Association between Dental Caries in Adults and Evaluation of *Streptococcus Sobrinus* in Plaque Samples

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Abstract

Objective: *Streptococcus mutans (S. mutans)* and *Streptococcus sobrinus (S. sobrinus)* are known to play major roles in the development of dental caries. Several studies have demonstrated the superior cariogenic potential of *S. sobrinus*. To date, no simple assay (kit) has been developed for detecting *S. sobrinus* using plaque samples for caries risk assessment. Additionally, the association between the level of *S. sobrinus* and dental caries (caries risk) in adults is somewhat unclear. This study aimed to evaluate the association between *S. sobrinus* 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 assay for caries risk assessment. Design: Brushing-plaque samples were obtained from 225 healthy adult volunteers. Risk levels were categorized based on *S. mutans* /total streptococci (Sm/TS) and *S. sobrinus*/TS (Ss/TS) ratios. The presence of decayed, missing, and filled teeth (DMFT) was analyzed as a measure of caries history in public health. Results: DMFT in the detectable *S. mutans* group, non-detectable *S. mutans* group, detectable *S. sobrinus* group, and non-detectable *S. sobrinus* group were significantly different, respectively. DMFT for Ss/TS ratios in the high-risk, medium-risk, and low-risk groups were significantly different. DMFT were the highest in the groups that exhibited significantly higher Ss/TS ratio than Sm/TS ratio. Conclusions: The results of this study suggested a relationship between *S. sobrinus* levels and dental caries in adult subjects using plaque samples. Furthermore, the findings of this study showed that the quantity of *S. sobrinus* in dental plaque was more strongly associated with the severity of dental caries than the quantity of *S. mutans* and indicated that evaluation of the Ss/TS ratio was more useful than the total number of *S. sobrinus*.

Key Words: Dental caries, Streptococcus sobrinus, Epidemiology, Public health

Introduction

Dental caries is a bacterial infection characterized by the destruction of hard dental tissues by cariogenic bacteria living in the biofilm on the surface of teeth [1,2]. Total *streptococci*, which account for the majority of oral bacteria, are classified into four groups: *Mutans streptococci*, *Streptococcus mitis*, *Streptococcus salivarius*, and *Streptococcus sanguis* [3-5]. *Mutans streptococci* is an important pathogen for caries. Of the seven species of *Mutans streptococci* [3,4], *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*) are known to play major roles in the development of caries [6-10]. Despite reports that *S. mutans* and *S. sobrinus* are related to caries, most of the previous studies were performed in infants or school children [11-16]. There are very few reports associating these two microorganisms with caries in adults [17,18].

Several epidemiological studies have demonstrated the superior cariogenic potential of *S. sobrinus*, indicating that infection with this bacterium increases the risk of caries development [2,4,19]. Therefore, *S. sobrinus* is a significant cariogenic bacterium that requires a more detailed research. However, to date, several studies that have sought to assess caries risk only analyzed the presence or absence of *S. sobrinus* [1,2,4,5]. No reports exist on the dynamics of these bacteria when detected in plaque of adults who are at a specific risk of dental caries.

Simple and commercially available culture kits such as Dentocult SM™ have been used in clinical or epidemiological studies [3,4] and have been shown to detect *Mutans streptococci* at the chair-side without the need for expensive equipment. However, these kits use saliva as specimens and only allow for the detection of Mutans streptococci. Furthermore, the disadvantage of this method is that both *S. mutans* and *S. sobrinus*, which are important cariogenic bacteria, cannot be evaluated.

Some studies have proposed the use of a monoclonal antibody test using specific immunochromatography for the selective detection of *S. mutans* [4]. Measurement kits based on immunochemical methods in lieu of these culture methods have previously been available; however, due to issues such as measurement accuracy and high cost, their sale has currently been halted. Furthermore, to the best of our knowledge, no simple assay (kit) has been developed for the detection of *S. sobrinus* to date. Culture methods are generally used to provide a quantitative estimation of the number of cariogenic bacteria [1-5]. On the other hand, some studies have reported a method of evaluation based on the ratio of *S. mutans* to total streptococci (Sm/TS ratio) [20,21]. Unfortunately, the validity and reproducibility of the Sm/TS ratio have only been

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assessed in a limited number of samples, and no studies have used culture methods to investigate the standard value of this ratio of *S. sobrinus*/TS (Ss/TS)ratio) in relation to caries risk or the relationship between the standard value and dental caries.

