Published in final edited form as:

Arch Oral Biol. 2011 December ; 56(12): 1560–1568. doi:10.1016/j.archoralbio.2011.06.017.

Urease activity as a risk factor for caries development in children during a three-year study period: a survival analysis approach

E Morou-Bermudez^{1,*}, A Elias-Boneta¹, RJ Billings², RA Burne³, V Garcia-Rivas¹, V Brignoni-Nazario¹, and E Suárez-Pérez⁴

¹University of Puerto Rico, School of Dental Medicine, San Juan, PR

²University of Rochester, School of Medicine and Dentistry, Rochester, NY

³University of Florida, College of Dentistry, Gainesville, FL

⁴University of Puerto Rico, Graduate School of Public Health, San Juan, PR

Abstract

Recent cross-sectional studies suggest that reduced ability to generate alkali via the urease pathway in dental plaque may be an important caries risk factor, but it has not been assessed prospectively.

OBJECTIVE—To evaluate the effect of plaque and saliva urease activity on the risk for developing new caries over a three-year period in children.

METHODS—A panel of 80 children, three to six years of age at recruitment, was followed prospectively for three years. Plaque urease activity, saliva urease activity and dental caries were measured every six months. Survival analysis methodology was used to evaluate the effect of urease on caries development during the study period adjusted for gender, age, baseline caries levels, sugar consumption, amount of plaque, and mutans streptococci levels.

RESULTS—The risk for developing new caries increased in a dose-responsive manner with increasing levels of urease activity in saliva (adjusted HR_{Q4 vs. Q1}: 4.98; 95%CI: 1.33, 18.69) and with decreasing urease activity in plaque (adjusted HR_{Q4 vs. Q1}: 0.29; 95%CI: 0.11, 0.76). Multiple measurements of urease activity were conducted to overcome the variability of urease activity in this study. Baseline caries and mutans streptococci in saliva were also important predictors of caries risk.

CONCLUSIONS—Increased urease activity in saliva can be an indicator of increased caries risk in children, while increased urease activity in plaque may be associated with reduced caries risk. The reproducibility of urease measurements must be improved before these findings can be further tested and clinically applied.

Keywords

urease; caries risk factors; children

* Author Responsible for Correspondence: Evangelia Morou-Bermudez, DDS, Ph.D, Department of Oral Biology, School of Dental Medicine, University of Puerto Rico Medical Sciences Campus, PO Box 365067, San Juan, Puerto Rico 00936-5067, Tel: 787-758-2525 (ext 2523), Fax: 787-763-4868, evangelia.morou@upr.edu.

INTRODUCTION

The prevalence of dental caries has been steadily declining in the United States during the past two decades; however young children have not experienced the same improvement in their oral health that older children and adults have^{1, 2}. This observation suggests that the generalized caries preventive measures that are currently available are not as effective for preventing caries in young children and that additional risk factors may be important for caries development in this age group. These factors should be identified and addressed in order to design “targeted” caries management approaches for this vulnerable population³.

Dental caries develops as a result of prolonged acidification in the dental biofilm, which disrupts its ecological balance leading to an increase in the proportions of highly acidogenic and aciduric bacteria^{4,5}. However, it has recently been proposed, that caries development may not only be associated with increased acid production in plaque, but also with a reduced ability to generate alkali from endogenous sources^{6,7,8}. One of best-characterized alkali-generating pathways in the oral cavity is the urease pathway, which generates ammonia from urea^{8,9}. This pathway is expressed in a number of oral bacteria, in dental plaque and in saliva^{10, 11}. Recent pilot cross-sectional studies have demonstrated significant differences in plaque urease activity between caries-free and caries-active adults, but not in saliva urease activity^{12, 13}. One recent pilot study in children also found that caries-free children had significantly higher urease activity in their plaque compared to children with caries, when urease activity was measured under fasting conditions¹⁴. These cross-sectional studies support the hypothesis that reduced ability to generate alkali from urea may be associated with increased caries levels in adults, and possibly in children. However, as the temporal sequence of events in these cross-sectional studies cannot be determined, these studies cannot establish a cause-and-effect relationship between urease activity and dental caries. Furthermore, in these pilot studies the potentially confounding effect of other important caries risk factors was not evaluated.

To begin to understand the role of urease activity in dental caries better we conducted a longitudinal panel study with repeated measures on a group of 80 children, aged three to six years at recruitment. The first findings of this study regarding the distribution of urease in children, its trend and reproducibility over time, and its relationship with several caries risk factors were recently reported¹⁵. Plaque urease activity was negatively associated with sugar consumption, while urease in saliva was positively associated with salivary mutans levels and with age, demonstrating for the first time the complex interactions between urease and other caries risk factors. This manuscript presents the findings from this study regarding the relationship of urease with caries in children. The effect of urease on the risk for developing new caries lesions during the study period was evaluated directly using a survival analysis approach, adjusted for age, gender, overall caries experience, salivary mutans levels, sugar consumption and amount of plaque.

