The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/24925

Please be advised that this information was generated on 2019-09-02 and may be subject to change.
INFANT CARIES

Streptococcus mutans in children using nursing bottles

Cees M. Kreulen, DDS, PhD
Hans (J) J. de Soet, PhD
Remon Hogeveen
Jaap S.J. Veerkamp, DDS, PhD

Frequent and prolonged consumption of carbohydrate-rich substrates from a nursing bottle may cause a type of rampant caries in infants that is referred to as Nursing Bottle Caries (NBC) or Baby Bottle Tooth Decay (BBTD). This caries process affects surfaces that are usually at low risk. The last few years more attention has been paid to etiologic and preventive aspects, because with a decline of the caries prevalence, it became apparent that a small percentage of children is still highly affected by caries. Different criteria regarding diagnosis of the disease biases the outcomes of prevalence studies. Examples of criteria are: decay of the labial or lingual surfaces of at least two maxillary incisors without further restrictions to the location of other carious lesions or age of the patient; decay of at least three of the maxillary incisors; or maxillary incisors affected and further decay in the order of eruption. It is important to classify Nursing Bottle Caries in accordance with the latter, combining appearance in the eruption sequence, during which caries attacks, and the type of teeth affected.

Of the four etiologic factors in the caries process, diet and time have been addressed in detail. This regards the sweetened liquids in the nursing bottle, the frequency of use, and use at bedtime. Predeterminants for improper use of nursing bottles or other sweetened pacifiers are found to be related to cultural habits, socioeconomic class, and family composition and size. Moreover, other habits (breast feeding at will, sucking on a piece of chocolate during sleep etc.) have also been shown to result in Nursing Bottle Caries. Host factors are not well documented. Research on enamel mineralization is hard to perform, because healthy teeth from control patients of the same age cannot be obtained. Studies on salivary factors and immunological responses are still in their infancy. The fourth factor, cariogenic microorganisms, can be demonstrated by the infectious character of the disease and the type and numbers of bacteria involved. Several authors showed high (relative) levels of S. mutans to be a risk factor in the disease.

Children who use a nursing bottle beyond the regular dietary need do not necessarily develop Nursing Bottle Caries. This indicates special factors influencing the onset of the disease. Even within families the dental health of child-relatives may vary under similar Nursing Bottle Caries-provoking conditions. In these children at least two etiologic factors are apparently similar (diet and time). Differences may be found, therefore, in their microbiological flora, for instance, regarding S. mutans. The objective of this study is to compare Nursing Bottle Caries-patients and unaffected children within families, regarding the numbers and clonal types of S. mutans. Both children of a pair in the family had a bottle history. To assess the effect of treatment, the measurements are repeated in the Nursing Bottle Caries-patient after treatment.

Dr. Kreulen and Dr. Veerkamp are in the Department of Pediatric Dentistry and Dr. de Soet and Dr. Hogeveen are in the Department of Oral Microbiology, Academic Centre for Dentistry Amsterdam (ACTA), The Netherlands.
Centre for Special Dental Care (SFT) Amsterdam for providing the patients, and the families for cooperation in the study.
MATERIAL AND METHODS

In a period of nine months, subjects were selected from the patients of the Centre for Special Dental Care (SBT) in Amsterdam. Special Dental Care is a regional centre where children are referred to by general practitioners because of noncooperative behavior during regular dental treatment. During a routine admission the parents of Nursing Bottle Caries-patients were asked whether a brother or sister showed a similar dietary behavior (bottle history), that did not result in Nursing Bottle Caries. These families were invited to attend another session.

The mother, the Nursing Bottle Caries-patient and the unaffected brother or sister (control) of seven families were then dentally examined. The criteria for Nursing Bottle Caries were adopted from Veerkamp and Weerheijm. Brief medical histories were taken and the bottle behavior was recorded. Two pairs of children were apparently identical twins. From the patient, the control and the mother non-pooled plaque was sampled using a probe. Sample sites were: labial surface of left central incisor and buccal surface of a posterior tooth (the 65 with children and 26 with their mothers). In the Nursing Bottle Caries-patients, the anterior sample was taken from a carious site. Saliva was sampled from the tongue using a 10 μl-loop. The dental examination and sampling procedure of the Nursing Bottle Caries-patient was repeated after four to seven months posttreatment (sampling from mandibular incisors, if maxillary anterior teeth were extracted). Treatment included restorations and extractions, performed in a regular setting or using general anesthesia.

The plaque and saliva samples were kept in 0.9 ml RTF and processed within a few hours. The specimens were ultrasonic dispersed (10 times 1 sec). 0.1 ml of a tenfold dilution was plated onto the S. mutans selective medium TYCSB and incubated anaerobically at 37°C for two days. For assessment of the salivary number of colony forming units (CFU), colonies of S. mutans from the tongue samples were counted as identified by their morphology.

The plates with plaque samples were prepared for S. mutans type-screening. If present, more than thirty colonies per subject were randomly selected, colonies with different morphologies were selected deliberately. This selection procedure assures that more than 90 percent of the strain types of S. mutans present in the sample will be obtained with 95 percent confidence. The selected colonies were subcultured on blood agar and stored in skimmed milk at −80°C until further use.

