 15 remained caries-free at the age of 3-years. At level 2, all 13 children were caries-free at baseline. By the second examination, 3 of these 13 children had dropped out of the study, 6 had new caries, and 4 were caries-free. At level 3, 2 children had caries and 3 were caries-free at baseline. Among these 3 children, no one dropped out, 2 had new caries, and 1 was caries-free at 3-years. As shown in Fig. 3, there was a correlation between the salivary *S. mutans* level at baseline and the mean number of new caries at 18–36 months in those children who had been caries-free at baseline. A statistically significant difference was observed between level 1 and 2 (one-way ANOVA; $p<0.05$).

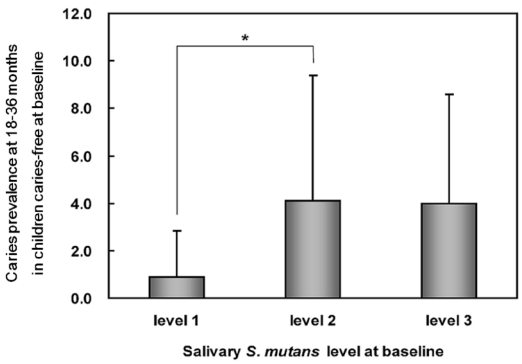

Fig. 3 Correlation between baseline *S. mutans* level and caries prevalence at 18–36 months in children free of caries at 18-months examination

*: one-way ANOVA; $p<0.05$

Discussion

The primary aim of this study was to determine whether the salivary *S. mutans* level before the window of infection was an indicator of risk for caries. In Japan, children are obliged to undergo medical and dental examinations at the ages of 18 months and 3 years under government policy, which enabled us to carry out this longitudinal study.

The salivary *S. mutans* level was determined by SALIVA-CHECK MUTANS® at baseline. It is difficult for small children to chew gum. Therefore, a swab was inserted into the mouth of each child in order to absorb saliva, which was then wrung out and transferred to a mixer. Under the cultivation protocol used, it took 2 days to determine the number of salivary *S. mutans*, so it was not possible for the clinical dentists to inform the parents/guardians of the results on that day. The SALIVA-CHECK MUTANS® kit offers a rapid *S. mutans* detection test, taking only 15 min to detect more than 5×10^5 CFU/ml *S. mutans* (level 3). With the use of Silver Enhancer reagent, it then takes an additional 15 min to determine levels 2 and 1, which correspond to 1–5×10^5 CFU/ml and less than 1×10^5 CFU/ml, respectively. In an earlier study, this test showed a good correlation other cultivation methods and commercial products such as Dentocult SM® and MUCOUNT®[8].

A correlation was observed between caries prevalence and salivary *S. mutans* level at 18 months, which is in agreement with the results of many earlier studies[5,6,11]. This rapid *S. mutans* detection test allows patients at high risk of dental caries to be screened in only a short time.

A strong correlation was observed between caries increment at 18–36 months and salivary *S. mutans* level at baseline (Fig. 2). A significantly greater level of caries was observed in the level 2 group at baseline than in the level 1 group. On the other hand, only 5 children showed level 3 at baseline, so no clear correlation could be inferred.

In Table 1, we focused on caries-free children at the 18-month examination. It shows that some children at each *S. mutans* level who were caries-free at baseline had their first caries experience during the next 18-month period of the study. Six of the 21 caries-free children at level 1 (29%) developed caries during this period, although they were caries-free at baseline. Six of 10 children at level 2 (60%) had new caries, and 2 of 3 (67%) at level 3. Compared with level 1, the number of children with new caries during this 18-months doubled at both level 2 and 3.

As can be seen in Fig. 3, a score of level 2 before the window of infection according to SALIVA-CHECK MUTANS® indicated that the child was at risk of future caries. Interestingly, high caries prevalence could be determined by level 3 and high caries risk by level 2.

Because of the high correlation between caries prevalence and caries increment, the SALIVA-CHECK MUTANS® offers a useful tool in a clinical setting, particularly for the pediatric dentist. It is easy to use and rapid in providing results. It needs no incubator or additional facilities, and can be used outside of a clinical setting. This makes it especially useful for community care such as in the screening of small children for risk of caries and regular dental examinations. *S. sobrinus* is also known to play an important role in the incidence of dental caries. However, *S. sobrinus* has not been widely accepted as a target

indicator of caries before the window of infection, and neither has mixed infection with *S. sobrinus* and *S. mutans*[13]. We believe that there is scope for further discussion on this point.

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