Effect of anticipatory guidance on the presence of cariogenic bacteria in preschool children

by

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Overview

This thesis is submitted as partial fulfillment of the requirements for the Master of Dentistry degree at the College of Dentistry, with principle investigator Dr. Nick Lekic. This research project addressed whether or not the level (high or low) of *Streptococcus mutans* in the saliva of preschool children is a reliable indicator of caries risk. The study assessed the level of *Streptococcus mutans* in the saliva of preschool children that were three to six years of age, before and approximately 6-months after receiving a health promotion intervention known as anticipatory guidance. The *Streptococcus mutans* bacterial level was assessed with an immunoassay system (Saliva-Check MUTANS) that utilizes monoclonal antibody technology (10, 14, 15, 16). Bacterial counts were determined at baseline as well as at a six month recall examination. After determining bacterial counts at the initial exam, anticipatory guidance was provided for each patient and their family. This study investigated whether the level of *Streptococcus mutans* is related to the presence of dental decay, the development of new dental caries, and whether or not the provision of anticipatory guidance results in a decrease in *Streptococcus mutans* bacterial counts.

Introduction

Dental caries is defined as a transmissible communicable disease that is caused by tooth-adherent bacteria, with their metabolism of carbohydrates, demineralizing teeth over a period of time (1). Dental caries has been recognized as an important childhood disease and a significant health care problem in Western countries (2). In children, dental caries has been found to be far more common than chronic medical conditions such as asthma and hay fever. (3). However, understanding of caries etiology, the provision of preventive programs has led to generally reduced rates of caries development (4). The American Academy of Pediatric Dentistry (AAPD) advises for the creation of a dental home with eruption of the first primary tooth and the latest by 1 year of age (5). Despite of these efforts, recent studies in the USA have shown an increased prevalence of caries in deciduous teeth over the past decade (6). This is particularly troublesome as early childhood caries (ECC) can lead to a number of growth and developmental problems and often results in the need for treatment under general anesthesia (7).

Dental caries is recognized as a multifactorial disease affected by a balance of pathologic and protective factors (8). These pathologic factors include carbohydrate intake, reduced salivary flow and the presence of cariogenic bacteria (8). Additional risk factors also include, reduced fluoride exposure, poor oral hygiene and lower socioeconomic status (9). The importance of specific microbiological factors in the causation of caries has also been recognized (10). In particular, studies have strongly linked severe early childhood caries to the presence of *Streptococcus mutans* (9). These bacteria are acidogenic, aciduric, and capable of secreting intra-and extracellular polysaccharides that facilitate microbial colonization on teeth (11). The presence of

increased levels of *Streptococcus mutans,* in younger children, has been found to be a useful caries risk indicator for pre-school children (12). The AAPD recognizes the presence of increased levels of *Streptococcus mutans* (>10^5 cfu/ml) as an indicator of high caries risk for children younger than the age of six (9). Studies have shown that the source for early transmission of *Streptococcus mutans* is commonly from the primary care giver (via vertical transmission), but may also from siblings or children of the same age group (via horizontal transmission) (1).

Screening for the presence *Streptococcus mutans* may allow for identification of children with increased caries risk that would benefit most from early and intensive intervention to prevent or minimize caries experience. Traditional testing for oral colonization of *Streptococcus mutans* has been primarily done with conventional selective media; however, chair-side microbial tests have been recently introduced (13, 14). Due to improved specificity to *Streptococcus mutans* and decreased post-operative development time, these are becoming more frequently used (10). Studies have proven the validity of the chair-side saliva tests with values as high as 97.6% for sensitivity, and 90.6 % for specificity (10, 15). Its validity as a diagnostic tool was also seen in its significant relation to the PCR-confirmed culture method ($p<0.01$) (16). Nevertheless, further studies are required to determine the efficacy of *Streptococcus mutans* counts as a diagnostic method in assessing the caries risk status in children.

Literature Review

Early Childhood Caries

The AAPD defines ECC as the presence of ≥ 1 decayed, extracted or restored surface in any deciduous teeth in children between birth and 6 years of age (17). In patients that are under the age of three years, any indication of a smooth surface cavity classifies them as having Severe-ECC (17). For children aged three, four or five, the finding of ≥ 1 cavitated, extracted or filled tooth surface (dmfs) on a primary maxillary anterior tooth, or a dmfs score of four, five and six respectively, also results in the classification of Severe-ECC (17). The presence of caries in the deciduous dentition has been shown to be an important factor for determining future caries in adult dentition, with a positive predictive value of 85.4% (18).

ECC is not only a disease affecting teeth. This form of caries has been associated with malnourishment, a decreased Body Mass Index (BMI) percentile for the child's age and may also be a potential indicator for Iron Deficiency Anemia (19). A recent study has also found an association between early childhood caries and decreased Vitamin D levels (20**).** Other studies have found the presence of caries in children to significantly increase the prevalence of sleep deprivation, anxiety and/or depression, attention deficit hyperactivity disorder and other behavioral problems in comparison to caries-free children (21). Children with ECC have also been shown to decrease school performance, coupled with an increase in school absence (1). A decreased quality of life with increased pain and decrease in food consumption has also been reported in children with caries as opposed to caries-free children (22). Untreated dental caries may also

cause serious life-threatening conditions, including facial cellulitis and Ludwig's angina (23). These conditions require more invasive treatment and hospitalization for their management and can be life threatening (23).

