

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

Ying Ji, Omar Marianito Maningo Rodis, Masahiko Hori, Yukie Nakai, Naoyuki Kariya, Seishi Matsumura and Tsutomu Shimono

Department of Behavioral Pediatric Dentistry,  
Graduate School of Medicine and Dentistry, Okayama University  
2-5-1 Shikata-cho, Okayama 700-8525, JAPAN

**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<sup>1</sup>. 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<sup>2</sup>. 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)<sup>3,4</sup>. 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<sup>®</sup> test, a caries activity test, can predict the occurrence of caries with good validity and reliability in young children<sup>5</sup>. The test is also effective for screening high-risk populations in young children<sup>6</sup>.

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.*<sup>7,8</sup> Moreover, using the PCR technique, Rodis *et al.*<sup>9</sup> investigated the presence of *S. mutans* or *S. sobrinus* in Cariostat-inoculated plaque samples obtained from Japanese mother-child pairs. Presently, there

Received on April 4, 2005

Accepted on July 8, 2005

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<sup>10</sup>. 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<sup>®</sup> 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 32.9/30.1 µg/OD and 33.9/33.1 µg/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.*<sup>7,8)</sup> Each PCR mixture (20 µl) consisted of 2 µl of 10×PCR buffer, 1.6 µl of dNTP mixture, 0.1 µl of *Taq* DNA polymerase (*Takara Taq*<sup>™</sup>), 5.9 µl of distilled Water (GIBCO<sup>™</sup>), 10 µl template solution and 2 µl 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<sup>7)</sup>. After amplification, the PCR products were analyzed by gel electrophoresis in a 1% agarose gel containing 10 mg/ml 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

Table 1 The relationship between detection of *S. mutans* and/or *S. sobrinus* and risk behaviors for children

| Factors                               | <i>S. mutans</i> and/or <i>S. sobrinus</i><br>Detection in children |         | <i>S. mutans</i> and/or <i>S. sobrinus</i><br>Non-detection in children |         | P value |
|---------------------------------------|---|---------|---|---------|---------|
|                                       | (n = 350)   |         | (n = 98)  |         |         |
|                                       | N   | (%)     | N   | (%)     |         |
| Gender of children                    |   |         |   |         |         |
| Male                                  | 171   | (48.9%) | 179   | (51.1%) | n.s.    |
| Female                                | 44  | (44.9%) | 54  | (55.1%) |         |
| Child's caries risk level             |   |         |   |         |         |
| High risk                             | 51  | (21.0%) | 192   | (79.0%) | n.s.    |
| Low risk                              | 47  | (22.9%) | 158   | (77.1%) |         |
| Mother's caries risk level            |   |         |   |         |         |
| High risk                             | 61  | (26.1%) | 173   | (73.9%) | <0.05   |
| Low risk                              | 37  | (17.3%) | 177   | (82.7%) |         |
| Mother's cariogenic bacteria          |   |         |   |         |         |
| Present                               | 68  | (26.8%) | 186   | (73.2%) | <0.01   |
| Absent                                | 30  | (15.5%) | 164   | (84.5%) |         |
| Daytime Caregivers                    |   |         |   |         |         |
| Kindergarten                          | 12  | (17.9%) | 55  | (82.1%) | n.s.    |
| Parents                               | 75  | (21.4%) | 276   | (78.6%) |         |
| Grandparents                          | 11  | (36.7%) | 19  | (63.3%) |         |
| Breast-feeding                        |   |         |   |         |         |
| No                                    | 77  | (19.9%) | 309   | (80.1%) | <0.01   |
| Fed while awake                       | 2   | (100%)  | 0   | (0%)    |         |
| Before sleeping or after going to bed | 19  | (31.7%) | 41  | (68.3%) |         |
| Bottle-feeding                        |   |         |   |         |         |
| No                                    | 77  | (22.4%) | 267   | (77.6%) | n.s.    |
| With milk or juice                    | 21  | (20.2%) | 83  | (79.8%) |         |
| Brushing by child                     |   |         |   |         |         |
| Yes                                   | 83  | (22.7%) | 282   | (77.3%) | n.s.    |
| No                                    | 15  | (18.1%) | 68  | (81.9%) |         |
| Brushing by mother                    |   |         |   |         |         |
| Yes or sometimes                      | 88  | (21.4%) | 323   | (78.6%) | n.s.    |
| No                                    | 10  | (27.0%) | 27  | (73.0%) |         |
| Taking meals at regular times         |   |         |   |         |         |
| Yes                                   | 59  | (19.7%) | 241   | (80.3%) | n.s.    |
| No                                    | 39  | (26.4%) | 109   | (73.6%) |         |
| Pre-chewing                           |   |         |   |         |         |
| No or sometimes                       | 78  | (20.2%) | 309   | (79.8%) | <0.05   |
| Yes                                   | 20  | (32.8%) | 41  | (67.2%) |         |
| Setting time for snack                |   |         |   |         |         |
| Yes                                   | 77  | (21.5%) | 281   | (78.5%) | n.s.    |
| No                                    | 21  | (23.3%) | 69  | (76.7%) |         |
| Frequency of snack                    |   |         |   |         |         |
| $\leq 2$ times/day                    | 84  | (21.3%) | 310   | (78.7%) | n.s.    |
| $\geq 3$ times/day                    | 14  | (25.9%) | 40  | (74.1%) |         |
| Eating snacks while playing           |   |         |   |         |         |
| No                                    | 46  | (17.2%) | 222   | (82.8%) | <0.01   |
| Yes                                   | 52  | (28.9%) | 128   | (71.1%) |         |
| Getting snacks from neighbors         |   |         |   |         |         |
| No                                    | 44  | (18.0%) | 201   | (82.0%) | <0.05   |
| Yes                                   | 54  | (26.6%) | 149   | (73.4%) |         |
| Caries                                |   |         |   |         |         |
| No                                    | 90  | (20.5%) | 350   | (79.5%) | <0.001  |
| Yes                                   | 8   | (100%)  | 0   | (0%)    |         |
| Attending maternity class             |   |         |   |         |         |
| Yes                                   | 4   | (14.3%) | 24  | (85.7%) | n.s.    |
| No                                    | 94  | (22.4%) | 326   | (77.6%) |         |