The clonal types of the isolates were distinguished using a random primed polymerase chain reaction (RP-PCR) on the isolated DNA of the bacterial cells. The cells stored in skim milk were put in a sixteen-hour culture in BH1-broth (1 ml) and harvested by centrifugation. 100 μl Triton X-100 was added to obtain lysis at 95°C. 5 μl of this suspension were added to a standard RP-PCR-mix, using the DNA-primers 5'CCGGCGC and 5'GTAAGGCCG. The RP-PCR settings were: 35 cycles of denaturation at 94°C (1 min), annealing at 32°C (30 sec) and elongation at 72°C (1 min). The amplimers were made visible by using a standard agarose gel-electrophoresis and staining with ethidium bromide. Differences between isolates were made visible by comparing the gel-electrophoresis patterns of the amplimers (Figure 1).

RESULTS

The educational and growth experiences of patient and control were similar. In two families both parents were employed, whereas in the other five, the male was working professionally. One family was of recent immigrant origin. There were no signs of hereditary or congenital hypomineralization of the enamel; hygiene practices within families were similar. In Table 1 more detailed information is shown on diet, medical histories, dietary and bottle habits. Four of the mothers had a DMFT>18, which made them highly caries-active individuals; three mothers were scored as moderate caries-active (7<DMFT<11). We were not able to find essential dissimilarities between the patient and the control regarding the frequency and the content of the bottle. Moreover, two of the older children (controls)
exhibited a bottle behavior that should expose the child to a higher risk for caries than the patient. The controls were affected with caries to a certain extent, but this could certainly not be identified as Nursing Bottle Caries. From six patients, at least the maxillary incisors were extracted, which reflects the severity of the cases with Nursing Bottle Caries.

The patients had 5.8 log(CFU)/ml (± 0.8) saliva as obtained from the tongue (Table). The controls had a mean of 2.9 log(CFU)/ml (± 2.1), which is statistically different compared to the patients (Wilcoxon matched-pairs signed-rank test, p = .02, two-sided). In the mothers, S. mutans was always found (mean 4.8 log(CFU)/ml (± 1.2); Table). Several months after treatment the CFU/ml of S. mutans decreased by more than ten times in five patients. In two patients, CFU were comparable to baseline sampling (> 109), one of those still using the bottle. In the group with decreased CFU, also one child did not wean from the bottle.

With the DNA typing procedure, only one clonal type of S. mutans could be found in each Nursing Bottle Caries-patient (at the various sampling site (incisors), the noncarious site (molars) and also on the tongue). The controls had more variation in their S. mutans-flora and harbored two to five strains of plaque on the incisors as well as on the molars.

**DISCUSSION**

The relationship between Nursing Bottle Caries and *S. mutans* has previously been shown by comparing Nursing Bottle Caries patients with healthy children, but it is unclear whether the unaffected individuals in these studies also used a sweetened nursing bottle.11,12 For the purpose of comparison, case-control studies are most appropriate. Matching for dietary behavior (especially prolongation and frequency of bottle use), and also for age, gender, general health and SEC-factors, however, is tedious. Families were reported where the use of a sweetened milk drink was more regular than the elder sibling,13 for example, during day up until 1.5 years; bedtime up until 2 years; during day and bedtime; more regularly during day and bedtime; the children did not wean from the bottle.14

The controls had 5.8 log(CFU)/ml (± 0.8) saliva as obtained from the tongue (Table). The controls had a mean of 2.9 log(CFU)/ml (± 2.1), which is statistically different compared to the patients (Wilcoxon matched-pairs signed-rank test, p = .02, two-sided). In the mothers, S. mutans was always found (mean 4.8 log(CFU)/ml (± 1.2); Table). Several months after treatment the CFU/ml of S. mutans decreased by more than ten times in five patients. In two patients, CFU were comparable to baseline sampling (> 109), one of those still using the bottle. In the group with decreased CFU, also one child did not wean from the bottle.

With the DNA typing procedure, only one clonal type of S. mutans could be found in each Nursing Bottle Caries-patient (at the various sampling site (incisors), the noncarious site (molars) and also on the tongue). The controls had more variation in their S. mutans-flora and harbored two to five strains of plaque on the incisors as well as on the molars.

**DISCUSSION**

The relationship between Nursing Bottle Caries and *S. mutans* has previously been shown by comparing Nursing Bottle Caries patients with healthy children, but it is unclear whether the unaffected individuals in these studies also used a sweetened nursing bottle.11,12 For the purpose of comparison, case-control studies are most appropriate. Matching for dietary behavior (especially prolongation and frequency of bottle use), and also for age, gender, general health and SEC-factors, however, is tedious. Families were reported where the use of a sweetened nursing bottle was habitual (without affecting all children). Hence, study within these families seems to meet some of the matching problems. Even then assumptions of similar behavior have to be included, since detailed information on the older child is retrospective in nature. Nevertheles, the two twin-families in the present study, where more factors are stable, provide adequate support for the reliability of the data. Additionally, research is hindered by the relatively advanced age of the children, since parents of Nursing Bottle Caries-children usually seek professional support in an advanced stage of the process.