Patients with ECC require dental treatment, combined with their pre-cooperative age, a lack of adequate cognitive and emotional development or maturity for comprehension; this treatment is commonly done under general anesthesia (24). ECC is the main cause for a hospital day surgery in most pediatric hospitals (25). Lengthy waiting lists for treatment are not uncommon, resulting in the child remaining in pain for an extended period of time (25). Cost of treatment is substantial, for Manitoba children who are federally insured, the cost reaches $3.5-4 million annually (26). These children are frequently transported for treatment from distant rural communities, further adding to the overall treatment cost (26).

ECC has been reported to be prevalent and between 1% to 12% in infants residing in developed countries (27). In Canada, children at higher risk for development of early childhood caries include the First Nations, immigrants, refugees, low-income families and those in rural areas where dental access is limited (25). One Manitoba study reported prevalence of caries to be lower than 5% in the general public and 50% to 80% in groups that are of increased risk, including immigrants and aboriginal Canadians (27).

Caries Risk Assessment

Caries risk indicators determine the presence of factors, which may show that the child is at risk for developing cavities (9). A risk-based approach to caries management enables identification of patients who are most vulnerable to the development of this disease with a goal of minimizing causative factors and maximizing protective factors (9, 28). The AAPD Caries Risk Assessment Tool has become the principle guide utilized by pediatric oral health care providers for early assessment of children (9). The three main factors for caries risk assessment in children include biological, protective, and clinical findings (9).

The frequency of daily sugar consumption, in particular at nighttime, through beverages containing sugar, has been found to increase a child's caries risk (29). Children with delayed weaning from the bottle and with prolonged nighttime feeding with sugar containing products may have decay rates as high as 86 % in the maxillary primary incisors (29, 30). Although no study has found human breast milk as cariogenic, its combination with sugar in an infant's diet may increase their caries risk (29). One systematic review reported that prolonged breast-feeding on demand at night for greater than a year following the eruption of the primary teeth may be associated with ECC, however they also reported that higher level evidence and more consistent data is required (31). Interestingly, recent studies have shown that although sugar consumption is involved in the formation of caries, the increased exposure to systemic fluoride has lessened its affect (32). In addition to a child's diet, a mother/primary caregiver with ≥ 10 carious lesions has been found to significantly increase a child's caries incidence (33).

Other biological factors identified by the AAPD as increasing a child's caries risk are low socioeconomic status and special health care needs (9).

Preventive dental care for children of all ages, including infants through adolescence, as well as children with special health care needs, primarily is directed at reducing caries risk (1). Oral disease can be prevented with the recognition and decrease of causative factors (i.e. presence of cariogenic bacteria, feeding habits, and/or plaque accumulation) while at the same time increasing the effect of protective factors (i.e. topical and systemic fluoride exposure, sealant application) (1). Using this concept, patients can be determined as low, moderate, or high-risk groups related to the expression of these factors (9). Patients that are at higher risk should be followed-up more frequently (i.e. every 3 months) with professional application of topical fluoride, whereas patients in the low risk group can be seen less frequently for routine recall appointments (i.e. every 12 months) (9). As per the Canadian Dental Association (CDA) guideline on fluoride use in children, those that are of high caries risk and residing in an area without fluoridated water may consider fluoride supplements (34). This would include all children with a total dietary fluoride intake that is not > 0.07 mg F/kg (34).

The presence of previous carious experience has been described as the most accurate predictor for caries risk children (35). In infants or toddlers where there may not have been sufficient time for a cavity to form, the presence of white spot lesions or compromised enamel places the child as high risk (36). Presence of plaque has also been associated with increased caries risk. One study found that caries risk could be accurately identified in 91 % of children by their plaque levels alone (37). The AAPD classifies a child under the age of six with visible plaque present as moderate caries risk (9). The

CDA recommends supervised brushing in children under the age of six, using utilizing a pea-sized quantity of dentifrice, and in children under the age of two a "smear-sized amount" (34). Physiological salivary flow rate is a well-recognized intrinsic host factor in prevention of caries formation. Decreased salivary flow rate, beneath 0.8-1 ml/min, has therefore been established as a high risk factor for caries initiation (38). Presence of *Streptococcus mutans*, in plaque and saliva of young children that are caries-free, has been associated with increased caries risk (7, 39, 40). The AAPD also recognizes the presence of defective restoration(s) and intra-oral appliance(s) as a moderate caries risk factor (9).

Streptococcus mutans

Oral microbes may invade an infant's oral cavity soon after birth (41). Studies have shown that via vertical transmission from the mother to the infant, and with "on demand" feeding and increased sugar consumption, bacteria may be present in pre-dentate infants (42). Following the eruption of deciduous teeth the quantity and complexity of bacterial colonies increases (41). The two main factors that affect transmission and colonization of *Streptococcus mutans* are bacterial and host (41). Bacterial factors include bacterial strains and biofilm. Host factors may involve genetics, surfaces available for colonization, salivary flow, immunologic factors, diet and oral hygiene (41).

Streptococcus mutans (MS) has been reported to be one of the main pathogens involved in the formation of ECC (43). The average age for a child to be colonized with *Streptococcus mutans* is 15.7 months (44). The principle mechanism of *Streptococcus*

mutans in caries formation is the digestion of sucrose to fructan and glucan and the subsequent production of acids that demineralize tooth structure (45). Fructan provides the bacterium with intracellular energy, meanwhile the water-soluble glucan enables extra-cellular adhesion of the bacteria to the tooth (45). Bacteriologic studies have demonstrated in patients with ECC, that *Streptococcus mutans* commonly surpassed 30 % of the collected plaque flora (46). This was profoundly different in comparison to children with negligible or no caries presence where the bacteria was found in less than 0.1% plaque (46). *Streptococcus mutans* has been collected in over 90 % of children with increased caries regardless of their socio-economic backgrounds (41). The presence of *Streptococcus mutans* has also been shown as a reliable indicator of future caries status, with a pooled risk ratio (95% Confidence Interval) of 3.85 in studies where the bacteria was measured in plaque, and 2.11 in studies where *Streptococcus mutans* was assessed in saliva (7). That said, further well-designed studies are required to confirm these results (7). Particularly as there are different techniques that may be used in measuring the levels of *Streptococcus mutans* in a child's oral cavity.