Chi-square test; n.s. = not statistically significant

software by the code numbers of the subjects for analysis. Chi-square tests with Spearman's rank-order correlation and odds ratios (95% CI) were used for statistical analysis. The children's group and the mother's group were each divided into two subgroups according to their Cariostat grades. Scores of 1.0 or lower were judged as low activity risk and 2.0 or higher as high activity risk for the children, and scores of 1.5 or lower were judged as low activity risk and 2.0 or higher as high activity risk for the mothers. Likewise, the children and mothers were divided by the presence or absence of *S. mutans* and *S. sobrinus* bands. Subjects with neither *S. mutans* nor *S. sobrinus* were assigned to the non-detection group and subjects with either *S. mutans* or *S. sobrinus* or with both *S. mutans* and *S. sobrinus* were assigned to the detection group.

**Results**

**Clinical examinations**

The total of 448 children included 233 boys (52%) and 215 girls (48%). Caries prevalence in the children was 1.8% (8/448). *S. mutans* or *S. sobrinus* was detected in all of the children with dental caries ( $P < 0.001$ ) (Table 1).

**Microbiological examinations**

1) Caries activity of children and their mothers  
 The large percentage of high-activity children with high-activity mothers was larger than the percentage of high-activity children with low-activity mothers ( $P < 0.001$ ) (Fig. 1).

2) Detection of *S. mutans* and/or *S. sobrinus* in children and their mothers

Neither *S. mutans* nor *S. sobrinus* was detected in 79% (354/448) of the children. Only *S. mutans* was detected in 16.7% (75/448) and only *S. sobrinus* was detected in 3.1% (14/448) of the children. Both *S. mutans* and *S. sobrinus* were detected in 1.1% (5/448) of the children (Fig. 2).