MATERIALS AND METHODS

Study Design and Study Group

This study employed a prospective panel design with repeated measures every six months over a three-year period (Fig.1). The panel consisted of 80 healthy Hispanic children, aged 3 to 6 years at recruitment. The rationale for selecting this particular age group was that the most drastic changes in the caries status of children are likely to occur around the time of eruption of the first permanent molars¹⁶. As such, all of the children in the study group transitioned from an entirely deciduous dentition to a mixed dentition during the study period. The panel was balanced at baseline with respect to age, gender and caries

experience. This was achieved using a balanced-block recruitment strategy¹⁷. All children lived in Puerto Rico, which does not have an optimally fluoridated public water supply.

The socio-economic profile of the study panel has been previously described in detail.¹⁵ Briefly, 40.5% of the children had private dental insurance, 54.4% had public health insurance, and 5.1% had none. About 28% of the children attended private schools, which is common in Puerto Rico. 65.8 % lived with both parents, 32.9% lived in single-parent families and 1.3% lived with a person other than the biological parent. 55.7% of the children had at least one parent with University Level education. Parent education level was the only socio-economic factor that had an association with urease levels as previously reported.¹⁵

Sample collection and analysis

The details of sample size and composition, patient recruitment, consent procedures, sample collection and laboratory assays have been recently described in a separate manuscript¹⁵. Briefly, whole unstimulated saliva samples (about 3 mls) were collected initially using a sputum trap attached to the dental suction. Supragingival plaque was collected from all available smooth surfaces and pooled. All samples were collected between 8–10 AM. The children were asked to refrain from brushing their teeth the night before and in the morning before the study to allow for sufficient plaque to accumulate. The plaque and saliva samples were kept on ice during collection and were immediately transferred to the laboratory where they were snap-frozen and stored at -80°C. Prior to freezing and storing, a small amount of saliva (100 µl) was serially diluted and plated onto Mitis Salivarius agar plates (Beckson-Dickinson, Sparks, MD) containing bacitracin (MSB) as previously described^{14, 15}. Urease activity in plaque and saliva samples was measured using a spectrophotometric reaction and expressed as units (mg of protein)⁻¹, where 1 unit was defined as 1 mmole urea hydrolyzed min⁻¹ as previously described^{12–15}. The amount of plaque collected from each child was measured by weighing the plaque collection tubes before and after the sample collection and expressed in milligrams (wet plaque). Sugar consumption was measured using a method based on a 24-hour diet recall record, originally described by Nizel and Pappas¹⁸, and used by us in two previous closely related pilot studies^{14, 15}. After sample collection the teeth of the children were brushed using a soft toothbrush and toothpaste to remove the remaining plaque and a dental examination was performed as described below.

Dental examination and caries scoring

The dental exam was performed with an enhanced visual method using a Fiber-Optic Trans-Illumination apparatus (FOTI) (SCHOTT North America Inc., Southbridge, MA)¹⁹. Caries lesions were scored using a combination of visual and FOTI criteria, as described by Cortes, Ellwood and Ekstrand²⁰: d0: sound surface, d1: non-cavitated lesion in the enamel visible only on dry surface (FOTI: gray shadow confined in enamel), d2: non-cavitated lesion in the enamel visible on wet surface (FOTI: gray shadow confined in enamel), d3: non-cavitated lesion with dentin involvement (FOTI: orange/brown shadow from the dentine), d4: cavitated lesion, and d5: pulp involvement. As a consequence of the young age of the children, it was not always possible to differentiate clearly between d1 and d2 lesions and for that reason these scores were combined together as “enamel lesions”. In this study, dental caries was defined at the level of the dentin (d3). This disease definition includes non-cavitated lesions (“white-spots”) as long as there is dentinal involvement, which can be easily determined with FOTI²⁰. “Enamel lesions” (d1 and d2) were not considered as “failures” in the survival analysis, as these types of lesions are almost invariably detected in children when using FOTI, and that was also true in this particular panel as described in the results. A combined d₃mfs+D₃MFS index was calculated to classify the children into “high-risk” (above the median) and “low-risk” (below the median) based on their overall baseline caries experience. The median baseline d₃mfs+D₃MFS was 2.

All dental exams were conducted by the same examiner throughout the study period. The examiner was trained and calibrated in caries detection and scoring prior to the study by an expert reference “gold standard” examiner during a separate calibration exercise on 22 children (inter-examiner kappa: 0.83), and also periodically throughout the study by the same reference examiner. Intra-examiner reliability was periodically re-assessed during the study period on 22 of the study subjects (intra-examiner reliability: weighted kappa=0.86, 95% CI: 0.83, 0.89).