In a study comparing an antibody-based chair-side immunoassay test to the conventional laboratory technique, it was determined that the immunoassay correctly and quickly determined *Streptococcus mutans* levels in saliva. This supports its usefulness for the chair-side determination of child's caries risk (10); along with a reported sensitivity/specificity of 97.6/90.6%). Another study showed significant correlation between PCR-confirmed culture medium and the chair-side saliva-check for *Streptococcus mutans*. In fact, the chair-side immunoassay test was determined to be a reliable measurement with total significance at $p<0.01$ (16). In addition to the previously

mentioned advantages of the immunoassay monoclonal antibody test, it does not require cultivation, thereby allowing it to be used in a clinic setting and also reduces the contamination risk when used in a dental office (16). Within the chair-side immunoassay tests, differences in the level of *Streptococcus mutans* between stimulated and non-stimulated saliva have not been found to be significant (14).

Anticipatory Guidance/Oral Health Promotion

Anticipatory guidance is a means of offering practical, developmentally appropriate oral-health related information about a children's health so as to assist parents with the significant milestones of childhood (47). These age-specific oral health topics encompass brushing and flossing, diet, trauma prevention, habits, substance abuse, effects of oral jewelry, as well as a child's communicative and overall development (47). In addition to the members of the dental team, anticipatory guidance may be provided by physicians, nurses and other staff and can be offered in a variety of ways (48). This includes verbal discussion, written material, interactive media and models, and role play (48). Together, health promotion and anticipatory guidance have been proven as an effective measure in reducing the presence of dental plaque and therefore a child's caries risk (47).

Early detection of elevated *Streptococcus mutans* and plaque levels has allowed for targeted implementation of risk-based prevention management of (very young children aged 2-5 year olds) with a subsequent reduction in caries rate (12). Administration of anticipatory guidance aimed at families of children that are MS-positive, has been shown to decrease the caries risk in higher socioeconomic status

families (39). In addition, identifying *Streptococcus mutans* levels may be helpful in monitoring parental compliance with home care recommendations since they correlate positively with dietary sucrose intake (39).

Collectively, anticipatory guidance is a multifactorial model that when established appropriately and implemented consistently has been found to create a positive long-term effect on the pediatric dental patient (12, 49, 50).

Objectives

Objectives of this study are:

1. Determine the relationship between providing anticipatory guidance and *Streptococcus mutans* levels in preschool children.
2. Quantify the relationship between the presence of *Streptococcus mutans* bacterial counts and the development of new dental decay in preschool children.

Hypothesis

Provision of anticipatory guidance for preschool children will result in a statistically significant decrease in *Streptococcus mutans* levels in high *Streptococcus mutans* subjects.

Material and Methods

Sample Selection

The sampling frame for this study included subjects that presented for an appointment at the University of Manitoba Pediatric Dentistry Graduate Clinic (PDC) or

Children's Dental World (CDW), a private pediatric dental office in Winnipeg, Manitoba. These two sites were chosen to help ensure that children with a full range of caries experiences and *Streptococcus mutans* counts were involved in the study.

A total of 100 children were obtained by selecting subjects from both clinical centers. This was achieved by having a study coordinator approach every second patient subject age 3-6 on the day that they were scheduled for an initial or recall examination. In the event that any of the selected subjects were not eligible, or refused to consent to participate in the study, the next subject on the list was selected and every second thereafter for the day. This selection process was repeated over multiple days until the targeted study number was reached.

Inclusion/Exclusion Criteria

Inclusion criteria included subjects that were healthy and between 36 and 72 months of age, as at this age level patients presented with a complete primary dentition and were of similar maturity regarding daily oral hygiene measures. Exclusion criteria included the following: subjects undergoing active medical treatment that limited their daily life function making them unable to participate in the study (i.e. cardiovascular, renal treatment, patients under chemotherapy); subjects with special health care needs (unable to maintain oral hygiene independently); subjects with more than 10 primary teeth extracted and/or restored with stainless steel crowns; and subjects with chart notes indicating that the child had previously received anticipatory guidance.

Informed Consent

Primary caregivers of subjects that met the inclusion criteria were provided with a detailed description of the study to review prior to giving informed consent for their child's participation in this study. All questions and concerns were addressed and then after receiving primary caregiver's consent, the baseline examination was completed.

Baseline Examination and Interview

Baseline examination of each subject took place at one of the two previously mentioned clinical sites. After obtaining informed consent, the principle investigator performed each dental examination in a standard dental clinic environment using a dental explorer, mirror, and overhead light. Examiner validity and reliability were addressed by having a single dentist examiner for all subject exams, and by calibrating this single examiner to World Health Organization standards. Calibration was conducted under the supervision of a clinical instructor at the University of Manitoba Graduate Program, in Pediatric Dentistry. Variables collected during the baseline examination included patient demographics, socio-economic factors, deft, DMFT, Tureski modification of the Quigley Hein Plaque index and more relevant information (see Appendix).

