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# Effect of *Mutans Streptococcal* Colonization on Plaque Formation and Regrowth in Young Children – A Brief Communication

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#### Abstract

**Objectives:** Visible plaque on the maxillary anterior teeth of young children has been identified as a risk indicator for early childhood caries. The present study examined whether this plaque is related to the colonization of children's teeth with Mutans streptococci (MS) or toothbrushing frequency. **Methods:** Thirty-nine children, aged 12 to 36 months, had plaque scores, and plaque samples were taken from the labial surfaces of the maxillary incisors at baseline and repeated 3 days after suspended oral hygiene (plaque regrowth). **Results:** A positive correlation was found between the baseline percent MS and regrowth plaque score and between baseline and regrowth plaque scores. Plaque scores of those that brushed zero to one time a day were not different from those who brushed two or more times a day. **Conclusions:** This study suggests that the presence of plaque on the anterior teeth of young children is consistent and related to MS colonization.

Key Words: plaque, mutans streptococci, children

#### Introduction

Personal oral hygiene measures are generally considered the mainstays of oral disease prevention, and dental professionals include oral hygiene instruction for patients, especially for those who present with high levels of dental plaque. While the importance of this aspect of health education is not in question, many clinicians may be making the assumption that higher levels of plaque are solely the result of the patient's poor skill or motivation in removing plaque deposits. In contrast to this conventional wisdom, there is evidence that individuals may experience higher levels of plaque for reasons other than oral hygiene effort.

Differential plaque formation between individuals is well documented. Studies have consistently placed subjects into "high" or "low" plaque-forming groups (1,2). While several factors play a role in differential plaque formation rates, no one variable alone is believed to be responsible for these differences (2).

*Mutans streptococci (MS)*, the group of organisms most often etiologically associated with the initiation of dental caries, produce extracellular polysaccharides that may aid in dental plaque formation (3). The water-insoluble glycosidic linkage unique to *MS*-derived polymers, makes *MS*-containing plaque more resistant to removal (4), and *MS* mutants defective in synthesizing these polymers have a decreased ability to form plaque and induce caries (5).

With regard to the association of dental caries with visible dental plaque on the teeth, several reports have shown that the presence of visible plaque on incisors in young children is highly correlated with caries prevalence (6,7).

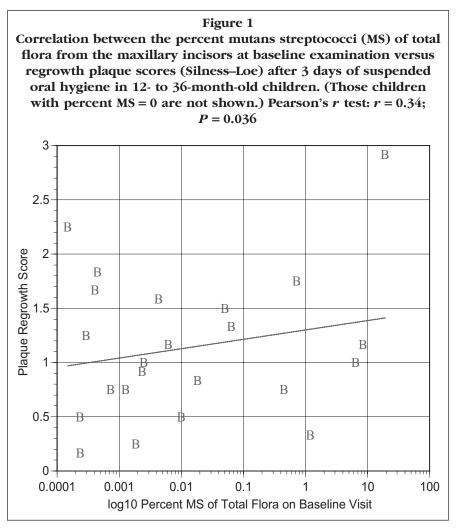
Linking these concepts together, we hypothesized that MS colonization in young children promotes the formation and accumulation of dental plaque. The principal objective of this investigation was to determine if such MS colonization was related to the presence of visible dental plaque and plaque regrowth in 12- to 36-month-old children. Additional objectives were to investigate the relationship between toothbrushing frequency and baseline plaque levels, and to determine if a relationship exists between baseline and regrowth values of both plaque levels and percent MS in dental plaque.

#### Methods

After the Institutional Review Board approval was obtained, 39 children, 12 to 36 months old, were recruited for this prospective clinical study from the University of Maryland Dental School and three affiliated clinics. Inclusion criteria were the presence of all four maxillary primary incisors; exclusion criteria included cavitations and/or restorations on the facial surfaces of the maxillary incisors, as well as any underlying medical conditions which would have contraindicated participation in the study. Children who had taken systemic antibiotic therapy within 2 weeks of the first visit were either excluded or rescheduled.

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Participation consisted of two visits, spaced 3 days apart. During the baseline visit (Visit 1), informed consent was obtained and a sevenitem questionnaire was administered that asked about parental toothbrushing frequency and dietary behavior. Mean plaque scores for each child were obtained by one trained examiner using a modified Silness-Loe Plaque Index (8), scoring mesiobuccal, only the straight buccal, and distobuccal surfaces of the four maxillary incisors. Following plaque scoring, a sterile cotton swab was run along the labial gingival margin of the four incisors to obtain the plaque sample for culture. To maximize the sample yield, any plaque adhering to the explorer during the plaque scoring was also wiped onto the sampling swab. Following the clinical data collection, a toothbrush prophylaxis using fluoride-free dentifrice was performed by one dentist to bring each subject to a zero plaque level. Caregivers were then instructed to suspend oral hygiene efforts for 3 days until after the follow-up visit (Visit 2). They were also instructed to temporarily remove any toothbrushes from the child's reach to avoid the child from brushing his/her teeth without the caregiver's knowledge. At Visit 2, the caregivers were asked if they had complied with the abstinence in oral hygiene for the prior 3 days. Regrowth plaque scoring and the microbiologic protocol were repeated identically to that for the baseline sample. A toothbrush prophylaxis was also repeated.

