Evaluation of the Proportion of Cariogenic Bacteria Associated with Dental Caries

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Abstract

Background: Public oral health surveys have shown that the prevalence of dental caries in adults is increasing worldwide, resulting in increased workload and costs of dental and other clinical oral health services. Total streptococci are broadly grouped into mutans streptococci and three other species. They constitute a majority of bacteria found in the mouth. Of the total streptococci, the group of bacteria that primarily causes dental caries is mutans streptococci, which consists of seven species. *Streptococcus mutans* (*S. mutans*) is the mutans streptococcus most strongly associated with dental caries. To date, no simple culture assay (kit) has been developed for detecting *S. mutans* using plaque samples for caries risk assessment. This study aimed to evaluate the association between *S. mutans* and dental caries in adults based on the number and proportion of cariogenic bacteria in toothbrush plaque samples by culture methods to obtain basic data to develop clinical and chairside culture assays for caries risk assessment.

Materials and Methods: Plaque samples from 164 adult volunteers were obtained using sterile toothbrushes. The ratio of *S. mutans* to total streptococci (Sm/TS ratio) was determined by counting the number of colonies by culture methods. The extent of dental caries and the relative risk based on bacterial counts were assessed.

Results: The differences between Sm/TS ratios (%) in caries-free, medium-caries, and caries-active groups were statistically significant (*p*=0.001). The difference in the number of decayed, missing, and filled teeth (DMFT) in the four risk groups, each defined by Sm/TS ratio (%), was statistically significant (*p*=0.01). The risk associated with Sm/TS ratio was more significantly associated with the number of DMFT than with the number of *S. mutans*, and this parameter was useful in the selection of high-risk dental caries subjects.

Conclusions: The results of the present study suggested a significant association between the levels of *S. mutans* and dental caries using dental plaque samples in adults and indicate that quantification of Sm/TS ratio is effective for detecting subjects with the severity of dental caries. This approach has the potential for previous study of the development of simple culture assay for a risk assessment that may be incorporated into future clinical or epidemiological studies measures for the improvement of oral health worldwide.

Keywords Dental caries; Plaque samples; *Streptococcus mutans*; Proportion of cariogenic bacteria; Culture method; High-risk dental caries subjects; Epidemiological study

Introduction

Public oral health surveys have shown that the prevalence of dental caries in adults is increasing worldwide, resulting in increased workload and costs of dental and other clinical oral health services [1-3].

Colonization of the tooth surface with *S. mutans*, which is both acidogenic and aciduric, is considered to be a major cause of dental caries [2,4,5]. For initiating carious lesions in the enamel, *S. mutans* forms colonies on the tooth surface, creating an environment that poses a high risk of dental caries [6]. However, much of the existing research on the frequency and distribution of *S. mutans* and its correlation with dental caries has focused on infants and children [7-23], and there are very few reports concerning adults [24-26].

To practice oral healthcare that focuses on the prevention of oral diseases, it is necessary to provide appropriate health instructions after predicting the risk of morbidity [2,27]. This can be accomplished by methods such as dental caries activity tests that are used to evaluate the risk of dental caries [28-30]. Among these tests, examinations using bacteriological methods are usually assessed on the basis of the quantification of the number of *S. mutans*. In contrast, some reports [31,32] have utilized bacterial counts to determine the proportion of cariogenic bacteria (*S. mutans* in total streptococci, *S. mutans*/total streptococci [Sm/TS] ratio), which in turn helps to assess the risk of
dental caries. However, to the best of our knowledge, no study has comparatively evaluated the individual risk of dental caries in a given population based on the quantification of the number of *S. mutans* and the Sm/TS ratio.