In the mothers, 42.6% (191/448) had neither *S. mutans* nor *S. sobrinus*, 47.3% (212/448) had either *S. mutans* or *S. sobrinus*, and 10.0% (45/448) had both *S. mutans* and *S. sobrinus*. The percentage of children with bacteria whose mothers had *S. mutans* and/or *S. sobrinus* was larger than the percentage of children with bacteria whose mothers had no bacteria ( $P < 0.01$ ) (Table 1), and the percentage of children with bacteria whose mothers had high caries activity was larger than the percentage of children with bacteria whose mothers had low caries

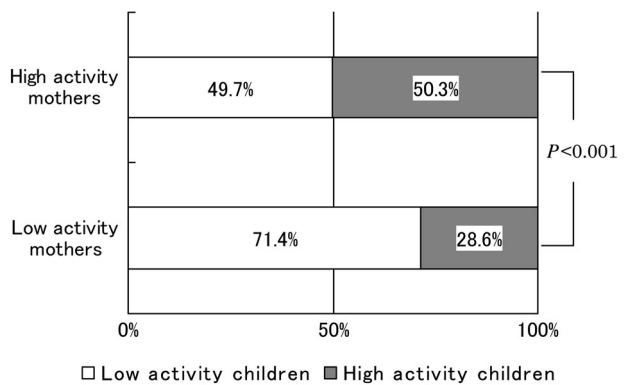


Fig. 1 Relationship of caries activity levels between children and their mothers

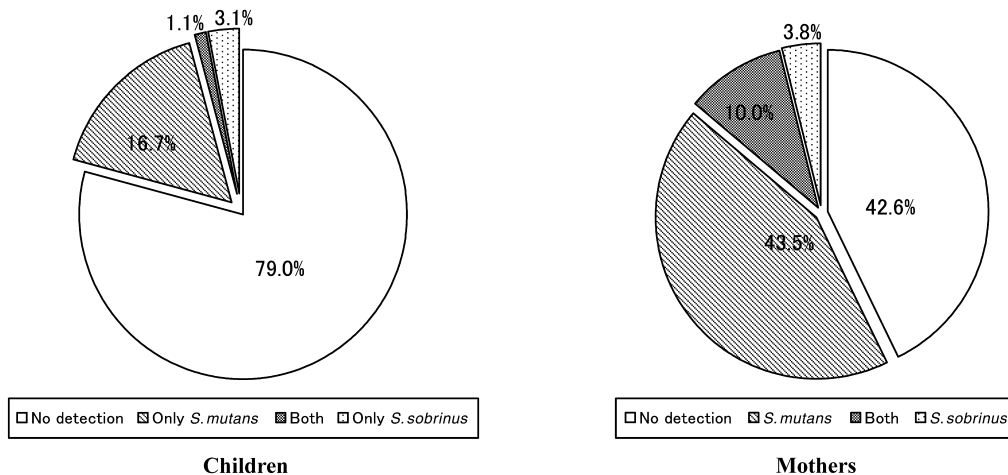


Fig. 2 Distribution of *S. mutans* or *S. sobrinus* detection in children and their mothers

activity ( $P<0.05$ ).

### Relationship between detection of *S. mutans* and/or *S. sobrinus* and feeding habits

#### 1) Breast feeding

The percentage of children with *S. mutans* and/or

*S. sobrinus* who received breast-feeding before sleeping or after going to bed was significantly larger than the percentage of children with *S. mutans* and/or *S. sobrinus* who received more than one feeding on waking or those who had already been weaned ( $P<0.01$ ) (Table 1).

