Distribution and Evaluation of *Streptococcus sobrinus* in Saliva Samples

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**Introduction**

*Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*) have important roles in the development of dental caries in humans (1–4). Various studies have reported on the association of both *S. mutans* and *S. sobrinus* with more extensive caries (5–7), although several studies have demonstrated that the detection of *S. sobrinus* is more closely related to caries activity than is the detection of *S. mutans* (8, 9). In any case, the detection of these two species in the oral flora during early childhood is potentially useful for the diagnosis and prevention of dental caries.

With a few exceptions, *S. mutans* and *S. sobrinus*, among mutans streptococci, can be isolated from the hu-
man oral cavity (1). Thus, it is imperative to elucidate the properties and behaviors of these two species from the perspective of clinical dentistry and the prevention of caries. Both S. sobrinus and S. mutans have equal ability to ferment mannitol. They synthesize insoluble glucan from sucrose, forming a robust biofilm on the smooth surface of the wall of a glass tube, thereby conferring resistance to bacitracin (1, 4). However, S. sobrinus varies in serotype, hemolytic nature, glucan aggregation ability, and other aspects, which distinguishes its properties and functions from those of S. mutans (10). In addition, S. sobrinus exhibits significant cariogenicity correlating with smooth surface caries. Despite the need to illustrate the behavior of S. sobrinus and its correlation with caries, little research has been performed to assess the utility of the distribution and reference levels of S. sobrinus in caries activity tests and risk diagnoses.

The quantitative determination of the number of cariogenic bacteria is generally based on culture methods, although some studies have utilized the S. mutans /total streptococci (Sm/TS) ratio (11, 12). However, limited data are available on the validity and reproducibility of the Sm/TS ratio, and no culture-based investigation has been performed to determine the relationship between the standard value of the S. sobrinus/TS (Ss/TS) ratio and the risk of dental caries. The prevention of transmission of cariogenic bacteria is incorporated in food and nutrition education and support in maternal and child health. To further manage and prevent such transmission, health workers of interprofessional collaboration should also include infants in their healthcare plan, determine the number of S. mutans and S. sobrinus in the saliva of adults in their 20s to 30s, and manage the cariogenic risks. This preliminary study aimed to investigate and comparatively assess the Ss/TS ratio and caries bacteria level using saliva in their 20s to accumulate further primary data to develop a simple culture assay for caries risk assessment.

Materials and Methods

Subjects and sample preparation

We conducted a cross-sectional study with an adult population sample at the Nihon University School of Dentistry in Matsudo, Japan. In total, 36 adult volunteers aged 21–29 years who had good physical condition and oral health were enrolled. Participants with any systemic disease and who used medications affecting salivary secretion or antibiotics were excluded. The selected participants were instructed not to eat, drink, use a mouthwash, or smoke 3 h before their appointment. They were informed regarding the study objectives in advance, and all of them provided informed consent. This study was conducted with the approval of the Ethics Committee of the Nihon University School of Dentistry, Matsudo, Japan. Using the following methods, oral samples of stimulated saliva were successively collected from each subject. Saliva was stimulated by chewing paraffin gum and secreted over a period of 5 min while being collected in a sterile bottle, chilled on ice, and used as a stimulated saliva sample (11, 13, 14).

Bacterial analysis

Mitis Salivarius Agar (Difco, Detroit, MI, USA) containing 20 % sucrose, 0.25 U bacitracin, and 1 % tellurite and 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 (15). Within 3 h of sampling, clinically isolated samples were disrupted by sonication (50 W, 20 s) using an 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). Following anaerobic incubation for 48 h, the number of S. sobrinus colonies on plates was counted. S. sobrinus could be visually distinguished according to colony morphology on the agar plates. In cases where differentiation was difficult, enzyme-linked immunosorbent assay (ELISA) was used. The Ss/TS ratio was determined by counting the colonies (11, 14).

Statistical analysis

Descriptive and statistical analyses were performed using IBM SPSS version 26.0 (SPSS IBM Corp, Chicago, IL, USA). The Mann–Whitney U-test was used to compare the values between the two groups, whereas the Bonferroni test was used to compare values among the three groups; the Spearman correlation coefficient was used for correlation analysis. Data are presented as mean ± standard deviation (SD), and p values of <0.05 were considered statistically significant.

Results

The mean number of S. sobrinus $[10^5$ colony-forming
unit (CFU) /mL] (mean±SD) in saliva samples was 6.705±17.174. The Ss/TS ratio(%) in saliva samples was 0.301±0.654.