Face to face interviews were used to collect information on variables such as subject demographics, use of topical fluorides, oral hygiene, diet, medical history, and dental history (see attached survey form).

During the baseline exam, immunoassay tests were conducted to assess the level of *Streptococcus mutans* through the use of Saliva-Check MUTANS ™. The test consisted of first providing the child with paraffin gum for one minute to stimulate salivary flow. Following this, the saliva was collected in a mixing container while

ensuring an adequate volume was collected without the presence of bubbles. One drop of reagent # 1 (alkaline solution) was then added and the container was tapped 15 times in a period of 10 seconds to mix the solution. Next, four drops of reagent # 2 were added following the same system of mixing the solutions. A change in color to light green was observed, indicating a pH change from alkaline to neutral. With a transfer pipette, an adequate amount of saliva was collected from the mixing container and placed on the sample window of the test device. The test device was then left at normal room temperature for a period of15 minutes. If the test was conducted properly a red line would appear in the Control (C) side of the window. The test was recorded as positive for *Streptococcus mutans* ($>5x10^5$ CFU/ml) if a red line appeared in the Test (T) side of the window. If following 15 minutes no red line presented in the test window, it was recorded as negative and indicative of low *Streptococcus mutans* levels ($<5x10^5$ CFU/ml). (10).

Study Interventions

Following the baseline examination, age–specific anticipatory guidance was provided to the caregivers (47). This anticipatory guidance involved providing caregivers and children with the previously mentioned age-specific health education information that would be important for the specific stages of development that the child would experience prior to their next recall appointment. This explanation was specific to each age group (3, 4, 5, 6 years of age). Information was provided with regards to the colonization of bacteria (impact on caries risk), oral hygiene/health (reviewed home oral care procedures) feeding management (reviewed diet outside the home and its caries

potential, discussed intake of sugary snacks) and fluoride usage (reassessed fluoride status; for more information see the study appendix). Anticipatory guidance also included discussion of oral development (described healthy periodontal tissue), habits (i.e. managing or stopping oral habits), and injury prevention (encouraged use of helmets, mouth guards and developed plans for oral trauma management; see reference 47).

Follow-up Examination

Six months following the baseline examination, the effectiveness of the anticipatory guidance measures provided was assessed during the subject's recall appointment. The same examination methodology and immunoassay test were used. If the scheduled recall appointment for that subject was longer than six months, a special follow-up appointment was arranged at the six month time interval. Efforts were made both verbally and in writing to contact subjects that did not schedule for or attend their recall appointment and arrange for a subsequent visit, unfortunately 40 subjects were still lost to follow-up

Data Handling

A researcher trained in the use of the data entry software (EpiInfo 6.0.) completed data entry. The authors provided statistical analysis of the data using SPSS 20.0 for Windows to report frequencies and to look for trends and associations between the dependent and independent variables. Bivariate analysis (ANOVA, Chi Square/Fishers/McNemar exact test) was used to identify associations between variables. For the purposes of data analysis several variables were adjusted to facilitate assessment.

This included the creation of a variable for "caries experience" which was done by mathematical summation of a subject's deft and DMFT scores. Several variables were also dichotomized for statistical analysis. This included, caregiver education level, caregiver annual income, change in *Streptococcus mutans* and caries experience from baseline to follow-up. The variable for caregiver dental health description of child was collapsed into 3 categories to equally distribute observed responses. A bio-statistician from the University's Bio-statistical consulting unit was utilized to ensure all conclusions drawn were statistically valid.

Results

Baseline Examination and Interview Results

A total of 100 subjects presented for the baseline examination (Table 1). However, caregivers of two subjects later contacted us to notify of their decision to no longer be involved in the study. For this reason, the total number of baseline participants is reduced from 100 to 98.

Patient Demographics

The 98 subjects presenting at baseline had a mean age of 4.8 years (SD 1.1) with a nearly even distribution of males and females (Table 1). The majority of subjects resided in fluoridated water communities (75.0%) and the majority (67.3%) used fluoridated toothpaste when brushing. The majority of subjects had also been to the dentist in the last 12 months (79.8%) and 51.3 % of subjects were from families presenting with and with an annual income that is greater than $60,000.

Dental health related variables

At baseline, most subjects reported brushing their teeth twice a day (56.7%) with caregiver help (76.7%; Table 3). The majority of subjects (61.1%) snacked less than once per day on sugary snacks. Almost half of subjects had not experienced dental treatment prior to this exam (50.5%). The majority of subjects and caregivers reported attending regular dental check-ups (79.8%); (Table 3 and Table 2, respectively).

The 98 subjects were found to have only mild plaque accumulation although the mean Plaque Index was low (0.48, SD 0.3) there was a considerable range (0.0-0.31) with a number of individuals showing plaque levels that were much higher. At the same time 38.8% of subjects displayed high caries experience, defined as a combined deft/DMFT $\geq$6) (Table 3). In regards to the presence of *Streptococcus mutans,* there was an equal distribution of subjects testing high and low with the bacterial test (Table 3).

