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Comparison of plaque samples and saliva samples using the CAT21 Test® (Cariostat method)

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Abstract The study compared CAT21® test scores (Cariostat score) of plaque and saliva samples of 117 kindergarten pupils to check the congruency of both sampling methods. The scores were also compared with that of the subject's "d" and "df" teeth. Test scores are based on color changes resulting from a decrease in pH brought about by presence of acid-producing microorganisms. Results revealed an early color change of the CAT21® test solution of the saliva samples compared to that of plaque samples. However, the difference in the color change in both sampling procedures became negligible after 48 hours of incubation. Results revealed a statistically significant correlation between CAT21® test scores of both sampling procedures and when compared with the mean "df" teeth.

Key words
Cariostat® method,
CAT21® test,
Dental plaque,
Saliva

Introduction

Attempts for an effective caries activity test were first made more than 100 years ago[1)] and were developed until the 1960's[2,3)]. But since dental caries is a multi-factorial entity, researchers have yet to formulate a simple, effective, and inexpensive caries activity test. Presently, a number of caries activity tests have been formulated with the idea of measuring caries-conductive factors of the Keyes' Venn diagram[4)]. Caries risk assessment may involve simply looking at the patient's clinical appearance[5)]. For example, one may say that a patient with two or more carious lesions may be considered at high risk of developing caries in the future[6)]. Moreover, factors such as oral hygiene, fluoride, *Streptococcus mutans* counts[7,8)], eating habits, salivary flow[9)], and past caries experience[10)], should also be considered. Presently, there are many kinds of caries activity tests available. There are tests designed to measure representative cariogenic bacteria counts in the saliva samples, *i.e.* Dentocult-SM® Strip mutans[8,11)], and Dentocult-LB® [8,11,12)] (Orion Diagnostica). However, problems regarding cost, practical application, and inability to let children understand its significance arise during collection of stimulated saliva. The Cariostat Method[13–15)] (CAT21 Test) developed by Shimono, is a colorimetric test that determines the acidogenecity of oral microorganisms in the plaque through changes in pH. Scoring is done by comparing the color results of the samples with the 4-scale (0, 1.0, 2.0, and 3.0) reference color chart provided with the CAT21® (Morita Co., JAPAN) kit. A score of "0" designates a low caries susceptibility risk while "3.0" having the highest caries susceptibility risk. Sampling is done through the use of a sterile cotton swab by wiping bucco-cervical surfaces of the maxillary teeth 2 to 3 times from one quadrant to the opposite quadrant. The swab is to be placed into the ampoule and incubated for up to 48 hours at 37°C. Scoring of this test is done in front of a fluorescent daylight type lamp of which resulting colors are compared with the reference colors provided. A blue result is said to have a pH of 6.1 ± 0.3, green having 5.4 ± 0.3, yellow-green with 4.7 ± 0.3, and finally

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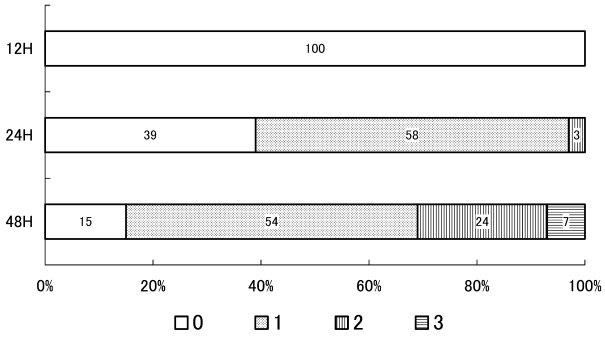

Fig. 1 CAT score distribution (plaque sampling method)

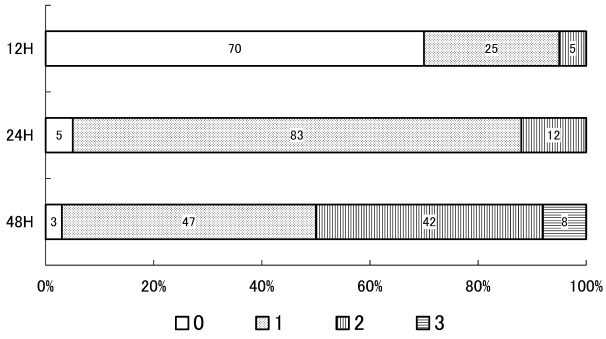

Fig. 2 CAT score distribution (saliva sampling method)

yellow, said to have a pH of 4.0 ± 0.3. The method is simple, effective, inexpensive, and can be used as a means of determining the caries activity status of an individual in the clinical setting as well as in the fieldwork setting in a large-scale basis.