Stimulated saliva samples are commonly used to assess caries activity in the oral cavity because quantitative measurement of such samples is easy [28,30]. However, cariogenic bacteria are primarily present in plaque on the tooth surface, and hence, these bacteria must be counted in the plaque to assess caries activity [27,33]. In addition, a previous study demonstrated that toothbrush plaque samples (plaque suspensions) collected after brushing all the teeth can be quantitatively measured [31], but this study only investigated the validity of using such samples. A parameter must have a confirmed association with the occurrence of dental caries to be a useful evaluation standard for the assessment of caries activity [2,27,28]. To date, no studies have comparatively evaluated the usefulness of toothbrush plaque samples for assessing the risk of dental caries based on the number of *S. mutans* and Sm/TS ratio.

Obtaining samples from children and elderly individuals with commonly utilized methods (i.e., sample saliva while masticating on chewing gum) is challenging. However, the use of plaque as a specimen is problematic in terms of quantification and reproducibility. In the present study, we used a quantitative plaque analysis method using brushing that easily obtains samples from children and elderly individuals.

Simple culture kits are commercially available and used in clinical or epidemiological studies [2,27,28]. These kits can conveniently detect and evaluate *mutans streptococci* at the chairside without expensive equipment or facilities, but these use saliva samples and detect only *mutans streptococci*, which comprises seven types of bacteria. Therefore, such kits cannot be used to evaluate only *S. mutans*, which are the most critical pathogenic bacteria that causes dental caries. A previous study reported a monoclonal antibody test using immunochromatography that selectively detects *S. mutans* [28]. Although a measurement kit using immunochemical methods instead of culture methods was made commercially available, the sale was discontinued because of imprecision and high cost.

This pilot study investigated basic data to develop a clinical and chairside culture assay (kits) with higher precision for a risk assessment using quantitative plaque and Sm/TS ratio analyses of cariogenic bacteria without polymerase chain reaction or immunohistochemical methods. The aim of this study was to evaluate associations between dental caries and levels of *S. mutans* in adults based on the Sm/TS ratio and using dental plaque samples harvested from sterile toothbrushes.

**Materials and Methods**

**Subjects and preparation of plaque samples**

A cross-sectional study was conducted in an adult population sample at Nihon University School of Dentistry at Matsudo. In total, 164 adult volunteers in good physical condition and oral health and aged 21–27 years were enrolled as experimental subjects. Investigation of caries status and collection of plaque samples were performed at our university. Subjects with any systemic disease, those using medications affecting salivary secretions, and those taking antibiotics were excluded from the study. The selected individuals were instructed not to eat/drink, use a mouth wash, or smoke 3 h prior to their appointment. Subjects were informed about the aim of this study well in advance, and informed consent was obtained from each of them. This study was conducted with the approval of the Ethics Committee of the Nihon University School of Dentistry, Matsudo. Oral samples of brushing-plaque were successively collected from each subject by the following methods. A large portion of plaque was scraped off all their teeth by vigorous brushing for 1 min using a sterile toothbrush and was collected into a sterile bottle through a mouth rinse for 30 s with 5 ml phosphate-buffered saline and used as brushing-plaque sample [31].

**Bacterial analysis**

Mitis Salivarius agar (Difco, Detroit, MI, USA) containing 20% sucrose, 0.25 U bacitracin, and 1% tellurite, supplemented with 20 g/L yeast extract, 10 g/L colistin, 10 g/L nalidixic acid, and 4 g/L gramicidin, was used as a selective medium for total streptococci and *Mutans streptococci*, respectively [34]. Within 3 h of sampling, clinically isolated samples were disrupted by sonication (50 W, 20 s) using ultrasonic apparatus (5202 Type, Otake Works, Tokyo, Japan), serially diluted with chilled brain heart infusion broth, and inoculated on selective media using a spiral plating system (Model-D, Gunze Sangyo, Inc., Tokyo, Japan). After anaerobic incubation for 48 h, the number of total streptococci and *S. mutans* colonies on plates were counted. *S. mutans* could be visually distinguished on the basis of the morphology of colonies on the agar plates. The ratio of *S. mutans* to the total streptococci was determined by counting their colonies and designated as Sm/TS ratio [31,32].