Table 2 The relationship between caries activity and risk behaviors for children

| Factors                               | Caries activity<br>High risk in children |         | Caries activity<br>Low risk in children |         | P value |
|---------------------------------------|--|---------|---|---------|---------|
|                                       | (n = 137)                                |         | (n = 205)                               |         |         |
|                                       | N  | (%)     | N                                       | (%)     |         |
| Gender of children                    |  |         |   |         |         |
| Male                                  | 66                                       | (38.4%) | 106                                     | (61.6%) | n.s.    |
| Female                                | 71                                       | (41.8%) | 99                                      | (58.2%) |         |
| Mother's caries risk level            |  |         |   |         |         |
| High risk                             | 46                                       | (28.6%) | 115                                     | (71.4%) | <0.001  |
| Low risk                              | 91                                       | (51.3%) | 90                                      | (49.7%) |         |
| Daytime caregivers                    |  |         |   |         |         |
| Kindergarten                          | 18                                       | (34.6%) | 34                                      | (65.4%) | n.s.    |
| Parents                               | 106                                      | (40.0%) | 159                                     | (60.0%) |         |
| Grandparents                          | 13                                       | (52.0%) | 12                                      | (48.0%) |         |
| Breast-feeding                        |  |         |   |         |         |
| No                                    | 109                                      | (36.9%) | 186                                     | (63.1%) | <0.01   |
| Fed while awake                       | 0  | (0%)    | 1                                       | (100%)  |         |
| Before sleeping or after going to bed | 28                                       | (60.9%) | 18                                      | (39.1%) |         |
| Bottle-feeding                        |  |         |   |         |         |
| No                                    | 100                                      | (38.0%) | 163                                     | (62.0%) | n.s.    |
| With milk or juice                    | 37                                       | (46.8%) | 42                                      | (53.2%) |         |
| Brushing by child                     |  |         |   |         |         |
| Yes                                   | 111                                      | (40.1%) | 166                                     | (59.9%) | n.s.    |
| No                                    | 26                                       | (40.0%) | 39                                      | (60.0%) |         |
| Brushing by mother                    |  |         |   |         |         |
| Yes or sometimes                      | 127                                      | (40.3%) | 188                                     | (59.7%) | n.s.    |
| No                                    | 10                                       | (37.0%) | 17                                      | (63.0%) |         |
| Taking meals at regular times         |  |         |   |         |         |
| Yes                                   | 83                                       | (35.6%) | 150                                     | (64.4%) | <0.05   |
| No                                    | 54                                       | (49.5%) | 55                                      | (50.5%) |         |
| Pre-chewing                           |  |         |   |         |         |
| No or sometimes                       | 109                                      | (37.1%) | 185                                     | (62.9%) | <0.01   |
| Yes                                   | 28                                       | (58.3%) | 20                                      | (41.7%) |         |
| Setting time for snack                |  |         |   |         |         |
| Yes                                   | 103                                      | (38.1%) | 167                                     | (61.9%) | n.s.    |
| No                                    | 34                                       | (47.2%) | 38                                      | (52.8%) |         |
| Frequency of snack                    |  |         |   |         |         |
| $\leq 2$ times/day                    | 122                                      | (40.1%) | 182                                     | (59.9%) | n.s.    |
| $\geq 3$ times/day                    | 15                                       | (39.5%) | 23                                      | (60.5%) |         |
| Eating snacks while playing           |  |         |   |         |         |
| No                                    | 85                                       | (40.9%) | 123                                     | (59.1%) | n.s.    |
| Yes                                   | 52                                       | (38.8%) | 82                                      | (61.2%) |         |
| Getting snacks from neighbors         |  |         |   |         |         |
| No                                    | 74                                       | (40.7%) | 108                                     | (59.3%) | n.s.    |
| Yes                                   | 63                                       | (39.4%) | 97                                      | (60.6%) |         |
| Attending to maternity class          |  |         |   |         |         |
| Yes                                   | 3  | (15.0%) | 17                                      | (85.0%) | n.s.    |
| No                                    | 134                                      | (41.6%) | 188                                     | (58.4%) |         |

Chi-square test; n.s. = not statistically significant

2) Bottle feeding

There was no difference between the percentages of bottle-fed children with *S. mutans* and/or *S. sobrinus* and those without *S. mutans* and/or *S. sobrinus* ( $P>0.1$ ) (Table 1).

**Relationship between caries risk levels and feeding habits**

1) Breast feeding

As shown in Table 2, caries activity was significantly higher in children who received breast-feeding before sleeping or after going to bed than in those who received more than one feeding on waking or those who had already been weaned ( $P<0.01$ ) (Table 2).

2) Bottle feeding

The difference was not statistically significant ( $P>0.1$ ) for bottle feeding (Table 2).