In this pilot study, we analyzed basic data on the development of a simple culture assay that can be used for a more accurate detection of *S. sobrinus* in clinics and at the chair-side. We evaluated the cariogenic bacteria ratio using a quantitative dental plaque analysis method instead of polymerase chain reaction or immunochemistry. To improve the precision of the assessment of bacteriological factors, the association between *S. sobrinus* levels and dental caries was evaluated based on the number and ratio of cariogenic bacteria, which were detected from cultures of brushing-plaque samples obtained from adults.

Materials and Methods

Subjects and preparation of plaque samples

Overall, 225 adult volunteers (aged 21-39 years) in healthy physical condition and with good oral health were enrolled as experimental subjects conducted at the Nihon University School of Dentistry at Matsudo. Investigation of caries status and collection of plaque samples were performed at the university. Subjects with any systemic disease, those using medications affecting salivary secretions, and those taking antibiotics were excluded from the study. 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 [20].

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 [22]. Within 3 h after sampling, the clinically isolated samples were dispersed by sonication (50 W, 20 s) using an ultrasonic apparatus (5202 Type; Otake Works, Tokyo, Japan), serially diluted with chilled brain heart infusion (BHI) broth, and inoculated on the selective media using a spiral plating system (Model-D; Gunze Sangyo, Inc., Tokyo, Japan). After an anaerobic culture for 48 h, the number of total streptococci and Mutans streptococci colonies on the agar plates was counted. *S. mutans* and *S. sobrinus* could be distinguished based on the morphology of the colonies on the agar and BHI agar plates. The ratios of *S. mutans* and *S. sobrinus* to the total streptococci on the agar plates were determined by counting their colonies and were designated as *S. mutans*/total streptococci (Sm/TS) and *S. sobrinus*/total

streptococci (Ss/TS) ratios. When bacteria selection was difficult, a stereomicroscope was used to identify the bacteria [20,21].

Investigation of caries experience and classification of the caries risk groups

The subjects' caries experience was determined according to World Health Organization standards [23]. The presence of decayed, missing, and filled teeth (DMFT) was determined and recorded for each subject. Risk levels were categorized into two groups based on the bacterial levels of *S. sobrinus* and Ss/TS in a descending order: high-risk and low-risk groups. DMFT for the high-risk and low-risk groups in those with detectable numbers of *S. sobrinus* and Ss/TS were calculated. Similarly, risk levels were categorized into the following two groups based on bacterial levels of *S. mutans* and Sm/TS ratios in a descending order: high-risk and low-risk groups. DMFT for the high-risk and low-risk groups in those with detectable numbers of *S. mutans* and Sm/TS ratios were calculated. Risk levels were classified into three groups based on bacterial levels of *S. sobrinus* and Ss/TS in a descending order: high-risk, medium-risk, and low-risk groups. DMFT for the high-risk, medium-risk, and low-risk groups in those with detectable *S. sobrinus* and Ss/TS were calculated

Statistical analysis

Descriptive statistics and statistical analyses were performed using a statistical software package (SPSS 22.0, Inc., Chicago, IL, USA). The Mann–Whitney U test was used to compare mean values between two groups, whereas the Bonferroni test was used to compare values among the groups. Data are presented as mean values ± standard deviation (SD). A p-value <0.05 was considered statistically significant.

Results

DMFT values in the detectable *S. mutans* group (n=210), non-detectable *S. mutans* group (n=15), detectable *S. sobrinus* group (n=48), and non-detectable *S. sobrinus* group (n=177) were 8.11 ± 5.84, 4.93 ± 5.09, 10.63 ± 5.09, and 7.16 ± 5.81, respectively.
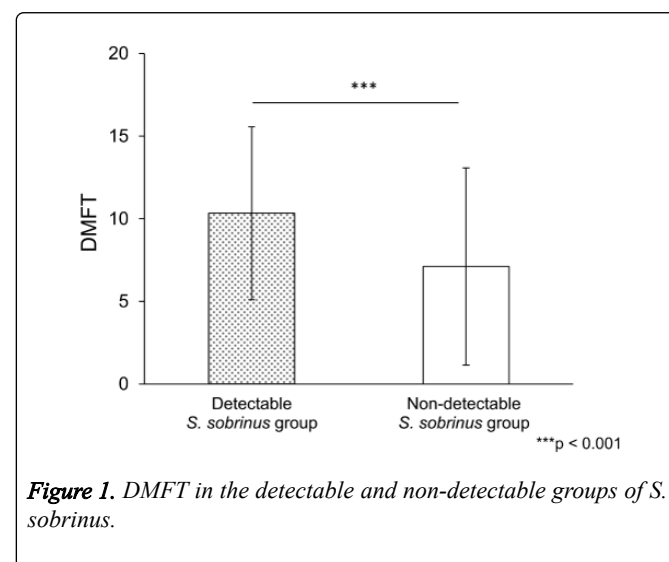

