Abstract: The aim of this study was to assess whether a clinical evaluation of oral cleanliness reflects subsequent caries incidence. Oral examination of 180 children (1- to 4-year-olds) was carried out twice in a six-month period. Caries prevalence at baseline (dfs) in 1- to 2-year-olds (group A) and 3- to 4-year-olds (group B) correlated significantly with oral cleanliness as well as salivary mutans streptococci count (MS). Caries increment (Δdfs) correlated significantly with oral cleanliness in group A but not in group B, while Δdfs significantly correlated with MS in group B and slightly correlated with that in group A. ANCOVA revealed that dfs was significantly higher at the second examination than at baseline in group B, even after adjusting for oral cleanliness. This finding was confirmed by Wilcoxon test when group B was divided in three categories (low, middle and high) based on oral cleanliness. This suggests that the relationship between Δdfs and oral cleanliness decreased with age and that the significant positive correlation found in group B by our point-prevalence survey is derived from the remainder of the positive correlation that occurred at a younger age. (J. Oral Sci. 45, 93-98, 2003)

Key words: caries incidence; preschool children; oral cleanliness; mutans streptococci.

Introduction

Tooth brushing is recommended in official dental health education material and by many dentists as one of the main means of preventing gingivitis and dental caries. A correlation between good oral hygiene and gingival health has been clearly demonstrated in both adults and children. It is generally accepted, therefore, that good oral hygiene is required to maintain gingival health, and that poor oral hygiene leads to gingival disease. In contrast, the relationship between oral hygiene and dental caries has not yet been clearly established, because studies have provided conflicting evidence. The relationship between oral cleanliness and caries in preschool and school children was summarized in a review by Sutcliffe (1), which stated that about half of existing prevalence studies showed a positive association between plaque and caries, whereas the remainder showed either no association or a weak negative association. Although almost all incidence studies have shown that caries tends to increase with decreasing oral cleanliness, the results are seldom statistically significant (2-4).

One reason for a low correlation between caries and oral hygiene might be a bias toward selecting well-qualified subjects with sufficient numbers of susceptible teeth. The elderly seem more resistant to caries, in part because of their relatively mature teeth. Not so many studies have investigated the relationship between oral hygiene and dental caries in preschool children, whose teeth are susceptible. Almost all prevalence studies of preschool children have reported a positive association between plaque and caries (5-7). On the other hand, while few longitudinal surveys have been conducted (8,9), one such study suggested a distinct association between caries...
Fluoride has a proven effect against caries. Fluoride consistently present in the oral environment, in the form of fluoridated water and fluoride tablets or dentifrices that are used regularly, could obscure an association between oral hygiene and caries; therefore, the results of studies should be interpreted with caution. However, no study has yet investigated the relationship between oral hygiene and caries incidence in preschool children who have limited access to fluoride.

Mutans streptococci (MS) are reputed to be the main etiologic agent of dental caries in humans (10,11). Caries-susceptible individuals can likely be identified by a correlation between the presence of MS and caries incidence (12-14). Because MS detected in saliva is thought to be shed from plaque biofilm and correlates well with plaque MS levels, salivary MS counts are useful for assessing caries risk.

In this study, we analysed both the amount of plaque and the salivary MS count in relation to caries incidence in preschool children who had limited access to fluoride. Thus, we assessed whether oral cleanliness as well as salivary MS count is related to subsequent caries incidence.

Materials and Methods

Subjects
This study was initiated with 240 preschool children (113 males, 127 females), 1 to 4 years of age, from two nursery schools in Tokyo, Japan, a non-fluoridated area. Two age groups were examined at 6-month intervals between 1994 and 1996 to obtain baseline data: 1- to 2-year-olds (group A, n = 86) and 3- to 4-year-olds (group B, n = 154). Six months after baseline, 180 children were reexamined (group A, n = 52; group B, n = 128); the remaining 60 children were absent from the second examination, either because they missed their appointment or because they had left the schools and could not be contacted. Consequently, 60 children were eliminated from the data for the following statistical analysis. We obtained permission from the parents of all of the children to collect microbiologic samples and perform dental examinations.