Analytical procedures

A survival analysis methodology, including Incidence Rates, Kaplan-Meier plots and Cox Proportional Hazards models, was utilized to estimate the effect of urease on the risk of developing new caries during the study period. The log-rank test was used to test the equality of survivor functions. The “event” or “failure” in this analysis was defined as a subject with at least one new caries lesion or restoration (please refer to the previous section for definition of caries). As all children transitioned from an entirely deciduous dentition to a mixed dentition during the study period, new lesions were identified individually by comparing the scores for each surface in the seven study visits. Only lesions or restorations on surfaces that had a score 0–2 in the previous exams or on newly erupted surfaces were considered “failures”. This definition of “failure” allowed all subjects to be included in the analysis, irrespective of existing caries at the beginning of the study. As each child could experience multiple “events” during the study period (new caries), a “multiple failures-per-subject” survival approach was employed in STATA²¹ following the counting process approach of Andersen and Gill²². In this approach, caries was considered as an “ordered” failure event which met two basic assumptions: a) each failure event is equal and b) each subject experienced only one event at a given time (multiple new caries lesions in one subject at a given time point were considered as one failure event). This survival approach analyzes the time to each event, considering “at risk” all subjects under observation at a given time. The failure times were correlated within each subject, in contrast with traditional survival analysis, which assumes an independence of failure times²¹.

The main predictor variables in this study were urease activity in plaque and urease activity in saliva. The reproducibility of urease measurements between the study visits was low, especially for plaque urease, as discussed previously¹⁵. To define the baseline values of these predictors more accurately, the average of multiple urease measurements was used. As a consequence, two different types of models were produced, depending on the definition of urease: Model 1–2 was based on the urease average in visits 1 and 2 (baseline and six months), while Model 1–3 was based on the urease average in visits 1, 2 and 3 (baseline, six months and one year). The purpose was to assess the caries rate with a more precise starting value in urease activity both in plaque and in saliva. Once these averages were calculated, the Hazard Ratios were estimated between quartiles of these averages.

The models were adjusted for two types of covariates: constant and time-dependent. Gender, age at recruitment (three years, four years, five years and six years), and initial caries status (“high-risk vs. low risk”) were defined at baseline, and were considered constant variables, like urease. Sugar consumption, plaque wet weight and salivary mutans levels were included in the models as time-dependent covariates. In preliminary analysis, these variables were also considered as baseline, constant variables. Their effect was similar, but less significant than when used as time-dependent variables. For that reason, in the final models these potential confounders were included as time-dependent variables. Data were analyzed using the STATA version 10 program (StataCorp LP, College Station, TX).

Ethical Approval

The study was approved by the Institutional Review Board (IRB) of the University of Puerto Rico Medical Sciences Campus (UPR-MSC). The children assented to participate in the study using an age-appropriate assent form. The parents or legal guardians of the children consented to the voluntary participation of the children by signing an informed consent approved by the IRB of the UPR-MSC. The children received the benefit of a free dental examination by a pediatric dentist every six months and referrals for treatment as needed.

RESULTS

The composition of the study panel at baseline was balanced with respect to gender, age and caries experience, as previously reported¹⁵: 52.5% were females; 23.27% were three years-old, 31.32 % four years-old, 21.03% five years-old and 24.38% six years-old at time of entry into the study. 55% of the children were classified as “low caries risk” at baseline ($d_3mfs + D_3MFS \leq 2$) and 45% were “high risk” at baseline ($d_3mfs + D_3MFS > 2$). The mean combined $d_3mfs + D_3MFS$ index at baseline was 7.56, and the median was 2; 44% of the children were caries-free at the d3 level at baseline and 91% had “enamel lesions” (d1 and d2). The socio-economic profile of the study panel at baseline was balanced with respect to parent education level, type of school attended (public vs. private) and type of medical and dental insurance (public vs. private) as previously reported.¹⁵

The average annual attrition rate was approximately 13%, which was lower than the expected 20% attrition rate. A total of 52 children completed all seven study visits, for a total of 193.1 person-years. The composition of the panel at the end of the three-year period was similar to the original panel with respect to age and gender¹⁵, but there was a somewhat higher attrition of subjects with high caries levels at baseline compared to the “low-risk” subjects¹⁵. The proportion of children that were caries-free decreased to 38.5% at the end of the three-year study period, the mean combined $d_3mfs + D_3MFS$ increased to 8.44 and the median to 3. New “enamel lesions” (d1 and d2) were detected in as many as 79% of the children in the subsequent visits, and the prevalence of these lesions increased to 98%.