Figure 1. DMFT in the detectable and non-detectable groups of S. sobrinus.

The difference in DMFT values between detectable *S. sobrinus* and non-detectable *S. sobrinus* group was statistically significant (p<0.001; *Figure 1*). The difference in DMFT values between the detectable groups of *S. mutans* and *S. sobrinus* was statistically significant (p<0.01; *Figure 2*).

Figure 2. DMFT in the detectable groups of S. mutans and S. sobrinus.
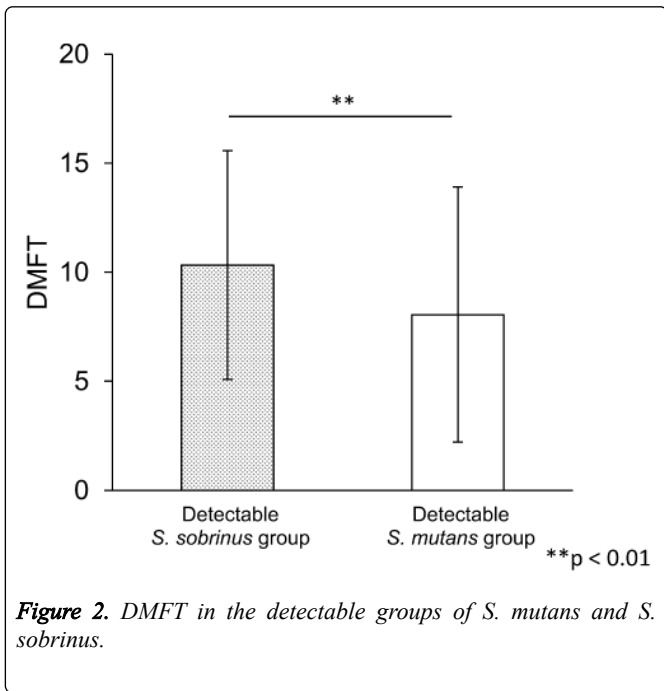
As shown in *Figure 3*, statistically significant DMFT values were noted in the high-risk (n=24) and low-risk (n=24) groups of subjects with Ss/TS ratios, and in the high-risk (n=24) and low-risk (n=24) groups of subjects with the numbers of *S. sobrinus* were 13.42 ± 3.97, 7.83 ± 4.58 and 13.54 ± 3.97, 7.71 ± 4.42, respectively (p<0.001; *Figure 3*).