**Relative risks for *S. mutans* and/or *S. sobrinus* detection and high caries activity in children**

As shown in Table 3, when grandparents were the daytime caregivers, the likelihood of *S. mutans* and/or *S. sobrinus* detection in children was 2.3 times higher (95% CI = 1.1–5.1). When the children were breast-fed while awake, before sleeping or after going to bed, the likelihood of detection was increased by 2.1 times (95% CI = 1.1–3.7) and high caries activity was increased by 2.5 times (95% CI = 1.3–4.7).

Table 3 Ranking of significant variables associated with PCR-detected of *S. mutans* and/or *S. sobrinus* and high caries activity of children

| Variables  | <i>S. mutans</i> and/or <i>S. sobrinus</i> |         | Caries risk          |         |
|--|--|---------|----------------------|---------|
|  | Adjusted OR (95% CI)                       | P value | Adjusted OR (95% CI) | P value |
| Daytime caregivers<br>Grandparents   | 2.3<br>(1.1–5.1)                           | <0.05   | 1.5<br>(0.7–3.5)     | n.s.    |
| Breast-feeding<br>Fed while awake<br>Before sleeping or after going to bed | 2.1<br>(1.1–3.7)                           | <0.05   | 2.5<br>(1.3–4.7)     | <0.01   |
| Bottle-feeding<br>With milk or juice                                       | 0.9<br>(0.5–1.5)                           | n.s.    | 1.4<br>(0.9–2.4)     | n.s.    |
| Brushing by child<br>No  | 0.7<br>(0.4–1.4)                           | n.s.    | 0.9<br>(0.6–1.7)     | n.s.    |
| Brushing by mother<br>No   | 1.4<br>(0.6–2.9)                           | n.s.    | 0.9<br>(0.4–1.9)     | n.s.    |
| Taking meals at regular times<br>No  | 1.5<br>(0.9–2.3)                           | n.s.    | 1.8<br>(1.1–2.8)     | <0.05   |
| Pre-chewing<br>Yes   | 1.9<br>(1.1–3.5)                           | <0.05   | 2.4<br>(1.3–4.4)     | <0.01   |
| Setting time for snack<br>No   | 1.1<br>(0.6–1.9)                           | n.s.    | 1.5<br>(0.9–2.4)     | n.s.    |
| Snacking while playing<br>≥3 times/day                                     | 1.3<br>(0.7–2.5)                           | n.s.    | 0.9<br>(0.5–1.9)     | n.s.    |
| Eating snacks while playing<br>Yes   | 2.0<br>(1.2–3.1)                           | <0.01   | 0.9<br>(0.6–1.4)     | n.s.    |
| Getting snacks from neighbors<br>Yes                                       | 1.7<br>(1.1–2.6)                           | <0.05   | 0.9<br>(0.6–1.5)     | n.s.    |
| Attending to maternity class<br>No   | 1.7<br>(0.6–5.1)                           | n.s.    | 4.0<br>(1.2–14.1)    | <0.05   |

P value were calculated by Spearman’s rank-order correlation analysis; n.s. = not statistically significant

Children who took meals at irregular times had 1.8 times higher (95% CI = 1.1–2.8) activity of caries than did children who ate at regular times. Moreover, mothers who chewed their children's food before giving it to their children were 1.9 times more likely (95% CI = 1.1–3.5) to have children in the detection group and 2.4 times more likely (95% CI = 1.3–4.4) to have higher risk children than were mothers who did not chew food for their children. In addition, the prevalence of *S. mutans* and/or *S. sobrinus* detection was increased by 2.0 times (95% CI = 1.2–3.1) when the children ate snacks while playing. Children who received snacks from neighbors had a 1.7 times higher (95% CI = 1.1–2.6) risk of detection than did children who did not receive snacks from neighbors. Children whose mothers did not attend maternity class had 4.0 times higher (95% CI = 1.2–14.1) caries activity than did children whose mothers received dental education from the Katano Health Center's maternity class.