A total of 141 “failures” were observed during the three-year study period representing an overall incidence rate of 0.73 (subjects with new caries)/person-years (Table 1). The caries incidence rate was similar in boys and girls; however, it was significantly higher in children who were six years old at baseline (0.96/person-year) compared to the younger children (0.66/person-year, $P=0.022$). The caries incidence rate was about three times higher in children who had caries levels above the median for the group at the beginning of the study, compared to the children who had baseline caries levels below the median ($P<0.001$). The caries incidence rate was also about three-fold higher in children who had detectable mutans streptococci in their saliva ($\geq 1,000$ CFU/ml) at baseline compared to those who did not ($P<0.001$). The effect of urease on caries incidence rate was evaluated initially at different quartiles of plaque and saliva urease. It was determined that the urease threshold for detecting differences in incidence rates was the 75th percentile for plaque urease (3.3–3.4 units/mg), and for saliva urease the 25th (0.04–0.06 units/mg). Children with average plaque urease levels above the 75th percentile during the first two or three visits had lower caries incidence rates compared to children with lower plaque urease, but this difference was not statistically significant ($P>0.05$). However, children with average salivary urease levels above the 25th percentile during the first two or three visits had two to four times higher caries incidence rate compared to children with the lower saliva urease levels ($P=0.006$, and $P<0.001$, respectively). No differences were observed in caries incidence rates by urease levels when these levels were measured only at baseline ($P>0.05$, data not shown).

Kaplan-Meier plots were used to evaluate the rate of caries development over the three-year study period. Based on incidence rates, the Kaplan-Meier plots indicate that children who at baseline had caries levels above the median (Fig. 2A), and those who had detectable mutans streptococci in their saliva (Fig. 2B) developed caries at faster rate compared to children with lower caries levels and those with no detectable mutans at baseline ($P_{\text{Log-rank test}} < 0.001$). The development of caries was also faster in children who had average saliva urease levels above the 25th percentile during the first two (0.04 units/mg-Fig. 3A) or three (0.06 units/mg-Fig. 3B) visits (Model 1–2 $P_{\text{Log-rank test}} = 0.016$, Model 1–3 $P_{\text{Log-rank test}} < 0.001$). Children with average plaque urease levels above the 75th percentile (3.3 units/mg) in the first two or three visits developed caries somewhat slower compared to the children with lower plaque urease levels (Figures 4A and 4B), but this difference was not statistically significant ($P_{\text{Log-rank test}} > 0.05$).

Cox Proportional Hazards models were used to evaluate the effect of urease on the risk of developing new caries lesions during the study period. When the average of two measurements (Model 1–2), or three (Model 1–3) was used to define baseline urease categories, it was observed that the risk of developing new caries decreased progressively up to 70% (adjusted Hazard Ratio_{Q4 vs. Q1}: 0.29; 95% CI: 0.11, 0.76) with increasing levels of plaque urease but increased progressively, up to five-fold (adjusted Hazard Ratio_{Q4 vs. Q1}: 4.98; 95% CI: 1.33, 18.69), with increasing levels of saliva urease (Table 2). The effect estimates for both plaque and saliva urease were stronger when the average of the first three measurements were used instead of two, even though the length of the observation period and number of observations decreased. The most significant predictor for caries development in all models was the caries status of the children at baseline (adjusted Hazard Ratio^{“high risk” vs. “low risk”}: 3.01, 95% CI: 1.50, 6.08). The risk of developing new caries also increased progressively with increasing levels of mutans streptococci in saliva (adjusted Hazard Ratios 1.27–4.09, depending on the salivary mutans levels). Children who were four or five years-old at recruitment had lower risk of developing new caries compared to the children who were only 3 years-old at baseline. However, older children, i.e., those who were six years-old at recruitment did not have a significantly lower caries risk compared to the younger ones (Table 2). No associations were observed between gender, sugar consumption, or plaque amount with the risk of developing caries in this study (Table 2).

DISCUSSION

Bacterial urease activity in dental plaque and in saliva generates alkali, which can increase the plaque pH and can protect non-cariogenic oral bacteria against acidification^{6–11}; consequently it has been hypothesized that oral urease activity may be a caries-inhibiting factor, and that loss of this activity may lead to caries development. Recent pilot clinical studies have reported significantly higher plaque urease activity in caries-free vs. caries-active subjects, but not significant differences in saliva urease activity, suggesting a potentially significant caries protective role for plaque urease but not for urease in saliva. These were cross-sectional studies that compared adult subjects with extreme caries activity to caries-free subjects^{12, 13}. One recent cross-sectional study reported similar findings in a small group of children; however his study was not evaluating the relationship of urease with caries directly¹⁴.