**Investigation of caries experience and the classification of caries risk groups**

Subjects’ caries experience was determined according to WHO standards [35], and the presence of decayed, missing, and filled teeth (DMFT) was calculated and recorded for each subject.

Based on classification (mean ± standard) for DMFT (dental caries), *S. mutans* counts and the Sm/TS ratio of subjects from the caries-free group (DMFT=0) were compared with those of subjects from the caries-medium and caries-active groups used as controls. In addition, risk levels were categorized into four categories from highest to lowest bacterial levels, with bacterial levels as classification standards (in the descending order: risk 4, risk 3, risk 2, and risk 1). The number of DMFT for risk 4 (n=41), risk 3 (n=41), risk 2 (n=41), and risk 1 (n=41) were calculated (intergroup comparison).

**Statistical analysis**

Descriptive statistics and statistical analyses were performed using statistical software (SPSS 22.0, Inc., Chicago, IL, USA). The Mann–Whitney U test was used for comparison between the two groups. The Bonferroni test was used to perform comparisons among the three study groups and the four risk category groups. A probability (p) value of <0.05 was considered statistically significant.

**Results**

The detection rate of total streptococci for all subjects (n=164) was 100%. The mean detected bacterial number (10⁷ CFU/ml) of total streptococci was 9.95 ± 12.35 (mean ± SD), and the detection rate of *S. mutans* was 92.07%. The mean detected bacterial number (10⁵ CFU/ml) of *S. mutans* was 25.61 ± 85.49. The mean Sm/TS ratio (%)...
was 2.33 ± 5.03%. The mean number of DMFT for all subjects was 7.59 ± 6.01.

On the basis of the mean number of DMFT (mean number, 7.59), the subjects were classified into caries-free (DMFT=0, n=21), medium-caries (DMFT=7 and 8, n=18, control group), and caries-active groups (DMFT ≥ 16, n=19, control group). The number of total streptococci in the caries-free, medium-caries, and caries-active groups were 10.78 ± 16.34, 9.54 ± 6.94, and 9.22 ± 10.23, that of S. mutans were 1.64 ± 2.72, 9.21 ± 14.77, and 58.87 ± 131.34, and Sm/TS ratios were 0.32 ± 0.49, 1.31 ± 2.11, and 4.95 ± 3.75, respectively. The intergroup differences were statistically significant (p<0.001) for the Sm/TS ratio (Bonferroni test, Table 1).

### Table 1: Comparison of number of total streptococci, number of S. mutans, and Sm/TS ratio (%) between the control (caries-active and caries-medium) and caries-free groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Numbers of Total Streptococci (10^7 CFU/ml)</th>
<th>Numbers of S. mutans (10^5 CFU/ml)</th>
<th>Sm/TS ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caries active group</td>
<td>9.22 ± 10.23</td>
<td>58.87 ± 131.34</td>
<td>4.95 ± 3.75</td>
</tr>
<tr>
<td>Caries medium group</td>
<td>9.54 ± 6.94</td>
<td>9.21 ± 14.77</td>
<td>1.31 ± 2.11</td>
</tr>
<tr>
<td>Caries-free group</td>
<td>10.78 ± 16.34</td>
<td>1.64 ± 2.72</td>
<td>0.32 ± 0.49</td>
</tr>
</tbody>
</table>