Baseline statistical analysis

At baseline no significant association was determined between the subject's age and any of the demographic variables obtained in this study ($p>0.05$). However, a significant association was noted between caries experience and the reported caregiver annual income ($p<0.01$). Subjects from families that presented with an annual income reported to be less than $30,000 had a mean caries experience of 7.0 (SD 5.0), more than double that of subjects from families with an annual income greater than $30,000 who were observed to have a mean caries experience of 3.1 (SD 3.7). Caregiver education was not, however, significantly related to baseline caries experience ($p>0.05$). At the same time there was also not a significant association ($p>0.05$) among the presence of

Streptococcus mutans and caregiver annual income. With regards to the presence of *Streptococcus mutans* and baseline caries experience, there was a significant association ($p<0.001$). Subjects with low bacterial counts were observed to have a mean caries experience of 2.1 (SD 3.4), approximately one third that of subjects with high bacterial counts that showed a mean caries experience of 6.8 (SD 4.6)

Follow-up Examination

Of the 98 subjects that were included in the initial examination, 58 (59.2%) returned for the follow-up examination. Despite the described efforts made to have subjects attend the follow-up examination, 40 subjects (40.8%) were lost to follow-up.

Demographics

With respect to the 58 subjects that completed the study, the majority of subjects (71.4%) resided in areas with fluoridated water representing a total of 13 different Manitoba communities (see Table 4). For these subjects, nearly all caregivers reported that their child used toothpaste when brushing, with 80.8% using fluoridated toothpaste. The caregivers also reported that 83.9% of children had seen a dentist within the previous 12 months. Collectively, although there was variation between the subjects on many demographic variables, the preschool children were healthy, generally from middle-class families, and were routinely followed by a pediatric dentist.

Dental health related variables

At the follow-up examination, the subjects again presented with low plaque levels with a mean Plaque Index of 0.3 (SD 0.2), which was similar to the baseline findings. At the same time, the caries experience was slightly higher in comparison to the baseline examination with a mean of 4.12 (SD 4.6). It was also noted that 18 subjects (31%; Table 5) presented without any caries experience, which was similar to baseline findings. In total, 46 subjects remained with the same caries experience as seen at baseline, while 12 subjects exhibited new carious lesions.

Streptococcus mutans levels following the anticipatory guidance provided at baseline were low on follow-up examination for the majority of subjects (67.2%) (Table 5).

Follow-up statistical analysis

The subject's gender and age were not significantly associated with an increase in caries experience at follow-up ($p>0.05$).

When *Streptococcus mutans* levels at follow-up were compared to those at baseline, it was observed that 17 subjects exhibited reduced levels of *Streptococcus mutans,* 22 maintained the same level, and 8 were found to have higher levels. The observed changes were analyzed with a McNemar Test and although not significantly ($p=0.10$) reduced from baseline it gives some evidence that the provided anticipatory guidance did have an effect in reducing the bacterial presence over a short period of time. The subject's gender was not associated significantly ($p>0.05$) with a presence of

Streptococcus mutans or a change in *Streptococcus mutans* levels between baseline and follow-up. However, the subject's age was significantly related to a change in *Streptococcus mutans* levels ($p<0.05$), with 50 subjects having a mean age 4.74 (SD 1.1) showing either same or decreased bacterial counts. In comparison to 8 subjects with a mean age 5.63 (SD 0.5) had *Streptococcus mutans* levels increase from baseline.

As demonstrated at baseline, the presence of *Streptococcus mutans* was again significantly related to caries experience ($p<0.01$) at follow-up. Subjects with low bacterial counts had a mean caries experience of 3.0 (SD 4.3), yet a twofold increase was seen in the subjects with high *Streptococcus mutans* with an observed mean caries experience of 6.4 (SD 4.7). Interestingly, 4 of the 5 subjects that were caries-free but had high levels of *Streptococcus mutans* at baseline, presented six months later with new carious lesions at the follow-up exam. A statistically significant relation was also noted between the presence of new carious lesions at follow-up and a change in *Streptococcus mutans* levels from baseline ($p<0.05$). Subjects with an increase in *Streptococcus mutans* levels were found significantly more likely to develop new caries. This was observed in 4 subjects that had increased *Streptococcus mutans* levels and presented with new carious lesions in comparison to 42 subjects who did not experience a change in the caries status and had bacteria counts remain the same or decrease.

Differences from those lost to follow-up

Demographic variables

Characteristics of subjects who returned for the follow-up examination were slightly different from those subjects who were lost to follow-up. In regard to

demographic variables, those returning for follow-up examination were significantly ($p<0.05$) more likely to be from families reporting an annual income >$30,000 (84.0% vs 60.7%) (Table 6). No significant differences were noted regarding age, gender, or caregiver education.

Dental related variables differences

Subjects who were lost to follow-up had significantly higher plaque levels ($p<0.05$) compared to subjects who returned for the follow-up examination (0.7 vs. 0.3). Subjects lost to follow-up also showed a trend towards increased caries experience (5.6 vs. 3.7), and high levels of *Streptococcus mutans* (55% vs. 32.8%) (Table 7). However, neither was found to have statistically significant difference ($p>0.05$).

Discussion

This study was designed to determine the relationship between the levels of *Streptococcus mutans*, anticipatory guidance, and caries incidence. The main finding of this study was that increased levels of *Streptococcus mutans* were correlated with higher caries prevalence at baseline, and caries incidence at follow-up. Furthermore following anticipatory guidance, the levels of *Streptococcus mutans* were reduced at the subsequent follow-up examination, approaching but not reaching statistical significance, and caries incidence was significantly lower in subjects demonstrating low S*treptococcus mutans* levels. While similar results have been reported in previous studies (43, 44, 46), this study is one of the first to show the benefit of anticipatory guidance specifically on the levels of *Streptococcus mutans*.

Results from this study have shown no correlation among the presence of *Streptococcus mutans* and the subject's demographic or socio-economic background. Studies haves also shown a high correlation among the levels of *Streptococcus mutans* and cavity rates in young patients of various ethnic and socio-economic origin (51, 52). In this study, while there was a significant correlation between caries experience and the annual income at baseline, the number of caregivers who refused to answer this question was quite large, thereby reducing the validity of the data obtained.