The present study is, to our knowledge, the first to examine the role of urease in caries development in children, prospectively. The design of the study involved repeated urease and caries measurements over a three-year period on a single panel of children with different caries levels at baseline, including caries-free children. Caries detection was performed using a sensitive, yet simple method, which facilitated the detection of non-cavitated lesions (“white spots”) even in small children, as well as the differentiation between “white spots”

restricted to the enamel, versus those where dentin was already involved. For many investigators and clinicians, “white spot” lesions in children younger than six years of age are considered “caries”. However, in this study, these lesions were considered as “failures”, i.e., caries, only when there was a clear indication using FOTI that dentin was involved, for the reasons explained in the Methods. The study considered several known caries risk factors as potential confounders, such as age, gender, baseline caries status, mutans streptococci levels, sugar consumption and plaque amounts. We have recently reported some interesting findings regarding the complex interactions observed between urease activity and the aforementioned risk factors in this study¹⁵. For all these reasons, it is not surprising that this study yielded some original and unexpected findings compared to the previous pilot clinical studies, which were cross-sectional, utilized adult populations with significant caries activity, and did not control for potential confounders.

The most unexpected and important finding of this study was that saliva urease activity had a significant effect on the risk of developing caries, and that this effect was not protective, but in fact caries promoting. As urease activity in saliva increased, caries incidence rates increased up to four times, and the risk of developing new caries increased progressively up to five times, even when the models were adjusted for baseline caries, age, and mutans streptococci levels. A possible explanation for this finding is that there may be competition between ureolytic bacteria in plaque and in saliva for limiting urea. Saliva is the principal source of urea in the oral cavity²³. As urease activity in saliva increases, salivary urea is more rapidly hydrolyzed and the amount of urea available to plaque bacteria may become limited, leading to a more acidic plaque. Another explanation for the association of saliva urease with increased caries risk can be the positive relationship between urease activity in saliva and salivary mutans streptococci levels in this study¹⁵. This observation was attributed to the knowledge that subjects with high salivary mutans levels had more acidic salivary pH, which is known to de-repress urease expression in *Streptococcus salivarius* in saliva^{24–26}. Interestingly, this relationship was observed in non-fasting subjects, but not in fasting subjects, and this may be the reason why it was not observed in the previous cross-sectional studies that used fasting subjects only.

The findings of the present study support the proposed protective effect of plaque urease on caries development, evidenced by progressively decreasing caries incidence rates and hazard ratios as plaque urease activity levels increased. However, the association of plaque urease activity with caries development in this study was not as significant as previously suggested in the cross-sectional studies, especially when only one measurement was used to define urease categories, or when urease was analyzed as a time-dependent variable in the Cox Proportional Hazards models. The magnitude and significance of this effect increased as multiple measurements were used to define baseline urease activity, such as the average of the first two, or first three visits. That was true for both plaque and saliva urease activity. The reason for this observation is most likely the poor reproducibility of urease activity at the six-month recall visits in this study, especially in plaque urease¹⁵. A possible reason for the lower reproducibility of plaque urease was that plaque samples were pooled from different surfaces; therefore, their composition may have been different at each visit. Another source of variability in urease measurements in this study was whether the children had eaten prior to sample collection or not. Fasting prior to sample collection was difficult to control at the level of the study design due to the young age of the children, but it was measured during the study and controlled for in the analysis. Fasting had a strong impact on the reproducibility of plaque urease, compared with urease in saliva¹⁵. Notably, subjects in the prior cross-sectional studies that found a significant difference in plaque urease activity between caries-free and caries-active had fasted prior to sampling. It appears, therefore, that it may be possible to observe a much stronger effect of plaque urease on caries development

if a more standardized protocol for plaque and saliva collection is employed, such as collecting plaque from specific tooth sites, and under fasting conditions.

In conclusion, the findings of this prospective study suggest that increased urease activity in the saliva of small children may be associated with progressively increased caries risk. This finding may be attributed to a competition between ureolytic bacteria in the plaque and in saliva for salivary urea, or it may reflect the positive relationship between urease and mutans levels in saliva. Work in progress may clarify the mechanism of action. However, in either case, this observation could have significant clinical implications, as this study suggests that urease activity in saliva could be an equal or better predictor of caries risk in children than salivary mutans levels. Measuring urease activity in saliva is much faster and can be cheaper compared to the microbiological tests for mutans detection in saliva. Increased urease activity in dental plaque could be associated with decreased caries risk in children, but in order to demonstrate this association clearly and to apply it in clinical practice it will be necessary to improve the reproducibility of urease measurements in plaque. Using plaque urease as a risk indicator for caries would also require that the measurements be performed under fasting conditions, which may be a difficult requirement for children, but apparently the use of salivary urease does not have this requirement. An attractive and interesting approach that we are currently exploring is the possibility of combining low plaque urease activity and increased saliva urease activity into a single predictor for caries risk in children.