***: p<0.001

The number of S. mutans (10^5 CFU/mL) in the four risk groups [risks 4, 3, 2, and 1 (n=41 each)] were 91.60 ± 154.18, 8.83 ± 3.93, 1.79 ± 0.79, and 0.23 ± 0.27, respectively. Moreover, the number of DMFT for the high-risk (risks 3 and 4, n=82) and low-risk (risks 1 and 2, n=82) groups (as defined by the number of S. mutans) were 10.34 ± 5.89 and 4.83 ± 4.76, respectively, demonstrating a significant difference between the groups (p<0.001). The Sm/TS ratios (%) of the four risk groups (risks 4, 3, 2, and 1 (n=41 each)) for caries risk were 7.51% ± 8.08%, 1.37% ± 0.45%, 0.38% ± 0.18%, and 0.05% ± 0.05%, respectively. The number of DMFT relating to the Sm/TS ratio in the caries-high-risk (risk 3 and 4, n=82) and caries-low-risk (risk 1 and 2, n=82) groups were 10.68 ± 4.49 and 5.94 ± 4.24, respectively, demonstrating a significant difference between the groups (p<0.001). The number of total streptococci (10^7 CFU/mL) in the four risk groups [risks 4, 3, 2, and 1 (n=41 each)] were 25.45 ± 16.17, 8.61 ± 2.18, 4.26 ± 0.82, and 1.50 ± 0.76, respectively. The number of DMFT relating to the number of total streptococci in the high-risk (risk 3 and 4, n=82) and low-risk (risk 1 and 2, n=82) groups were 7.78 ± 5.51 and 7.39 ± 6.49, respectively. However, the differences between the groups were not significant (Mann–Whitney U test, Figure 1).
The number of DMFT relating to the number of total streptococci in the four risk groups [risks 4, 3, 2, and 1 (n=41 each)] were 7.76 ± 5.85, 7.80 ± 5.23, 8.61 ± 6.88, and 6.17 ± 5.91, respectively, indicating no significant differences among these classes (NS, Bonferroni test, Figure 4).

Discussion

Total streptococci are broadly grouped into mutans streptococci and three other species, i.e., S. mitis, S. salivarius, and S. sanguis. They constitute a majority of bacteria found in the mouth. Of the total streptococci, the group of bacteria that primarily causes dental caries is mutans streptococci, which consists of seven species [27-29, 36-40]; seven cariogenic streptococcal species, namely S. mutans, S. sobrinus, S. rattus, S. cricetus, S. downei, S. maccace, and S. ferus, are responsible for dental caries formation, however, high caries activity has been shown to be strongly associated with the prevalence of S. sobrinus and S. mutans. S. mutans is detected in more than 90% of mouths, while S. sobrinus is detected in about 20%-30% by the culture technique. Accordingly, S. mutans was investigated in this study. Results from the comparative control groups of the present study conducted in adults suggested a weak association between total streptococci and presence of dental caries.

S. mutans infections in humans can begin with eruption of teeth [2, 27]. In most infants, infection is transferred from the mouths of family members, particularly the mother [41]. S. mutans then colonizes the tooth surfaces and increases with age. S. mutans is detected in a vast majority (>90%) of adults regardless of their country, race, sex, eating habits, or presence of dental caries [27]. Several clinical studies have reported a clear relationship between S. mutans levels in the saliva and the development of dental caries in populations with a relatively high risk of dental caries [42, 43]. To evaluate caries risk in terms of the bacterial factor, it is important to evaluate not only the total amount of S. mutans and S. sobrinus, but also the amounts of each. Several methods for detection and identification of S. mutans and S. sobrinus have been reported for selective media and subsequent biochemical or serological tests [27,28,34]. Polymerase chain reaction and the real-time quantitative polymerase chain reaction technique were found to be more sensitive for detection of S. mutans and cariogenic species compared to the traditional culture-based methods [27,28]. In this study, for comparison with simple culture commercial kits utilized in clinical studies and at the chair side, these similar agar medium (culture medium) is used. Based on the results of such studies, at present a simple kit for a risk assessment that uses such contemporary culture method to detect S. mutans is being researched and developed. In addition, the proportion of salivary S. mutans is significantly higher in patients with active caries than in those who are caries-free. Similar results have also been demonstrated in the dental plaque of children [44,45].