Until now, no single variable has proven to be successful in predicting caries development for the majority of populations that have been studied[3,4]. Caries activity testing is very effective in establishing the caries risk of children. Many caries activity test now use saliva samples because a fixed amount of caries-causing bacteria can be collected only from a fixed amount of saliva. However, this can be difficult when dealing with young children. Therefore, it is an advantage to collect dental plaque in children. However, determining the weight of the plaque is impossible.

Thus, this study was made to compare results of plaque and saliva samples in correlation to the caries status using the CAT21 Test.

Materials and methods

The study comprised 117 kindergarten pupils with ages ranging from 5 to 6 years old. All children underwent oral examination using a mouth mirror in good lighting condition while taking note of the "def" teeth. Informed consent was obtained from all of the subjects and their parents to participate in the study. Oral examination was done by one person while lectures, orientations, recording of data, and sampling were done by the other members of the field work team. Plaque sampling was done immediately after by swabbing the bucco-cervical surfaces of the maxillary teeth using a sterile cotton swab, placing it into the test solution, and incubating at 37°C for up to 48 hours. After each of the children finished plaque sampling, they were all given an orientation regarding saliva sampling. The children were asked to chew on a pellet of unflavored gum for 3 minutes to stimulate salivation. Sampling was done through a mini collection tube into which the children can expectorate saliva while chewing. A cotton swab was then completely dipped into the collection tube and placed into the test solution and incubated at 37°C for up to 48 hours. Scoring was done after a predetermined incubation period using the modified 7-scale grading system, a 0.5 interval grade based on the 4-scale system. Test score results were noted at 12, 24 and 48 hours of incubation and were grouped as follows: 0 (CAT 0 and 0.5), 1 (CAT 1.0 and 1.5), 2 (CAT 2.0) and 3 (2.5 and 3.0). Results were then correlated with the sampling method and "def" teeth. For the comparison of the mean dft scores per group, the Analysis of Variance (ANOVA) and the *t*-test was used.

Results

The average net weight of the plaque sample was 0.058 g while that of the saliva sample was 0.128 g. A high correlation was seen between results of 24 hours and 48 hours incubation in plaque and saliva sampling ($r = 0.373$: $P < 0.001$, $r = 0.519$: $P < 0.001$ respectively). There was a noted decrease in pH as indicated by an early color change in the saliva samples, as compared to that of the plaque samples, after an incubation period of 12 hours. At this time, a total of 30.0% of saliva samples already had a test score of either 1.0 or 2.0 (Fig. 2) while all of the plaque samples exhibited no color change (Fig. 1). The change became more evident after 24 hours of incubation as indicated by the increase in the total number of saliva samples that obtained a test score

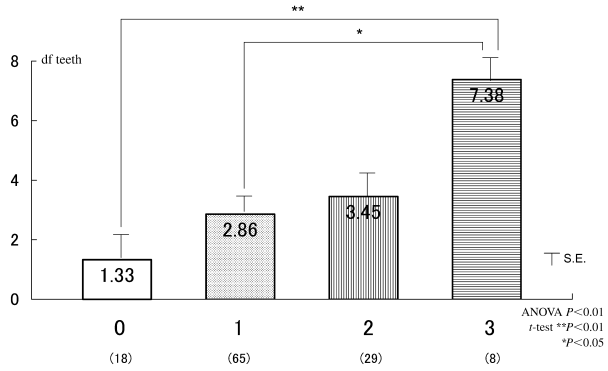

Fig. 3 Relationship between CAT scores (plaque) and mean df teeth (48 H)