Acknowledgments

The authors would like to acknowledge Dr. Walter Psoter, NYU College of Dentistry, for his advice in the design of this study and in the analysis of the data, and also for the critical evaluation of this manuscript. Dr. Ralph Katz, NYU College of Dentistry, for his advice in the design of this study. Dr. Dorota Kopycka-Kedzierawski, University of Rochester School of Medicine and Dentistry, and Dr. Sona Rivas-Tumanyan, University of Puerto Rico School of Dental Medicine for the critical evaluation of this manuscript. Also, the staff of the Clinical Research Center at the University of Puerto Rico Medical Sciences Campus and the UPR students Emanuel Rosado, Omar Chacon, Joel Caraballo, Joel Diaz, Guillermo Ramirez, Valerie Garcia, Monica Rodriguez and Aixa Morales for their help in this study. The study described was partially supported by a Career Development Award, K23 DE015285 (PI: Morou-Bermudez, Evangelia) from the National Institute for Dental and Craniofacial Research, by RCMI grant #G12 RR 03051, and by an RCMI Clinical Research Infrastructure Initiative (RCRII) Award 1P20 RR 11126, from the National Center for Research Resources (NCRR). This publication was also made possible by Grant Number 1U54RR026139-01A1 from NCRR, a component of the National Institutes of Health (NIH). Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH.

REFERENCES

1. U.S. Department of Health and Human Services. Oral Health in America: A Report of the Surgeon General. Rockville, MD: U.S. Department of Health and Human Service, National Institute of Dental and Craniofacial Research, National Institutes of Health; 2000.
2. Dye BA, Tan S, Smith V, Lewis BG, Barker LK, Thornton-Evans G, Eke PI, Beltran-Aguilar ED, Horowitz AM, Li CH. Trends in oral health status: United States, 1988–1994 and 1999–2004. *Vital Health Stat 11*. 2007 Apr;248:1–92. PubMed PMID: 17633507. [PubMed: 17633507]
3. Tinanoff N, Douglass JM. Clinical decision making for caries management in children. *Pediatr Dent*. 2002; 24:386–392. [PubMed: 12412953]
4. Marsh PD. Dental plaque as a biofilm and a microbial community-implications for health and disease. *BMC Oral Health*. 2006; 6 suppl I:S14. [PubMed: 16934115]
5. Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. *J Dent Res*. 2011 Mar; 90(3):294–303. Epub 2010 Oct 5. Review. PubMed PMID: 20924061. [PubMed: 20924061]
6. Burne RA, Marquis RE. Alkali production by oral bacteria and protection against dental caries. *FEMS Microbiol Lett*. 2000 Dec 1; 193(1):1–6. [PubMed: 11094270]