Figure 3. DMFT in the high-risk and low-risk groups based on numbers of S. sobrinus and Ss/TS ratio (%).
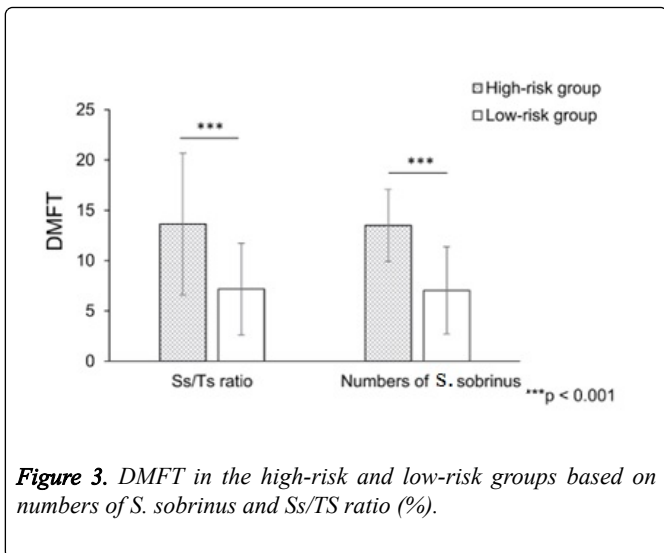
DMFT in subjects classified into three groups based on Ss/TS ratio (%), the high-risk (n=16), medium-risk (n=16), and low-risk (n=16) groups, were 15.00 ± 3.25, 10.75 ± 3.26, and 6.13 ± 4.26, respectively. The differences in values between these groups were statistically significant (p<0.01, p<0.001, respectively; *Figure 4*).
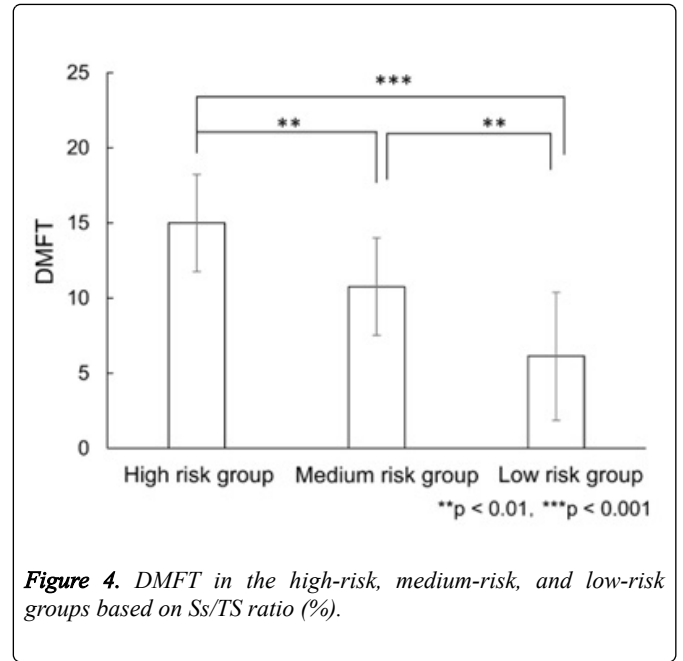

Figure 4. DMFT in the high-risk, medium-risk, and low-risk groups based on Ss/TS ratio (%).
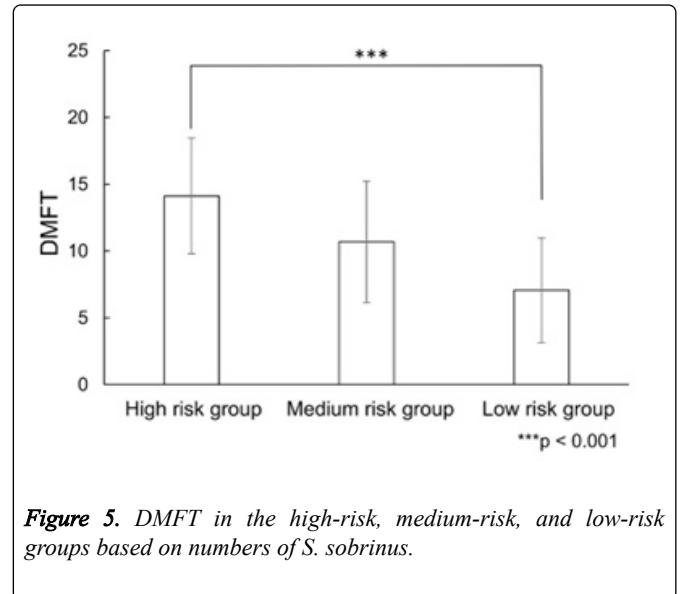

Figure 5. DMFT in the high-risk, medium-risk, and low-risk groups based on numbers of S. sobrinus.
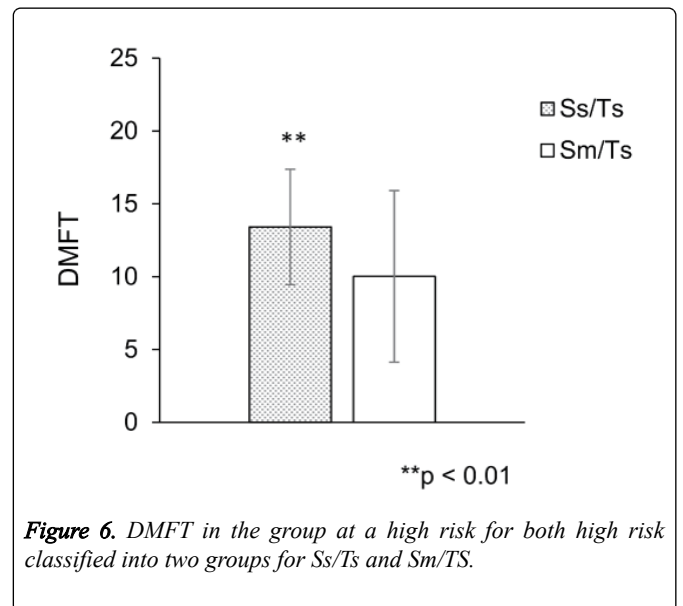