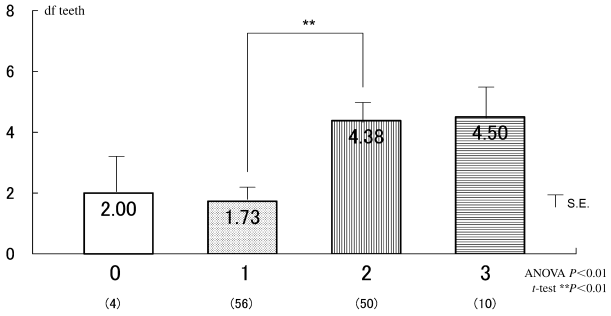

Fig. 4 Relationship between CAT scores (saliva) and mean df teeth (48 H)

of 1.0 (Fig. 2). A total of 83.0% of saliva samples were observed to have a score of 1.0 as compared to that of 58% of the plaque samples. Moreover, a total of 12.0% of saliva samples had a score of 2.0 compared with just a mere 3.0% of plaque samples. After 48 hours of incubation of the saliva samples, 42% had a score of 2.0 and 47% had a score of 1.0. In contrast, 24% of the plaque samples had a CAT score of 2.0 and 54% had 1.0. However, the difference decreased when compared at the 12-hour and 24-hour incubation period. Samples with a score of 3.0 became almost similar in either of the saliva and plaque samples. A high significant correlation was seen between df teeth and CAT score of plaque sampling after 48 hours incubation ($r=0.236$: $P<0.01$).

The mean df teeth increased with every increase of the CAT score of plaque sampling and a significant difference was seen with each group (ANOVA $P<0.01$). There was a difference in the mean df teeth in the 0 and 3 group, and 1 and 3 group (t-test $P<0.01$, $P<0.05$ respectively). Moreover, the mean df teeth was 1.33 and 7.38 for the CAT score 0 and 3, and the difference of df teeth was 6.05 in two groups (Fig. 3). Likewise, a high significant correlation was seen between df teeth and saliva sampling ($r=0.270$: $P<0.01$). The mean df teeth increased with every increase of the CAT score (ANOVA $P<0.01$). There was a significant difference between the mean df teeth and CAT score 1 and 2 but groups 0 and 1, and 0 and 3 were almost similar (Fig. 4).

Discussion

Because of the multi-factorial trait of dental caries, there is yet no single caries assessment test that can accurately predict future occurrence of tooth decay. Researchers have been searching for factors that can enable them to predict who would develop tooth decay even before it starts. Though many of these factors have been investigated for their relevance in the development of caries, still many authors are quick to note that there is still much to be done. Often used sampling procedures for assessing caries risk status of an individual are plaque and saliva. Saliva sampling is used in almost all caries risk assessment tests and its validity has been widely investigated. However, when applied to young children, clinicians are often faced with difficulty in taking samples. Okazaki[16] reported the difficulty of collecting at least 1.0 ml of saliva in about 1/5 of senior kindergarten class and 2/3 of junior kindergarten class. Since it is important to establish caries risk of young children through caries activity testing, plaque sampling therefore becomes an advantage. The CAT21® test utilizes plaque sampling and in order to consider if there are any differences or similarities of this sampling method with saliva sampling, we made this study. The test liquid, which is initially a dark blue solution containing 20% sucrose and pH indicators, changes color from blue to green, or yellow, depending on the ability of the bacteria present to produce acid which lowers down the pH, thus bringing about the color change.

This test has the ability to determine the acidogenecity of the plaque. Matsumura[15] reported that 10^3 CFU/ml, 10^5 CFU/ml, 10^8 CFU/ml of mutans streptococci put into the test ampoule during 12 and 24 hours incubation can be affected by the amount of colony forming units but after 48 hours,

the pH reaches about 5.0 and becomes stable. Thus, amount of CFU will have little effect when using the Cariostat method after 48 hours. This study however was based on reference strains and not clinical samples.

Results have shown an early change in color with that of the saliva samples compared with the plaque samples. It is thought that this early change in the pH may be due to the rapid spread of bacterial colonies due to their well-dispersed configuration in the saliva. This was within the 12-hour incubation period and which may prove a fast exponential growth rate as compared with the test solution containing plaque samples. While plaque samples started to exhibit a change of color after the 12-hour incubation period accounting to almost 60%, those of the saliva samples already had a 95% color change. As bacteria reach the peak of its growth phase, cell functions begin to deteriorate and cell division stops. As this happens, certain organisms stop acid-production while aciduric bacteria continue to grow and produce acid. This can be seen during the 48-hour incubation period of which the difference of the resulting color in both sampling procedures became smaller. This is of great importance considering the fact that saliva sampling in young children is difficult. Moreover, most studies regarding the method and its validity as a predictive tool in assessing high risk group was done with children as the main subjects.