7. Kleinberg I. A mixed-bacteria ecological approach to understanding the role of oral bacteria in dental caries causation: an alternative to *Streptococcus mutans* and the specific-plaque hypothesis. *Crit Rev Oral Biol Med*. 2002; 13:108–125. [PubMed: 12097354]
8. Burne RA, Chen YY. Bacterial ureases in infectious diseases. *Microbes Infect*. 2000 Apr; 2(5):533–542. Review. PubMed PMID: 10865198. [PubMed: 10865198]
9. Kleinberg I. Effect of urea concentration on human plaque pH levels in situ. *Arch Oral Biol*. 1967 Dec; 12(12):1475–1484. PubMed PMID: 5237332. [PubMed: 5237332]
10. Sissons CH, Hancock EM, Perinpanayagam HE, Cutress TW. The bacteria responsible for ureolysis in artificial dental plaque. *Arch Oral Biol*. 1988; 33(10):727–733. PubMed PMID: 3075450. [PubMed: 3075450]
11. Salako NO, Kleinberg I. Incidence of selected ureolytic bacteria in human dental plaque from sites with differing salivary access. *Arch Oral Biol*. 1989; 34(10):787–791. PubMed PMID: 2610614. [PubMed: 2610614]
12. Shu M, Morou-Bermudez E, Suarez-Perez E, Rivera-Miranda C, Browngardt CM, Chen YY, Magnusson I, Burne RA. The relationship between dental caries status and dental plaque urease activity. *Oral Microbiol Immunol*. 2007 Feb; 22(1):61–66. PubMed PMID: 17241172. [PubMed: 17241172]
13. Nascimento MM, Gordan VV, Garvan CW, Browngardt CM, Burne RA. Correlations of oral bacterial arginine and urea catabolism with caries experience. *Oral Microbiol Immunol*. 2009 Apr; 24(2):89–95. Erratum in: *Oral Microbiol Immunol*. 2009 Jun; 24(3):264. PubMed PMID: 19239634; PubMed Central PMCID: PMC2742966. [PubMed: 19239634]
14. Toro E, Nascimento MM, Suarez-Perez E, Burne RA, Elias-Boneta A, Morou-Bermudez E. The effect of sucrose on plaque and saliva urease levels in vivo. *Arch Oral Biol*. 2010 Mar; 55(3):249–254. Epub 2010 Jan 21. PubMed PMID: 20096398; PubMed Central PMCID: PMC2853032. [PubMed: 20096398]
15. Morou-Bermudez E, Elias-Boneta A, Billings RJ, Burne RA, Garcia-Rivas V, Brignoni-Nazario V, Suarez-Perez E. Urease activity in dental plaque and saliva of children during a three-year period and its relationship with other caries risk factors. *Archives of Oral Biology*. 2011
16. Kopycka-Kedzierawski DT, Billings RJ. A longitudinal study of caries onset in initially caries-free children and baseline salivary mutans streptococci levels: a Kaplan-Meier survival analysis. *Community Dent Oral Epidemiol*. 2004 Jun; 32(3):201–209. PubMed PMID: 15151690. [PubMed: 15151690]
17. Beltrán-Aguilar ED, Estupiñán-Day S, Báez R. Analysis of prevalence and trends of dental caries in the Americas between the 1970s and 1990s. *Int Dent J*. 1999 Dec; 49(6):322–329. PubMed PMID: 10907429. [PubMed: 10907429]
18. Nizel, AE.; Papas, AS. *Nutrition in Clinical Dentistry*. 3rd edition. W.B. Saunders Company; p. 277–308.
19. Pine, CM. In: Stookley, GK., editor. *Fiber-optic trans-illumination (FOTI) in caries diagnosis; Early detection of dental caries: Proceedings of the 1st annual Indiana Conference*; Indianapolis: Indiana University School of Dentistry; 1996. p. 51–65.
20. Cortes DF, Ellwood RP, Ekstrand KR. An in vitro comparison of a combined FOTI/visual examination of occlusal caries with other caries diagnostic methods and the effect of stain on their diagnostic performance. *Caries Res*. 2003 Jan–Feb; 37(1):8–16. PubMed PMID: 12566633. [PubMed: 12566633]
21. Cleves, M. [Retrieved on March 31, 2011] StataCorp. *Analysis of Multiple Failure-Time Survival data*. 1999 November. updated July 2009, from <http://www.stata.com/support/faqs/stat/stmfail.html>
22. Andersen PK, Gill RD. Cox's regression model for counting processes: A large sample study. *Annals of Statistics*. 1982; 10:1100–1120.
23. Kopstein J, Wrong OM. The origin and fate of salivary urea and ammonia in man. *Clin Sci Mol Med*. 1977 Jan; 52(1):9–17. PubMed PMID: 23916. [PubMed: 23916]
24. Sissons CH, Perinpanayagam HE, Hancock EM, Cutress TW. pH regulation of urease levels in *Streptococcus Salivarius*. *J Dent Res*. 1990 May; 69(5):1131–1137. PubMed PMID: 2110582. [PubMed: 2110582]

25. Chen YY, Burne RA. Analysis of *Streptococcus salivarius* urease expression using continuous chemostat culture. *FEMS Microbiol Lett.* 1996 Jan 15; 135(2–3):223–229. PubMed PMID: 8595861. [PubMed: 8595861]
26. Li YH, Chen YY, Burne RA. Regulation of urease gene expression by *Streptococcus salivarius* growing in biofilms. *Environ Microbiol.* 2000 Apr; 2(2):169–177. PubMed PMID: 11220303. [PubMed: 11220303]

Figure 1.
Overview of study design

Figure 2. Caries rate by A: Caries status at baseline ($P_{\text{Long-rank test}} < 0.001$), and B: Salivary mutans levels at baseline ($P_{\text{Long-rank test}} < 0.001$).

Figure 3.

Caries rate by saliva urease. (A) Baseline saliva urease categories were defined using the average of visits 1 and 2 ($P_{\text{Long-rank test}}=0.016$). (B) Baseline saliva urease categories were defined using the average of visits 1, 2 and 3 ($P_{\text{Long-rank test}}<0.001$).

Figure 4.

Caries rate by plaque urease. (A) Baseline plaque urease categories were defined using the average of visits 1 and 2 ($P_{\text{Long-rank test}} > 0.05$). (B) Baseline plaque urease categories were defined using the average of visits 1, 2 and 3 ($P_{\text{Long-rank test}} > 0.05$).