Following the provision of anticipatory guidance at the initial examination, the proportion of subjects testing high for *Streptococcus mutans* was reduced by approximately 15 % six months later. These findings support those of Seow et al. (2003) who reported that single dental health education intervention could result in almost a 25% of reduction of *Streptococcus mutans* in children that are under two years of age (53). Similar findings were seen in a study by Pienihakkinen et al. (2002) addressing the clinical outcomes of a caries prevention plan that was risk-based, demonstrating that in young children this can be an efficient method to prevent dental caries (12). In this present study and in others, it was shown that early detection of *Streptococcus mutans* can be used as a means for early identification of children at high caries risk. Oral hygiene instructions have shown to be effective, over a short period of time, as demonstrated by reduced counts of *Streptococcus mutans*. This finding was more apparent among children with higher initial counts (43). However, studies that examined children after a longer period of time found the levels of *Streptococcus mutans* to be higher than after the shorter follow-up period (43).

The recall examination in our study was completed after 6 months and improvements regarding the level of *Streptococcus mutans* as well as the reduced caries incidence may well be transient in nature. Further follow-ups will be necessary to assess the overall effectiveness of the anticipatory guidance provided and to see if the information offered to the families has been translated into appropriate and long-term improvement on the child's health (46).

In this study 4 out of 35 subjects, who at the initial examination were without caries and had increased levels of *Streptococcus mutans,* exhibited caries lesions at their recall visit. These results are supported in the study by Ansai et al. (2000) which reported that in children under two years of age *Streptococcus mutans* levels correlated more closely with caries at an annual recall examination compared to the time of the initial exam (54). This supports the likelihood that the 4 subjects who in our study had high levels of *Streptococcus mutans* and no detectable caries at their initial exam were in fact already at a high risk for caries development.

In view of this it is reasonable to accept that the assessment of *Streptococcus mutans* levels is an increasingly important element in determining caries risk. This study supports the use of a simple and quick immunoassay diagnostic test (i.e. Saliva-Check Mutans) for chair-side use as part of today's dental practice. Results from this and other studies have shown that the evaluation of *Streptococcus mutans* levels may be an important predictor regarding future caries experience. For this reason, conventional diagnostic concepts should consider including this microbiological test as part of routine examinations to assist in preventing dental caries. However, further well-designed studies will be required to support the findings from this study.

Conclusions

Results from this study have shown that anticipatory guidance, offered to caregivers and children at an initial examination, led to a statistically non-significant reduction in the proportion of children with high *Streptococcus mutans* levels upon recall six mothers later. Children with low S*treptococcus mutans* levels were also less likely to develop new dental caries. These findings suggest that *Streptococcus mutans* counts could be used to assess caries risk and encourages clinicians to administer anticipatory guidance to their patients to help avoid the development of new caries.

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Study # ____

APPENDIX

Parents/Caregiver Interview Questionnaire Form
And
Oral Examination Forms
Subjects Aged 3-6 Years

Protocol for Interviewers:

- Explain the purpose and process for the interview & exam; obtain consent
- Assign each exam/interview a unique code, i.e. in sequence of being seen.
- Record this code on **each** page in the box provided
- The person interviewed should be the child's *primary* caregiver
- Ask the questions as written on the interview form, clarifying any meaning as needed
- Record all answers; circle, check or print the parent/caregiver's response
- Explain to patent / caregiver that he/she may refuse to answer any question, and/or that he/she may not know the answer
- Thank the caregiver for their time and participation

Parent's/Caregiver's:

Study code number**:** _____

Relationship to child _____

A. Patient Demographics

1. In what Town/City/Community does your child normally

   live?_____(write here)

   8 Don't know 9 Refused

2. How old is your child as of today?

   ☐ 3 years
   ☐ 4 years
   ☐ 5 years
   ☐ 6 years

8 Don't know 9 Refused

B. Medical and Dental History

5. Which of the following best describes your child's dental health?

☐ Very good
☐ Good
☐ Fair
☐ Poor

8 Don't know 9 Refused

6. How long has it been since your child last visited the dentist/dental therapist?

☐ 0-12 months
☐ 13-24 months
☐ 25 months or more

8 Don't know 9 Refused

7. Has your child experienced tooth decay in the past 12 months?

☐ Yes
☐ No

8 Don't know 9 Refused

8. Has your child ever received dental treatment?

☐ No
☐ Yes, in a dental / dental therapist clinic
☐ Yes, in the hospital under general anesthesia (put to sleep)

8 Don't know 9 Refused

9. Do you normally help your child brush his/her teeth?

☐ Yes
☐ No

8 Don't know 9 Refused

10. How often does your child brush his/her teeth?

☐ Less than once a day

☐ Once a day
☐ Twice a day or more

8 Don't know 9 Refused

11. Does your child use toothpaste when brushing his/her teeth? If Yes, what type of toothpaste does your child use.

☐ No
☐ If Yes please indicate which one: _____

8 Don't know 9 Refused

12. Is your child being bottle-fed?

☐ Yes
☐ No

8 Don't know 9 Refused

13. How often does your child consume sugary snacks such as candy, cake, coke, etc?

☐ Once or less per day
☐ Twice a day
☐ Three or more times a day

8 Don't know 9 Refused

14. Do you have your own natural teeth?

☐ Yes, I have all or most of my own teeth
☐ Yes, I have a few of my own teeth
☐ No, I do not have my natural teeth, but I do have dentures/false teeth
☐ No, I do not have my natural teeth, nor do I have false teeth

8 Don't know 9 Refused

15. Which of the following best describes when you personally obtain dental care?

☐ Regularly, for routine checkups
☐ Only when I am in pain
☐ Never, or rarely

8 Don't know 9 Refused

16. Which of the following most accurately describes your level of education?

☐ High school or less
☐ College degree
☐ Above college level

8 Don't know 9 Refused

17. Please indicate your household income for the year 2011

☐ ≤ $30,000
☐ > $30,000 - $60,000
☐ > $60,000

8 Don't know 9 Refused

THANK the parent/caregiver for taking the time to answer these questions!