## Discussion

Dental caries is an infectious disease caused by various factors, including cariogenic bacteria, fermentable carbohydrate in the diet, susceptible tooth and host, and time<sup>11,12</sup>. Knowledge of all risk factors related to caries in children is therefore important for determination of the optimal period for prevention and interceptive treatment. In this study, we found that only 1.8% of the children (8/448) had dental caries, much less than that in a previous study on factors related to dental caries in 18-month-old children<sup>13</sup>. This was probably due to the fact that the children in this study underwent dental health examinations at the ages of 6 months, 10 months, 18 months, 24 months and 36 months. Also, the mothers of the children with high caries activity had received dental guidance from the Katano Health Center.

It is now well recognized that MS transmission in children occurs with the mother as the primary main source of infection<sup>14–17</sup>, and poor maternal oral hygiene increases the likelihood of transmission of infection from mother to infants. Hence, in this study, a large number of children of mothers in the detection group showed a higher rate of *S. mutans* and/or *S. sobrinus* detection and higher caries activity than did children whose mothers were in the non-detection group or low risk level group. In addition, it was found that the mother is the primary

caregiver of bacteria to children by salivary contact<sup>2</sup>. This study also identified that children of mothers who chewed food for the child were likely to have cariogenic bacteria and had a high caries risk level.

The results of this study also showed that some risk behavior factors of 18-month-old children are significantly related to the detection of *S. mutans* and/or *S. sobrinus* and high caries activity. First, breast-fed children had a higher rate of detection and higher caries activity than did children who had been weaned. Tsubouchi *et al.*<sup>18</sup> reported that whether or not the child was weaned at 18 months of age was most closely related to infant caries. Second, with regard to dietary habits, analysis of our data showed that children who snacked while playing had a two-times higher detection rate of *S. mutans* and/or *S. sobrinus* than did children who did not snack while playing. Moreover, the habit of taking three meals every day at irregular times was also significantly related to high caries activity.

Although the source of *S. mutans* is usually the mother, recent studies have shown that children can acquire *S. mutans* from both inside and outside the family<sup>19,20</sup>. We also found that being cared for by grandparents and getting snacks from neighbors were strongly correlated to the detection of *S. mutans* and/or *S. sobrinus*. Other caregivers or relatives of children besides their parents may be the source of transmission of cariogenic bacteria. Hence, further study is needed.

An important finding in our study is that a significant correlation was seen between caries risk level and dental maternity class attendance. Dental maternity class in the Katano Health Center is a free dental education program for pregnant mothers. In this program, a dental education video and computer graphics are shown to mothers for 20 minutes and then tooth brushing instructions for 40 minutes are given individually. We concluded that the children whose mothers have not attended maternity class are more likely to have a high risk level than are children whose mothers received instructions on the techniques.

The Cariostat test assesses the acid production of microorganisms in dental plaque. In Japan, many studies have demonstrated that Cariostat is a convenient method for predicting dental caries. PCR techniques are very sensitive for detecting these bacteria. There have been no previous study except for a study by Rodis *et al.*<sup>9</sup> on detection of *S. mutans* or *S. sobrinus* directly in Cariostat medium through

a PCR technique.

In conclusion, the results of the present study indicate that presence of *S. mutans* and/or *S. sobrinus* in children and caries risk level of children are significantly correlated with risk behaviors of children.

### Acknowledgments

We acknowledged all the staff of the Department of Behavioral Pediatric Dentistry, Okayama University Graduate School of Medicine and Dentistry, especially Dr. Mori Y. and Yamanaka K. for their assistance. We would also like to thank Nishimoto Y. dental hygienist of the Katano Health Center. This study was supported by the Katano Health Center.