Caries diagnosis
Caries prevalence at the baseline and at the second examination was determined by two experienced examiners. Caries was diagnosed on the basis of a visual classification described by the World Health Organisation (15); no radiographs were taken, and only manifest lesions in the primary teeth were considered. Before this investigation, the two examiners conducted a preliminary examination of five subjects and the agreement between their caries diagnoses was excellent ($\kappa = 0.88$).

Oral microbiologic sampling and processing
The Dentocult SM® Strip mutans test (16) was used to evaluate MS levels in unstimulated saliva. The strip was rotated on the surface of the tongue 10 times to sample saliva, and then was placed in culture medium and incubated for 48 hours at 37°C. After cultivation, the strip was dried, and examined under a microscope with a magnification $\times 10$. Levels were scored from 0 to 3 according to the evaluation chart provided by the manufacturer. One dentist experienced with the method scored all the strips.

Dental plaque assessment
We developed a novel plaque score, the total oral hygiene index (TOHI), to assess oral cleanliness. TOHI evaluates buccal, lingual and occlusal surfaces of all teeth, much like the Oral Hygiene Index does (17). The buccal and lingual score is obtained by dividing the sum of the debris score for buccal and lingual surfaces of all teeth by the number of surfaces examined. The occlusal score is obtained by dividing the sum of the debris score for occlusal surfaces of all teeth by the number of occlusal surfaces. TOHI is defined as the sum of all three surfaces’ debris scores.

Statistical analysis
Data were analysed using the SPSS® software package. The relationship between the variables was tested by Spearman’s correlation analysis. To analyse the difference of dfs between two examination times, dfs was transformed into $\sqrt{dfs}$ and analysis of covariance (ANCOVA) was carried out. The result of ANCOVA was confirmed by Wilcoxon test.

Results
The mean ± SE of dfs in group A and group B, respectively, was as follows: 2.00 ± 0.78 and 4.80 ± 0.62 at baseline; and 3.23 ± 1.09 and 7.18 ± 0.82 at the second examination. The mean ± SE for $\Delta$dfs was 1.23 ± 0.38 and 2.41 ± 0.34 for groups A and B, respectively. Caries prevalence increased from 23% at baseline to 35% at the second examination in group A, and from 57% to 65% in group B. The mean ± SE of TOHI at baseline was 0.87 ± 0.10 and 0.77 ± 0.05 in groups A and B, respectively. The distribution of salivary MS level in groups A and B, respectively, was as follows: level 0, 60% and 41%; level 1, 29% and 20%; level 2, 2% and 20%; and level 3, 10% and 19%.
Spearman’s correlation coefficients among dfs, salivary MS, and TOHI at baseline are shown in Table 1; those between Δdfs and salivary MS or TOHI at baseline are shown in Table 2. Caries prevalence (dfs) at baseline in both groups correlated significantly with oral cleanliness (r<sub>A</sub> = 0.244, P = 0.024, group A; r<sub>B</sub> = 0.232, P = 0.004, group B) and salivary MS level (r<sub>A</sub> = 0.504, P < 0.001, group A; r<sub>B</sub> = 0.411, P < 0.001, group B). Baseline TOHI correlated significantly with salivary MS level in both groups (r<sub>A</sub> = 0.236, P = 0.029, group A; r<sub>B</sub> = 0.205, P = 0.011, group B). The caries increment over 6 months (Δdfs) correlated significantly with oral cleanliness in group A (r<sub>A</sub> = 0.435, P = 0.001), but not in group B (r<sub>B</sub> = 0.146, P = 0.100). The Δdfs correlated significantly with salivary MS level in group B (r<sub>B</sub> = 0.316, P < 0.001) and slightly correlated with that in group A (r<sub>A</sub> = 0.253, P = 0.071).

To analyse this apparent contradiction in group B, we conducted ANCOVA concerning the difference of √dfs between the two examination times, adjusting for TOHI. That result suggests that √dfs was significantly (P = 0.033) higher at the second examination than at baseline in group B, even after adjusting for TOHI. This finding was confirmed by Wilcoxon test when group B was divided into three categories (low, middle and high) based on TOHI (Table 3).