Matsumura[15] studied young children 24–30 months old in Japan and reported the results of the method as correlated with mutans streptococci counts and "def" teeth. In this study, the difference of the mean df teeth with the CAT score 0 and 3 was 6.05 in the plaque samples, and 2.5 in the saliva samples. So, using this caries activity test, differentiating the low risk group and high-risk group with mean df teeth can be applied with a large number of the children. In this study, we considered that the difference of the mean df teeth was over twice in the plaque and saliva samples, thus making the CAT Test feasible for collecting plaque in children.

Conclusion

Although an early color change was observed in the saliva sampling procedure compared to the plaque sampling, this change became almost similar after the 48-hour incubation period. Because of a high correlation of df teeth in plaque sampling in contrast to saliva sampling, plaque sampling, which is easy, convenient and practical, especially when used in young children, becomes much more advantageous than saliva sampling.

References

1) Miller, W.D.: Microorganisms of the Human Mouth. S.S. White Dental Mfg. Co., Philadelphia, 1890.
2) Socransky, S.S.: Caries-susceptibility tests. *Ann NY Acad Sci* **153**: 137–146, 1968.
3) Bowen, W.H.: Caries activity tests. *Int Dent J* **19**: 267–272, 1969.
4) Keyes, P.H.: Present and future measures for dental caries control. *JADA* **79**: 1395–1404, 1973.
5) Powell, L.V.: Caries risk assessment: Relevance to the practitioner. *JADA* **129**: 349–353, 1998.
6) Core writing group members of the Ad Hoc Committee on Caries Prevention/Council on Access, Prevention and Inter-professional Relations: Treating caries as an infectious disease. *JADA* **128**: 2S–24S, 1995. (supplement)
7) Klock, B. and Krasse, B.: A comparison between different methods for prediction of caries activity. *Scand J Dent Res* **87**: 129–139, 1979.
8) Markku, L.: Saliva and dental caries: diagnostic tests for normal dental practice. *Int Dent J* **42**: 199–208, 1992.
9) Krasse, B.: Caries Risk: A practical guide for assessment and control. Quintessence Books, Chicago, 1985, p. 7.
10) Russel, J.I., McFarlane, T.W., Aitchison, T.C., Stephen, K.W. and Bruchell, C.K.: Prediction of dental caries in Scottish adolescents. *Community Dent Oral Epidemiol* **19**: 74–77, 1991.
11) Jensen, B. and Bratthall, D.: A new method for the estimation of Mutans Streptococci in human saliva. *J Dent Res* **68**: 468–471, 1989.
12) Crossner, C.-G.: Salivary Lactobacillus counts in the prediction of caries activity. *Community Dent Oral Epidemiol* **9**: 182–190, 1981.
13) Shimono, T. and Sobue, S.: A new colorimetric caries activity test. *Dental Outlook* **43**(6): 829–835, 1974. (in Japanese)
14) Matsumura, S., Shimamoto, T., Morisaki, I., Shimono, T. and Sobue, S.: Dental caries experience and dental caries activity by new caries susceptibility test (CARIOSTAT) of the children in the Okinawa Prefecture (Tarama Island of Miyako). *Jpn J Ped Dent* **18**: 612–617, 1980. (in Japanese)
15) Matsumura, S.: Bacteriological and epidemiological studies on the Cariostat for dental caries activity test. *Jpn J Ped Dent* **21**: 107–130, 1983. (in Japanese)
16) Okazaki, Y., Higashi, T., Murakami, T., Yamaoka, M., Okamoto, Y., Matsumura, M. and Shimono, T.: A study of the newly developed salivary buffer capacity test (CAT21 Buf Test) Part 1: The relationship between the salivary buffer capacity test and caries status in preschool children. *Jpn J Ped Dent* **39**: 91–96, 2001. (in Japanese)