### References

- 1) Loesche, W.J.: Role of *Streptococcus mutans* in human dental decay. *Microbiol Rev* **50**: 353–380, 1986.
- 2) Caufield, P.W., Cutter, G.R. and Dasanayake, A.P.: Initial acquisition of mutans streptococci by infants: evidence for a discrete window of infectivity. *J Dent Res* **72**: 37–45, 1993.
- 3) Holbrook, W.P., de Soet, J.J. and de Graaff J.: Prediction of dental caries in preschool children. *Caries Res* **24**: 44–52, 1993.
- 4) Mohan, A., Mores, D.E., O’Sullivan, D.M. and Tinanoff, N.: The relationship between bottle usage/content, age, and number of teeth with mutans streptococci colonization in 6–24 month-old children. *Community Dent Oral Epidemiol* **26**: 12–20, 1998.
- 5) Studi, H., Jen, C.H., Nishimura, M., Matsumura, S. and Shimono, T.: The determination of the predictive value of caries activity test and its suitability for mass screening in Indonesia. *Ped Dent J* **2**: 73–82, 1992.
- 6) Tsubouchi, J., Yamamoto, S., Shimono, T. and Domoto, P.K.: A longitudinal assessment of predictive value of a caries activity test in young children. *J Dent Child* **62**: 34–37, 1995.
- 7) Igarashi, T., Yamamoto, A. and Goto, N.: Direct detection of *Streptococcus mutans* in human dental plaque by polymerase chain reaction. *Oral Microbiol Immunol* **5**: 294–298, 1996.
- 8) Igarashi, T., Yamamoto, A. and Goto, N.: PCR for detection and identification of *Streptococcus sobrinus*. *J Med Microbiol* **49**: 1069–1074, 2000.
- 9) Rodis, O.M.M., Okazaki, Y., Kariya, N., Ji, Y., Kanao, A., Hayashi, M., Matsumura, S. and Shimono, T.: Presence of *Streptococcus mutans* or *Streptococcus sobrinus* in Cariostat®-inoculated plaque samples from Japanese mother-child pairs. *Ped Dent J* **15**: 98–102, 2005.
- 10) Tsubouchi, J., Yamamoto, S. and Shimono, T.: Longitudinal assessment of behavioral factors associated with dental caries in young children. *Ped Dent J* **7**: 13–18, 1997.
- 11) Newbrun, E.: Current concepts of caries etiology. In: *Cariology*. (3rd ed.) Quintessence Publishing Co., Inc., Chicago, 1989, p.29.
- 12) Milgrom, P., Riedy, C.A., Weinstein, P., Tanner, A.C.R., Manibusan, L. and Bruss, J.: Dental caries and its relationship to bacterial infection, hypoplasia, diet, and oral hygiene in 6- to 36-month-old children. *Community Dent Oral Epidemiol* **28**: 295–306, 2000.
- 13) Kubota, S., Kawasaki, K., Iijima, Y. and Takagi, O.: Factors affecting dental caries prevalence in 1.5-year-old children. *J J Dent Health* **41**: 192–205, 1991. (in Japanese)
- 14) Kohler, B. and Bratthall, D.: Intrafamilial levels of *Streptococcus mutans* and some aspects of the bacterial transmission. *Scand J Dent Res* **86**: 35–42, 1978.
- 15) Berkowitz, R.J. and Jones, P.: Mouth-to-mouth transmission of the bacterium *Streptococcus mutans* between mother and child. *Arch Oral Biol* **30**: 377–379, 1985.
- 16) Li, Y. and Caufield, P.W.: The fidelity of initial acquisition of mutans streptococci by infants from their mothers. *J Dent Res* **74**: 681–685, 1995.
- 17) Wan, A.K.L., Seow, W.K., Purdie, D.M., Bird, P.S., Walsh, L.J. and Tudehope, D.I.: A longitudinal study of *Streptococcus mutans* colonization in infants after tooth eruption. *J Dent Res* **82**: 504–508, 2003.
- 18) Tsubouchi, J., Higashi, T., Shimono, T., Domoto, P.K. and Weinstein, P.: A study of baby bottle tooth decay and risk factors for 18-month-old infants in rural Japan. *J Dent Child* **61**: 293–298, 1994.
- 19) Emanuelsson, I.R., Li, Y. and Bratthall, D.: Genotyping shows different strains of mutans streptococci between father and child and within parental pairs in Swedish families. *Oral Microbiol and Immunol* **13**: 271–277, 1998.
- 20) Kozai, K., Nakayama, R., Tedjosongko, U., Kuwahara, S., Sizuki, J. and Nagasaki, N.: Intrafamilial distribution of mutans streptococci in Japanese families and possibility of father-to-child transmission. *Microbiol and Immunol* **43**: 99–106, 1999.