### Discussion

In this study, caries prevalence at the baseline in group A (1- to 2-year-olds) was 23%, and that in group B (3- to 4-year-olds) was 57%. These are similar to the results of the Japan National Oral Health Survey in 1993, which reported that caries prevalence of Japanese children in 1993 was 20% in 1- to 2-year-olds, and 64% in 3- to 4-year-olds (18). Subjects in this study seem to have lived in a representative city having the average rate of caries occurrence in Japan.

The explanation of the relationship between oral cleanliness and caries begins with the widely accepted premise that dental caries occurs only after plaque has accumulated on susceptible tooth surfaces. This process is determined by the interdependence of plaque, sugar, and tooth susceptibility, which are three sine qua non variables of dental caries (19). Epidemiological evidence of the relationship between plaque and caries lacks consistency, and as a result the value of oral hygiene practices in preventing caries initiation has been doubted (20).

In this study, we used a point-prevalence survey at baseline and a longitudinal survey over six months. The point-prevalence survey showed significant correlations between plaque and caries prevalence in both age groups studied. Most previous prevalence studies have reported a positive association between plaque and caries prevalence in preschool children (5-7). About half of the prevalence studies of both preschool and school children have shown plaque and caries prevalence to be positively associated, but the remainder either showed no association or a weak negative association (1). Three studies (5,21,22) have suggested that poorer oral cleanliness in children is accompanied by increased caries prevalence. The results of the three studies taken together indicate that the youngest

### Table 1 Spearman’s correlation coefficients among dfs, salivary MS and oral hygiene at baseline

<table>
<thead>
<tr>
<th>Age group</th>
<th>Variables</th>
<th>Correlation coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>dfs, salivary MS</td>
<td>0.504</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(1-2 yr)</td>
<td>dfs, TOHI</td>
<td>0.244</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>salivary MS, TOHI</td>
<td>0.236</td>
<td>0.029</td>
</tr>
<tr>
<td>B</td>
<td>dfs, salivary MS</td>
<td>0.411</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(3-4 yr)</td>
<td>dfs, TOHI</td>
<td>0.232</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>salivary MS, TOHI</td>
<td>0.205</td>
<td>0.011</td>
</tr>
</tbody>
</table>

### Table 2 Spearman’s correlation coefficients between Δdfs and salivary MS and TOHI

<table>
<thead>
<tr>
<th>Age group</th>
<th>Variables</th>
<th>Correlation coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Δdfs, salivary MS</td>
<td>0.253</td>
<td>0.071</td>
</tr>
<tr>
<td>(1-2 yr)</td>
<td>Δdfs, TOHI</td>
<td>0.435</td>
<td>0.001</td>
</tr>
<tr>
<td>B</td>
<td>Δdfs, salivary MS</td>
<td>0.316</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(3-4 yr)</td>
<td>Δdfs, TOHI</td>
<td>0.146</td>
<td>0.100</td>
</tr>
</tbody>
</table>

### Table 3 Comparison of dfs in group B (3- to 4-year-olds) at two examination times with regard to TOHI level

<table>
<thead>
<tr>
<th>Examination time</th>
<th>TOHI</th>
<th>Baseline</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; exam.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (0 ~ 0.33)</td>
<td>4.22 ± 1.00&lt;sup&gt;*&lt;/sup&gt;</td>
<td>6.29 ± 1.31&lt;sup&gt;**&lt;/sup&gt;</td>
<td>&lt;0.001&lt;sup&gt;**&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>n = 41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle (0.34 ~ 0.63)</td>
<td>2.79 ± 0.78&lt;sup&gt;+&lt;/sup&gt;</td>
<td>5.47 ± 1.28</td>
<td>&lt;0.001&lt;sup&gt;+&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>n = 43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (0.64 ~ 1.39)</td>
<td>7.30 ± 1.26&lt;sup&gt;+&lt;/sup&gt;</td>
<td>9.68 ± 1.57</td>
<td>&lt;0.001&lt;sup&gt;+&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>n = 44</td>
<td></td>
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</table>

*<sup>:</sup> mean ± SE of dfs  
**<sup>:</sup> Differences according to the examination time: statistical evaluation using Wilcoxon test
children (3- and 4-year-olds) experience the greatest increase in caries with poor oral cleanliness, and the oldest subjects (12- and 14-year-olds) the smallest increase. The correlation between plaque and caries prevalence would, however, be relatively clearer in deciduous than in permanent dentition.