Further questions are for the Dentist Only

C. Baseline Dental Exam Date: _____

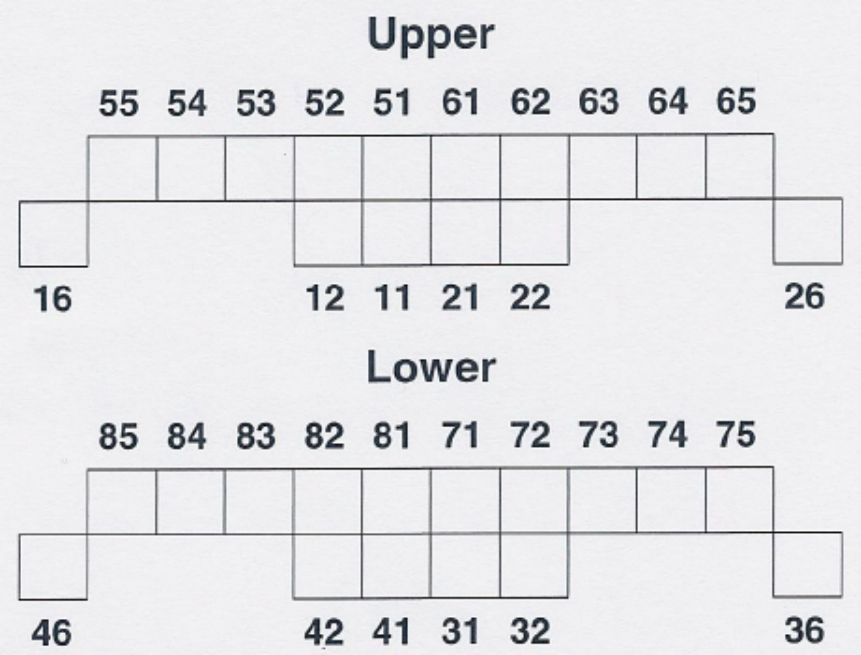
Level of *Streptococcus mutans* ☐ HIGH ☐ LOW

Plaque Index

16/55	11/51	26/65
Labial	Labial	Labial
Lingual	Lingual	Lingual
46/85	31/71	36/75

0 = no presence of plaque
1 = < 1/3 of tooth surface covered with plaque
2 = 1/3 – 2/3 of tooth surface covered with plaque
3 = Over 2/3 of tooth surface covered with plaque

Tooth Status



00 = sound
01 = decayed pit or fissure
02 = decayed smooth surface caries
03 = filed with no other decay
04 = filled and decayed
05 = missing due to caries
06 = missing for reasons other than caries
07 = restored for reasons other than
08 = to be extracted due to caries

D. Follow-up Dental Exam **Date: _____**

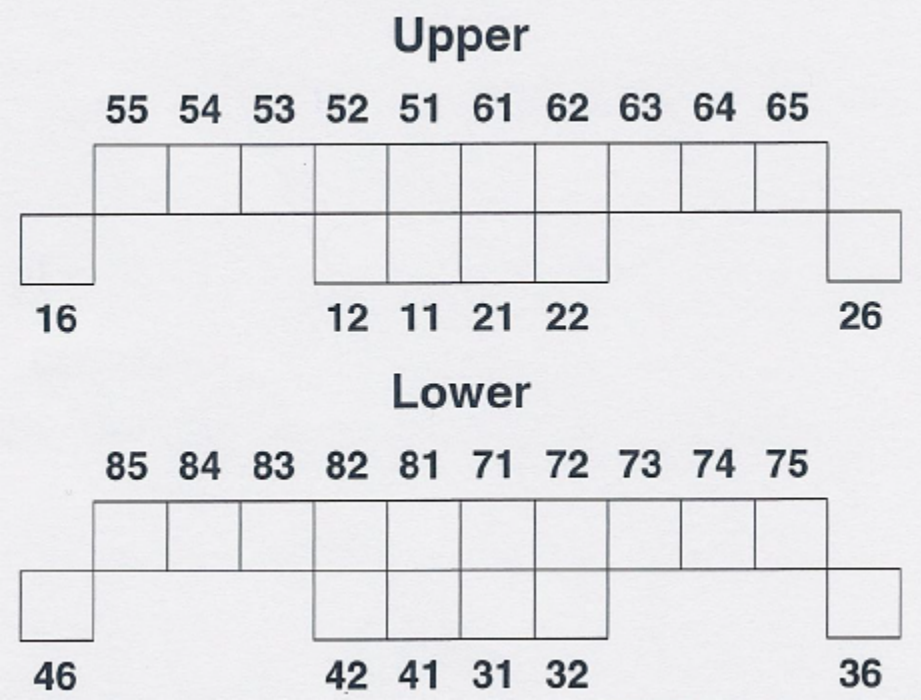
Level of *Streptococcus mutans* ☐ HIGH ☐ LOW