Saliva tests, which involve stimulating saliva production within a few minutes of chewing, are often performed in clinical studies [28,30]. Basically, mutans streptococci alone exist on the surface of teeth. When collecting stimulated saliva, dental plaque on the surface of teeth is rubbed off while chewing gum and wax, which allows the quantification of bacteria that have fallen off into the stimulated saliva [27,33]. When plaque is used as a sample, it is difficult to maintain a constant collection quantity, and the data obtained are unstable; therefore, saliva is more commonly used as a sample instead of plaque [2,28-30]. The issue with the previous method of measuring the quantity of bacteria from plaque was resolved by collecting plaque that is aggressively rubbed off the teeth surface by brushing to measure the quantity of bacteria therein [31].

The accurate evaluation of S. mutans levels is necessary to gain a precise understanding of the cariogenic strength of an individual’s plaque and to evaluate the proportion of S. mutans (cariogenic bacteria ratio) within it. Therefore, it is vital to identify people with highly cariogenic plaque in advance as being at a risk of dental caries, to create a plan to control and prevent dental caries, along with the implementation of control and prevention after treatment [2,27,30]. In the present study conducted in adults, when classified further into three subgroups (caries-active, caries-medium, and caries-free), the Sm/TS ratio was significantly associated with caries risk and was more useful than the number of S. mutans for the selection of high-risk subjects. There are various ways to prevent and manage S. mutans-related dental caries, depending on individual patient’s risk level [2,27]. Therefore, it is necessary to establish individual prevention and management programs that are suitable for the risk factors identified through an appropriate assessment [2,27]. It is extremely important to perform preliminary screening of patients who are at a high risk of developing dental caries. After a caries risk assessment, a caries prevention plan can be prepared to facilitate prevention and management of caries.

Once S. mutans infection occurs in the mouth, it is difficult to remove or reduce it. The most effective way of removing or reducing S. mutans infection/colonization is to physically treat the plaque with professional mechanical tooth cleaning and to chemically control cariogenic plaque in advance as being at a risk of dental caries, to create a plan to control and prevent dental caries, along with the implementation of control and prevention after treatment [2,27,30]. In other words, when considering the costs and invasiveness of the eradication treatment, it is important to accurately diagnose S. mutans infection levels and to perform treatment in high-risk subjects alone.

When subjects were classified into groups based on Sm/TS ratios and numbers of S. mutans, similar differences were observed in the experience of dental caries. However, in the more detailed four-group risk classification, the Sm/TS ratio was more significantly associated with the experience of dental caries and was useful for the selection of high-risk dental caries subjects. A major objective of diagnosing the risk of dental caries is to detect subjects who are at a high risk of dental caries so that they can receive preventive management. It would be
more effective to use the Sm/TS ratio for the detection of high-risk individuals in caries risk assessment.

This study involved no chronological analysis, and the sample size was insufficient. It is important for future studies to be conducted in a manner that would allow chronological sampling. In our current study, we were able to collect only a cross-section of the subjects’ colonization by *S. mutans*, a limitation that could hinder the interpretation of our findings of the subjects’ overall risk of developing caries. Furthermore, although our findings reached significance, they should be verified using a larger, more diverse sample size to represent regional differences because many of our subjects were localized to a single region. This study verified the use of quantitatively available plaque analysis methods and caries ratio analyses. Based on the results of such fundamental studies, a simple kit for a risk assessment that uses a culture method to detect *S. mutans* without using a stereomicroscope or immunochemical methods is being researched and developed.

**Conclusion**

In conclusion, the results of the present study suggested a significant association between *S mutans* levels and dental caries using toothbrush plaque samples in adults. In addition, the results indicated that it would be more effective to use quantification of the Sm/TS ratio because it is superior at detecting high-risk subjects with the severity of dental caries. This approach has the potential for previous study of the development of simple culture assay for a risk assessment that may be incorporated into future clinical or epidemiological studies measures for the improvement of oral health worldwide.

**Conflicts of Interest to Declare**

There are no conflicts of interest to declare.

**Ethical Approval**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed Consent**

Informed consent was obtained from all individual participants included in the study.

**Acknowledgments**

Not applicable.

**References**