High numbers of *S. mutans* in saliva correlate well with a high caries risk.21,22 Weinberger and Wright found
In this study, accordingly, tongue samples were used, because it is difficult to obtain a salivary sample just by expectorating in young children with rampant caries. The high caries risk for the Nursing Bottle Caries patients in comparison to the non-Nursing Bottle Caries children within families was confirmed by higher counts of \textit{S. mutans}. The correlation between CFU and caries risk was, however, not clear between families, since the control in family 5 harbored as many CFU as the patients in families 1 and 4. In six patients more than $10^5$ CFU were found. This is in accordance with the previously reported association between dmft and CFU in young children.\(^{24}\) Preferably, \textit{S. mutans}-CFU should decrease after prevention (weaning from the nursing bottle) and treatment of cavities. It is not sure yet whether the higher numbers of \textit{S. mutans} lead to Nursing Bottle Caries or that Nursing Bottle Caries itself contributes to the high prevalence of \textit{S. mutans}.

From the literature available, Berkowitz concluded that \textit{S. mutans} is usually not found in children before one year of age, indicating that the etiologic factor ‘microorganisms’ is absent during eruption of the primary incisors.\(^{25}\) This is in contrast with anecdotal observations where parents of Nursing Bottle Caries-children sometimes report that the maxillary teeth of their children ‘erupted already brown’. Moreover, Brown \textit{et al} did detect \textit{S. mutans} in teething children who had one to four teeth erupted.\(^{12}\) It may be suggested, therefore, that Berkowitz’ conclusion is merely based on healthy individuals who do not easily develop caries. Nursing Bottle Caries-children may acquire the cariogenic flora earlier. This is in agreement with data from Köhler \textit{et al}, who report that the moment of first colonization by \textit{S. mutans} is inversely related to the affection by caries.\(^{26}\) High CFU in the mothers promote this colonization process.\(^{12,27}\) High-CFU mothers in our study have children, however, with different CFU within their families (even within twins). Additional factors must be involved in the colonization and it can be hypothesized that the age when the first tooth erupts and the interaction with the child’s developing immune system determine the onset of the disease.\(^{28}\)

In principal it may be questioned whether only one strain of \textit{S. mutans} in the flora provides for a noncarious condition or whether various strains assure a stable, non-pathogenic bacterial plaque. Recently Alaluusua \textit{et al} observed that Nursing Bottle Caries-patients are colonized with several strains, which is contrary to our results.\(^{15}\) In that study no clear distinction was made between the species \textit{S. mutans} and \textit{S. sobrinus}. In patients where only \textit{S. mutans} was found, 50 percent harbored just one strain. Furthermore, \textit{S. sobrinus} is not likely to be found in healthy patients, and given the few isolates that were selected, it might not be unexpected to find only one strain in caries-free children in the Alaluusua-study, due to a moderate confidence in obtaining the strains of a sample.\(^{24}\) At a 90 percent detection level, our results reflect an inverse relationship between the number of clonal types of \textit{S. mutans} and Nursing Bottle Caries. This suggests a selection of strains in Nursing Bottle Caries patients. Selection for the strongest and most virulent strain of \textit{S. mutans} may occur when oral homeostasis is disturbed, possibly due to low oral pH. If only one type of strain is involved in all Nursing Bottle Caries-patients, this may be an opportunity for specific antimicrobial therapy.

The present results suggest a more complicated etiology for Nursing Bottle Caries than the bottle behavior alone. Etiologic host factors like enamel maturation and mineralization should ideally be documented. Regarding microorganisms, we expected high counts of \textit{S. mutans} in both patient and control, because of the rich carbohydrate diet. This was not confirmed, however, and the difference observed for CFU may be caused by salivary factors, such as the individual sugar retention time. Based on the present results, the microbiologic CFU-screening for the assessment of Nursing Bottle Caries-risk does not yield consistent predictive figures. It may be supplemented by clonal typing of \textit{S. mutans} to gain reliability. The similarities of the chromosomal DNA patterns of strains in this study need to be further explored, just as do their cariogenic potentials.

**REFERENCES**

MANAGED CARE

Leaders of the managed-care companies maintain that they can continue to deliver effective care to the American people at a sustainable cost only by closely controlling the practice patterns of hospitals and physicians and by limiting the choices available to their enrollees. But increased numbers of plan members have begun demanding that the federal and state governments use their regulatory powers to ensure that the plans do not engage in policies and practices detrimental to enrollees’ health. Finding the balance between the degree of control the managed-care plans need in order to give their members cost-effective care and the degree of assistance the members seek from regulatory authorities to be sure that they are not being exploited, and their health endangered, is a challenge that, once it has surfaced, will not be readily resolved or set aside.

Moreover, the rapid expansion of enrollment in managed care has not prevented the estimated overall spending for health care in 1995 from increasing by 5.4 percent to a level just short of $1 trillion ($858 billion). If one considers the period from 1980 to 1995, more than one trillion of dental caries in children. J Dent Res, 74:167-173, February 1995.