Table 1 shows the median (minimum, maximum) of the number of S. sobrinus \(10^5\) (CFU)/mL and the Ss/TS ratio(%) in saliva samples. We found a positive correlation between the number of S. sobrinus \(10^5\) CFU/mL and between the Ss/TS ratio(%) (r = 0.89; p < 0.001) in the saliva samples.

As shown in Table 2, statistically significant values were noted in the high-risk (n=18) and low-risk (n=18) groups of subjects with the number of S. sobrinus \(10^5\) CFU/mL (mean ± SD) were 13.054±22.842, 0.356±0.339 (p<0.001). Values in subjects classified into three groups based on the number of S. sobrinus \(10^5\) CFU/mL (mean±SD), the high-risk (n=12), medium-risk (n=12), and low-risk (n=12) groups, were 18.746±26.461, 1.213±0.617, and 0.158±0.146, respectively. The differences in values between these groups were statistically significant (p<0.05, respectively).

As shown in Table 3, statistically significant values were noted in the high-risk (n=18) and low-risk (n=18) groups of subjects with the Ss/TS ratio(%) (mean ± SD) were 0.578±0.847, 0.024±0.023 (p<0.001). Values in subjects classified into three groups based on the Ss/TS ratio(%) (mean ± SD), the high-risk (n=12), medium-risk (n=12), and low-risk (n=12) groups, were 0.809±0.965, 0.081±0.042, and 0.012±0.009, respectively. The differences in values between these groups were statistically significant (p<0.01, respectively).
Discussion

Enamel caries is broadly categorized into smooth surface and pit-and-fissure caries (1,4). While smooth surface caries has a propensity for decay at the cervix and adjacent surfaces, pit-and-fissure caries is characterized by decay in small, deep fissures. It has been reported that a high frequency of mutans streptococci can be isolated from enamel caries lesions. However, the viscous insoluble glucan synthesized from sucrose by mutans streptococci plays a significant role in the development of smooth surface caries (1,10).

Although S. sobrinus and S. mutans share similar abilities to synthesize insoluble glucan from sucrose, S. sobrinus has four different glucosyltransferases (GTFs), namely GTF-I, GTF-U, GTF-T, and GTF-S, for synthesizing glucan (10). These enzymes separate and isolate bacterial cells without binding to the cells in liquid medium without sucrose. Notably, both the amount of GTFs and the primary protein structure in S. sobrinus are significantly different from those of S. mutans. In particular, a joint action by GTF-I and GTF-T produces insoluble glucan with a considerable adhesive force. In addition, S. sobrinus is known to be significantly associated with smooth surface caries and has higher cariogenicity than S. mutans (8–10). Therefore, it is essential to clarify the distribution, level, and behavior of S. sobrinus.

In caries risk assessments, risk factors are often classified into several risk levels. The results of this study indicated that when classifying into two risk levels according to the Streptococcus sobrinus (Ss) bacterial count analysis, it is appropriate to classify Ss bacterial counts of ≥1.0 × 10^5 and ≤1.0 × 10^5 as high and low risks, respectively. Similarly, when classifying into three levels, Ss bacterial counts of ≥2.0 × 10^5, 0.5 × 10^5–2.0 × 10^5, and ≤0.5 × 10^5 are classified as high, medium, and low risks, respectively. In contrast, when classifying risks according to the ratio of Ss to total Streptococci (Ts), it was shown to be appropriate to classify the risk levels in either two categories (≥0.1 % and ≤0.1 % as high and low risks, respectively) or three categories (≥0.2 %, 0.02 %–0.2 %, and ≤0.02 %, as high, medium, and low risks, respectively). Our study results revealed a high correlation at r = 0.89 between the Ss bacterial counts and the Ss/Ts ratios for evaluation, it is advantageous to keep the coefficient of variation relatively low. The number of subjects in this study was low. We plan to examine more cases comprehensively and comparatively with other caries risk factors to collect data for the establishment of criteria for the development of a simple detection kit that can be used in dental check-ups in the field of preventive dentistry in the future.

In conclusion, the results of the present study suggest that cell cultures can be used to compare and estimate the number of S. sobrinus and the Ss/Ts ratio in saliva as well as to estimate reference values to assess whether individuals are at high risk of developing caries. In addition, with these results, we have accumulated further primary data for developing a simple culture kit using S. sobrinus for caries risk assessment.

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Conflict of Interest

There are no conflicts of interest to declare.

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