Figure 6. DMFT in the group at a high risk for both high risk classified into two groups for Ss/Ts and Sm/TS.

DMFT in subjects classified into three groups based on numbers of S. sobrinus, the high-risk (n=16), medium-risk

(n=16), and low-risk (n=16) groups, were 14.13 ± 4.33, 10.69 ± 4.53, and 7.06 ± 3.92, respectively; statistically significant differences in these values were noted between the groups (p<0.001; *Figure 5*).

In the ratio of cariogenic bacteria, DMFT of the group at high risk classified into two groups for Ss/TS (13.42 ± 3.97, n=24) was higher than that for Sm/TS (10.02 ± 5.89, n=105), demonstrating a significant difference (p<0.01; *Figure 6*).

Discussion

S. mutans and *S. sobrinus* have been strongly implicated in the development of dental caries in humans with numerous studies showing that the detection of these microorganisms is associated with more severe caries [13,24]. Moreover, several studies have indicated that *S. sobrinus* is more closely associated with caries activity than *S. mutans* [8,25,26]. Accordingly, detecting *S. sobrinus* may be important for the diagnosis and prevention of dental caries. In this study, for comparison with simple culture kits utilized in clinical studies and at the chair-side, similar agar medium (culture medium) is used. Till date, no study has described the evaluation criteria for bacterial number, and risk level of Ss/TS ratio in plaque for the diagnosis of dental caries risk using culture methods. *S. sobrinus* has been evaluated only in terms of its presence or absence in saliva, with no clear diagnostic criteria for bacterial counts. Using this study results as basic data, future studies involving a larger sample size are required to develop evaluation criteria for risk level assessment based on *S. sobrinus* counts and Ss/TS ratio and compare them with those of *S. mutans*.

There was a significant difference in the bacterial numbers of *S. sobrinus* and Ss/TS between the high-risk and low-risk groups. Moreover, we observed a significant difference in dental caries among subjects with detectable *S. sobrinus*. To date, caries activity and dental caries risk were examined only by analyzing the presence or absence of *S. sobrinus* in cultures [1-5]. The present study demonstrated a significant difference in dental caries depending on *S. sobrinus* levels among subjects with detectable *S. sobrinus*. Furthermore, the analysis of Ss/TS and number of *S. sobrinus* seems necessary to improve the accuracy of diagnosing dental caries risk and analyzing the number of subjects in whom *S. sobrinus* is detected.

An association classified into two groups was detected between the levels of *S. sobrinus* and DMFT; therefore, in the risk assay with classification into three groups, the evaluation of Ss/TS ratio would be more effective. In this study, DMFT were higher in subjects with high levels of *S. sobrinus* than in those with high levels of *S. mutans*. This suggests that evaluation of *S. sobrinus* levels is important for the identification of high-risk caries subjects. In future, larger studies should be performed to clarify the etiological relationship between cariogenic bacteria and caries risk assessment by prospectively investigating the association between caries activity and development of caries. The usefulness of a quantitative plaque analysis and caries ratio analysis was demonstrated. Based on these results, a simple assay for caries risk assessment is under development that will detect *S. sobrinus* by culture.

Conclusion

DMFT (dental caries) was greater in brushing-plaque samples obtained from adults with high levels of *S. sobrinus*. The results of this study suggested a relationship between *S. sobrinus* levels and dental caries in adult subjects with detectable bacteria in the plaque via culture methods. Furthermore, the findings of this study for the identification of high-risk caries subjects showed that the levels of *S. sobrinus* in dental plaque were more strongly associated with the severity of dental caries than the levels of *S. mutans* and indicated that the evaluation of Ss/TS ratio was more useful than the total number of *S. sobrinus*.

Conflict of interest

There are no conflicts of interest to declare.

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