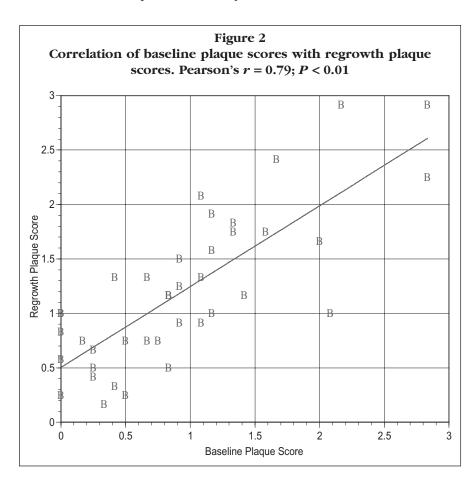
For the microbial analysis, the heads of cotton swabs containing the plaque samples were placed into 5-mL screw-top sample vials containing 1 mL of sterile saline. Sample vials were serially diluted and plated onto brain-heart infusion (BHI) agar (Difco, Detroit, MI) and also mitis salivarius kanamycin bacitracin (MSKB) agar (9). Dilutions were incubated at 37 °C in air containing 10 percent CO2 for 3 days (MSKB plates) or 1 week (BHI plates). Colony-forming units (CFUs) on the BHI plates were used to assess total bacteria counts, while CFUs on MSKB plates were used to assess MS counts. The percent MS of total flora was calculated for each subject visit from the MS and total bacterial count ratio.

For data analysis, independent *t*-tests were used to determine significant differences in plaque score based on toothbrushing frequency. Additionally, correlations (Pearson's *r*) were determined between baseline percent *MS* versus regrowth plaque scores, between the subjects' baseline and regrowth plaque scores, as well as for the subjects' baseline and regrowth percent *MS*.

## Results

Thirty-nine eligible subjects, with a mean age of  $24.7 \pm 8.22$  months, participated in this investigation. Twenty-one subjects were male and 18 were female. The mean baseline plaque score for all subjects was  $0.93 \pm 0.74$ , and the regrowth plaque score was  $1.20 \pm 0.70$ . Concerning initial plaque scores related to toothbrushing frequency, those children who reportedly brushed zero or one time a day had a mean plaque score of 1.11 ± 0.86 versus a mean plaque score of  $0.76 \pm 0.57$  (nonsignificant) for those that reportedly brushed two or more times a day.

A positive correlation was found between the percent MS of total flora cultured from the incisors at baseline versus the regrowth plaque scores [Pearson's r = 0.34(P = 0.036)] (Figure 1). The correlation between baseline percent MS of total flora versus regrowth plaque was repeated just for the MS positive subjects (n=23), yielding a correlation of r = 0.51(P = 0.013)(figure not shown).



Correlation analyses were also performed to determine if a relationship existed between an individual's baseline plaque score or percent MS of total flora score, and their subsequent 3-day regrowth plaque score or percent MS score. There was a positive correlation between the subjects' baseline plaque scores and regrowth plaque scores (Pearson's r = 0.79, P < 0.01) (Figure 2). Similarly, a correlation was found between the subjects' percent MS from the baseline samples and that cultured during the regrowth period (Pearson's r = 0.78, P < 0.01) (figure not shown).

#### Discussion

The objectives of this investigation were to determine whether there was a relationship between a child's level of *MS* colonization and the quantity of dental plaque formation, whether a child's plaque forming rates are constant, and whether reported oral hygiene procedures affect plaque levels. The results of this investigation may challenge the hypothesis that plaque levels in children primarily are a function of oral hygiene effort and/or skill. Such differences between the concept of whether plaque formation on teeth is primarily a result of oral hygiene techniques or the result of biologic factors (colonization of specific bacteria and constant correlations) are important regarding how we interpret the findings of plaque presence in young children as a risk factor for caries (6,7).

We found a positive correlation in these young subjects between their percent *MS* of total flora and their plaque scores, which is in accord with the concept that the *MS* colonization in a child's mouth affects his or her plaque levels. Physiologically, *MS* produce extracellular polysaccharides that may aid in dental plaque formation by adding to the mass of plaque (3) and by strengthening the attachment of these adsorbed bacteria to the enamel (4). It is interesting to speculate that, at least in young children, the presence of visual plaque on the maxillary anterior teeth may indicate an elevated level of *MS*.

Our results did not support an effect of toothbrushing frequency on visual plaque scores on the maxillary incisors in young children. No significant difference in plaque scores was observed with different parental toothbrushing frequency levels. Of course, it is possible that the small sample size may have resulted in insufficient statistical power to demonstrate an effect from brushing frequency on plaque levels in subjects, or that issues of questionnaire validity regarding oral hygiene procedures could confound such findings.

In the present study, a positive correlation between baseline and regrowth plaque scores is consistent with earlier works that could classify a given individual as a "high" or "low" plaque former (1,2). There was also a strong positive correlation between the baseline and regrowth percent MS. This correlation indicates that MS regrowth are minimally affected by the toothbrush prophylaxis performed by the examiner or the 3-day abstinence in oral hygiene measures. The public health implication of these results (the positive correlations between MS levels and plaque scores, baseline and regrowth plaque scores, and baseline and regrowth MS levels, but the absence of a relationship between plaque scores and toothbrushing frequency) is that the presence of visual plaque in young children is a caries risk factor that may not be affected by improving the oral hygiene behaviors performed by the children's caretakers.

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