The results of our longitudinal survey suggest that oral cleanliness as measured on buccal, lingual and occlusal surfaces of all teeth relates to subsequent caries in 1- and 2-year-olds but not in 3- and 4-year-olds, whereas caries incidence correlates with salivary MS levels in both groups. Almost all incidence studies in preschool and school children have shown increases in caries prevalence with poor oral cleanliness, although these results were seldom statistically significant (1). In a 1.5-year longitudinal study of children who were 19 months of age at baseline, visible plaque accumulation on the primary anterior teeth was associated with the development of caries (9). In contrast, the accumulation of plaque on the primary second molars in 2- and 3.5-year-olds correlated significantly with dfs scores of 5-year-olds, but the correlation never exceeded 0.21 (8). This study is not readily comparable to the two previous investigations, however, partly because the methods of plaque evaluation differ. We evaluated the buccal, lingual and occlusal surfaces of teeth in children who had limited access to fluoride, and in the 1- and 2-year-olds Δdfs and TOHI were very significantly correlated (Table 2). In addition, fluoride tablets or dentifrices have been generally used in the areas from which the previous studies recruited subjects. In one area, fluoride tablets were recommended if the drinking water contained less than 0.5 ppm fluoride (9); in the other, more than 73% of the children used fluoride tablets regularly and about 60% used fluoride dentifrices. In this study, however, no children used fluoride tablets and only 25% used fluoride dentifrices. Because of the limited use of fluoride by children in this study, the relationship between oral cleanliness and caries incidence (which is stronger in younger children, who have deciduous dentition) is similar to the two previous studies, even though the observation period of this study (6 months) was much shorter than that of the others (over 1.5 years).

The biological interaction among host, parasite, and diet suggests that tooth susceptibility may be one reason for the low correlation between plaque and caries incidence in elderly people. Numerous characteristics of saliva and teeth have been implicated in either promoting or inhibiting caries production. In general, new teeth seem to be more susceptible to poor oral cleanliness than older teeth. In this study, none of the variables for diet listed by the parents on the questionnaire (sugar substitute consumption, frequency of snacks and drinks, age at weaning) showed a significant association with caries increment by using a chi-square test (data not shown).

The classic hypothesis based on the biological interaction among host, parasite, and diet (19) partly explains the close relationship between oral cleanliness and caries increment in children less than 3 years of age, who, among other factors, have relatively susceptible tooth surfaces. Teeth in children more than 2 years old seem to be more resistant, apparently due in part to tooth maturation. Indeed, results from ANCOVA revealed that in subjects over 2 years old, even those with good oral cleanliness would develop new caries to the same degree as those with poor oral cleanliness, while Δdfs increased significantly with increased TOHI in children of less than 3 years. These data suggest that the positive correlation found in 3- and 4-year-olds in the point-prevalence survey might be residual from the positive correlation found when the subjects were younger.

In group B, however, Δdfs correlated significantly with salivary MS levels. In group A, Δdfs correlated only slightly with salivary MS levels. However, if we evaluated plaque MS levels, this slight correlation might be stronger, because of the tendency for Δdfs to have a lower correlation with saliva MS levels than plaque MS levels (23). To interpret these findings, we considered the hypothesis that dental caries is the result of an ecological shift in plaque, induced by changes in the local environment (24-26). Whereas a neutral pH would select for streptococci of the mitis group, such as Streptococcus sanguinis, Streptococcus gordonii, and Streptococcus oralis, a low-pH environment would support an overgrowth of aciduric species, such as mutans streptococci and lactobacilli (27,28). Mutans streptococci can produce large amounts of acid, even at relatively low pH (29,30). The development of a caries lesion depends on a back-and-forth battle between the forces of demineralization and remineralization. When hosts eat carbohydrates frequently, acidification by cariogenic microorganisms is likely. Delicate shifts in the plaque environment (compositional changes or even reorganisation of plaque) could finally decide the circumstances in plaque (31). The qualitative evaluation of plaque is very important for estimating subsequent caries development during most of early childhood, yet quantification of plaque might be valid only during an extremely short period of time.

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References