Table 1

Caries Incidence* by gender, age, baseline caries status, plaque urease, saliva urease and salivary mutans levels.

Groups	Incidence Rate*	P-value
Overall	0.73	
Gender		0.598
Males	0.70	
Females	0.76	
Age at Recruitment		0.044
<6 years	0.66*	
≥6 years	0.96	
Caries Status at Baseline		<0.001
“Low risk” ($d_3mfs + D_3MFS \leq 2$)	0.38*	
“High risk” ($d_3mfs + D_3MFS > 2$)	1.26	
Plaque Urease ^a (Average of visits 1–2)		0.161
≤3.3 units/mg (75%)	0.75	
>3.3 units/mg	0.53	
Plaque Urease ^b (Average of visits 1–3)		0.335
≤3.4 units/mg (75%)	0.73	
>3.4 units/mg	0.56	
Saliva Urease ^a (Average of visits 1–2)		0.013
≤0.04 units/mg (25%)	0.41*	
> 0.04 units/mg	0.79	
Saliva Urease ^b (Average of visits 1–3)		<0.001
≤0.06 units/mg (25%)	0.22*	
> 0.06 units/mg	0.83	
Salivary Mutans at baseline		<0.001
≤1,000 CFU/ml	0.27*	
>1,000 CFU/ml	0.87	

* Subjects with at least one new caries lesion per person-year

^a incidence rate was calculated after visit 2

^b incidence rate was calculated after visit 3

Table 2

Hazard Ratio (HR) estimation for caries development.

	Model 1-2*		Model 1-3**	
	Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
Gender ¹				
Female	0.78 (0.46, 1.30)	0.332	0.61 (0.33, 1.13)	0.116
Age at recruitment ²				
4 years	0.50 (0.24, 1.02)	0.058	0.24 (0.08, 0.70)	0.009*
5 years	0.65 (0.31, 1.39)	0.268	0.32 (0.10, 0.99)	0.048*
6 years	0.63 (0.32, 1.27)	0.195	0.52 (0.19, 1.45)	0.210
Caries Status at Baseline ³				
“High risk”	2.69 (1.54, 4.71)	0.001*	3.01 (1.50, 6.08)	0.002*
(d ₃ mfs+D ₃ MFS>2)				
Plaque Urease (units/mg) ⁴				
1.7–2.5 (Q2)	0.64 (0.32, 1.25)	0.189	0.77 (0.34, 1.74)	0.531
2.5–3.3 (Q3)	0.64 (0.35, 1.16)	0.142	0.56 (0.23, 1.35)	0.195
>3.3 (Q4)	0.62 (0.32, 1.19)	0.151	0.29 (0.11, 0.76)	0.012*
Saliva Urease (units/mg) ⁵				
0.04–0.1 (Q2)	1.04 (0.48, 2.26)	0.928	2.92 (0.82, 10.43)	0.099
0.1–0.2 (Q3)	1.44 (0.71, 2.93)	0.311	4.38 (1.23, 15.57)	0.023*
>0.2 (Q4)	1.38 (0.62, 3.07)	0.437	4.98 (1.33, 18.69)	0.017*
Time-dependent co-variables				
Sugar Score	1.03 (0.99, 1.08)	0.161	1.05 (0.98, 1.12)	0.613
Plaque Amount	1.01 (0.99, 1.02)	0.308	1.01 (0.99, 1.04)	0.251
Salivary Mutans ⁶				
10 ³ –10 ⁴ CFU/ml	1.72 (0.91, 3.24)	0.096	1.27 (0.50, 3.25)	0.613
10 ⁴ –10 ⁵ CFU/ml	2.01 (1.13, 3.58)	0.018*	1.60 (0.72, 3.57)	0.251
10 ⁶ –10 ⁶ CFU/ml	2.36 (1.28, 4.35)	0.006*	2.25 (0.94, 5.41)	0.070
>10 ⁶ CFU/ml	3.06 (1.50, 6.22)	0.002*	4.09 (1.58, 10.58)	0.012*

* Utilizes the average of urease at visits 1 and 2 as baseline. Observation period: 2.5 years. 249 observations, 65 subjects, 103 failures in multiple failure-per-subject data, R²=60.87

** Utilizes the average of urease at visits 1, 2 and 3 as baseline. Observation period 2 years. 190 observations, 56 subjects, 70 failures in multiple failure-per-subject data, R²=52.89

¹ referent category: male

² referent category: 3 years

³ referent category: “low caries” (d₃mfs+D₃MFS≤2)

⁴ referent category: <1.7 units/mg (Q1)

⁵ referent category: <0.04 units/mg (Q1)

⁶ referent category: <1,000 CFU/ml