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Risk behaviors and their association with presence of *S. mutans* or *S. sobrinus* and caries activity in 18-month-old Japanese children

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Abstract The purpose of this study was to investigate risk behaviors associated with the presence of *S. mutans* or *S. sobrinus* and caries activity. The subjects were 448 mother-child pairs who underwent dental health examinations between February 2004 and November 2004 when the children were 18 months old. Caries activity was assessed by the Cariostat test. The presence of *S. mutans* and *S. sobrinus* was detected using PCR techniques. Questionnaires regarding risk behaviors were completed by the mothers. A statistically significant correlation was found for the detection of *S. mutans* and/or *S. sobrinus* in children and mothers ($P<0.01$). High-risk mothers were more likely to have high-risk children ($P<0.001$). In children in whom bacteria were detected, breast-feeding was ranked as the most important risk factor ($P<0.01$), followed by eating snacks while playing ($P<0.01$), getting snacks from neighbors ($P<0.05$), being cared for by grandparents ($P<0.05$) and pre-chewing of children's food by mothers ($P<0.05$). In children with high caries risk, breast-feeding and pre-chewing were the most important risk factors ($P<0.01$), followed by taking meals at irregular intervals and mothers not attending maternity classes ($P<0.05$).

Key words
18-month-old,
Caries activity,
Risk behaviors,
S. mutans,
S. sobrinus

Introduction

Mutans Streptococci (MS) include the species *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*) and are the main bacteria responsible for dental caries in humans[1]. Caufield *et al.* suggested that the acquisition of MS in young children most likely takes place during a "window of infectivity" from 19 to 31 months of age[2]. MS infection is essential but not sufficient for the development of dental caries, which is exacerbated by risk behaviors related to dental caries (*e.g.*, dietary factors)[3,4]. In Japan, dental health check-ups for 18-month-old child have been mandatory since 1977. This age is very important, and it is critical to perform dental hygiene guidance for caregivers of children at this time.

The Cariostat® test, a caries activity test, can predict the occurrence of caries with good validity and reliability in young children[5]. The test is also effective for screening high-risk populations in young children[6].

The PCR (polymerase chain reaction) method have been used for the detection and identification of two human cariogenic species, *S. mutans* and *S. sobrinus*, using two primer pairs (SD10/SD20 and SOF14/SOR1623) designed by Igarashi *et al.*[7,8] Moreover, using the PCR technique, Rodis *et al.*[9] investigated the presence of *S. mutans* or *S. sobrinus* in Cariostat-inoculated plaque samples obtained from Japanese mother-child pairs. Presently, there

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have been no reports on the relationship between caries activity and risk behaviors using the PCR technique for detection of *S. mutans* or *S. sobrinus* bacteria.

Therefore, the aims of this study were to determine the relationship between the relative risk behaviors of *S. mutans* and/or *S. sobrinus* in Japanese mothers and their 18-month-old children and caries activity.

Subjects and Methods

Clinical examinations

The population for this study consisted of 448 mother-child pairs who underwent dental health examinations between February 2004 and November 2004 when the children were 18 months old. The study was conducted at a health center in Katano City of Osaka, Japan. The oral examinations for all children were conducted by the same dentist. The children were examined using dental mirrors and explorers under natural light. Caries were assessed in accordance with the criteria of the Health Policy Bureau, Ministry of Health and Welfare, Japan[10]. No radiographs were taken.

Microbiological examinations

The buccal surfaces of the maxillary teeth of all mothers and their children were swabbed by using sterile cotton-tipped applicators. Each applicator with the dental plaque sample was put into the Cariostat medium (Sankin Co., Japan) and incubated at 37°C for 48 hrs. After incubation, colorimetric changes were classified into seven grades using the original four grade standard color sample. After the judgment of scores, the samples were stored in a refrigerator at 4°C until DNA analysis. Within approximately one month, the samples were transferred in an ice box to a laboratory of the school of dentistry, at Okayama University for examination of the presence of *S. mutans* and *S. sobrinus* using a PCR technique.

Bacterial DNA extraction was performed using the Qiagen DNeasy® Tissue kit for purification of DNA from Gram-positive bacteria.

S. mutans ATCC 25175 and *S. sobrinus* ATCC 33478 were used as reference strains. The primer pairs SD10/SD20 and SOF14/SOR1623 were used in this study because they amplified species-specific amplicons with different lengths. The concentration of SD10/SD20 and SOF14/SOR1623 were 32.9/30.1μg/OD and 33.9/33.1μg/OD, respectively. The sequences were 5′-TAT GCT GCT ATT GGA GGT TC-3′ (positions 973 to 992)/5′-AAG GTT GAG CAA TTG AAT CG-3′ (positions 2225 to 2224) and 5′-TGC TAT CTT TCC CTA GCA TG-3′ (positions 134–153)/5′-GGT ATT CGG TTT GAC TGC-3′ (positions 1743–1726), respectively. This indicated that the present PCR method is useful for detection and identification of the two human cariogenic species *S. mutans* and *S. sobrinus*. PCR detection of *S. mutans* and *S. sobrinus* was performed using the procedure described by Igarashi *et al.*[7,8] Each PCR mixture (20μl) consisted of 2μl of 10×PCR buffer, 1.6μl of dNTP mixture, 0.1μl of *Taq* DNA polymerase (*Takara Taq*™), 5.9μl of distilled Water (GIBCO™), 10μl template solution and 2μl each of the primer pairs. The PCR conditions were denaturation at 95°C for 3 min, followed by 26 cycles of denaturation of 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min. The last cycle comprised of 94°C for 1 min, 55°C for 1 min and 72°C for 5 min[7]. After amplification, the PCR products were analyzed by gel electrophoresis in a 1% agarose gel containing 10 mg/m*l* ethidium bromide and visualized by ultraviolet light. The presence or absence of bands was noted.

Behavioral and dietary survey

A structured questionnaire was completed by the mothers and checked by a hygienist in face-to-face interviews. It was designed to collect information related to oral hygiene habits and dietary histories of the children. They received dental health check points as 18-month-old children. The 12-item questionnaire designed to assess child feeding practices, dietary history, snack food frequency, and oral hygiene practices of the child, included the following questions: “Who takes care of your child in the daytime?”, “Is your child currently breast-fed?”, “Is your child using a bottle now?”, “Does your child have his/her own toothbrush?”, “Do you clean your child’s teeth?”, “Does your child take three well-balanced meals a day?”, “Do you chew your child’s food before giving it to your child?”, “Do you have a set time for snacks for the child?”, “How often does your child eat snacks between meals in a given day?”, “Does your child eat snacks while playing?”, “Does your child get snacks from neighbors?”, and “Did you ever attend a maternity class?”.

Data analysis

All of the data were entered into the SPSS 11.0