Plaque Index

16/55	11/51	26/65
Labial	Labial	Labial
Lingual	Lingual	Lingual
46/85	31/71	36/75

0 = no presence of plaque
1 = < 1/3 of tooth surface covered with plaque
2 = 1/3 – 2/3 of tooth surface covered with plaque
3 = Over 2/3 of tooth surface covered with plaque

Tooth Status



00 = sound
01 = decayed pit or fissure
02 = decayed smooth surface caries
03 = filled with no other decay
04 = filled and decayed
05 = missing due to caries
06 = missing for reasons other than caries
07 = restored for reasons other than
08 = to be extracted due to caries

APPENDIX

Table 1: Demographic frequency distributions for 98 subjects at baseline

Gender	Male – 47 (48.0%) Female – 51 (52.0%)
Age	4.80 (SD 1.1)
Fluoridated Water Community	Fluoridated – 75 (81.5 %) Non-Fluoridated –17 (18.5 %) Not sure/missing - 6
Toothpaste use	Yes – 93 (97.9%) No – 2 (2.1%) Not sure/missing - 3
Fluoridated toothpaste	Yes – 66 (73.3%) No – 24 (26.7%) Not sure/missing - 10
Previous visit to the dentist	0 – 12 months – 75 (79.8%) 13-24 months– 16 (17.0%) ≥ 25 months – 3 (3.2%) Not sure/missing - 2
Caregiver education	High School or less – 29 (32.6%) College degree – 43 (48.3%) Above College level – 17 (19.1%) Not sure/missing - 9
Annual household income	Less than $30,000 – 19 (24.4%) > $30,000 – $60,000 - 19 (24.4%) > $60,000 – 40 (51.3%) Not sure/missing - 20

Table 2: Dental Health and History of 98 subjects at baseline

Toothbrushing frequency	Less than once a day – 8 (8.2%) Once a day – 34 (35.1%) Twice a day or more – 55 (56.7%) Not sure/missing - 1
Caregiver help with brushing	Yes – 75 (76.5%) No – 23 (23.5%) Not sure/missing
Caries experience in previous 12 months	No – 55 (61.8%) Yes -34 (38.2%) Not sure/missing - 9
Previous Dental Treatment	No- 49 (50.5%) Yes (dental clinic/therapist) – 13(32.0%) Yes, in hospital under GA – 17 (17.5%) Not sure/missing - 1
Daily frequency of snacking	Once or less per day – 58 (61.1%) Twice per day – 28 (29.5%) Three or more times per day – 9 (9.5%) Not sure/missing - 3
Caregiver description of child's dental health	Very good – 28 (29.5%) Good – 48 (50.5%) Fair – 15 (15.8%) Poor – 4 (4.2%) Not sure/missing - 3
Caregiver dental care	Regular check-ups – 75 (79.8%) Only when in pain – 15 (16.0%) Never, or rarely – 4 (4.3%) Not sure/missing - 3

Table 3: Frequency distributions for caries experience (def+DMFT) and *Streptococcus mutans* at baseline

Caries Experience	0	35 (35.7%)
	1-5	25 (25.5%)
	6-16	38 (38.8%)
SM Test Results	Low ($<10^5$ cfu/ml)	48 (49.0%)
	High ($>10^5$ cfu/ml)	50 (51.0%)

Table 4: Demographic frequency distributions for the 58 subjects presenting at follow-up

Gender	Male – 23 (40%) Female – 35 (60%)
Fluoridated Water Community	Fluoridated – 40 (71.4 %) Non-Fluoridated –16 (28.6 %) Not sure/missing - 2
Toothpaste use	Yes – 56 (98%) No – 1 (2%) Not sure/missing - 1
Fluoridated toothpaste	Yes – 42 (80.8%) No – 10 (19.2%) Not sure/missing - 6
Previous visit to the dentist	0 – 12 months – 47 (83.9%) 13-24 months – 6 (10.7%) ≥ 25 months – 3 (5.4%) Not sure/missing - 2
Caregiver education	High School or less – 17 (30.9%) College degree – 27 (49.1%) Above College level – 11(20.0%) Not sure/missing - 3
Annual household income	Less than $30,000 – 8 (16%) > $30,000 – $60,000 - 13 (26%) > $60,000 – 29 (58%) Not sure/missing - 8 Not sure/missing - 8

Table 5: Frequency distribution for caries experience (def+DMFT) and *Streptococcus mutans* for 58 subjects at the follow-up examination

Caries Experience	0	18 (31.0%)
	1-5	22 (37.9%)
	6-16	18 (31.0%)
SM Test Results	Low ($<10^5$ cfu/ml)	39 (67.2%)
	High ($>10^5$ cfu/ml)	19 (32.8%)

Table 6: Demographic frequency distributions for the 40 subjects presenting for baseline examination only

	Prevalence (n=40)
Gender	Male – 24 (60%) Female – 16 (40%)
Caregiver level of education	High School or less – 12 (35.3%) College degree – 16 (47.1%) Above college level – 6 (17.6%) Not sure/missing - 6
Caregiver annual income	Less than $30,000 – 11 (39.4%) > $30,000 - $60,000 – 6 (21.4%) > $60,000 – 11 (39.3%) Not sure/missing - 12

Table 7: Presence and Prevalence of Caries Experience for 40 subjects presenting for baseline examination only

Caries Experience	0	12 (30.0%)
	1-5	8 (20.0%)
	6-16	20 (50.0%)
SM Test Results	Low ($<10^5$ cfu/ml)	18 (45.0%)
	High ($>10^5$ cfu/ml)	22 (55.0%)