Community Dent Oral Epidemiol 2007; 35: 500–506

COMMUNITY DENTISTRY AND ORAL EPIDEMIOLOGY

Caries risk factors in the permanent dentition of Tanzanian children: a cohort study (1997–2003)

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Scheutz F, Matee MI, Poulsen S, Frydenberg M. Caries risk factors in the permanent dentition of Tanzanian children: a cohort study (1997–2003). Community Dent Oral Epidemiol 2007; 35: 500–506. © 2007 The Authors. Journal compilation © 2007 Blackwell Munksgaard

Abstract – *Objective:* The aims of the cohort study were to study the association between permanent dentition caries and malnutrition and other risk factors or indicators in a group of children with little or no access to restorative and preventive dental care. *Methods:* The study was conducted at two primary schools in Dar es Salaam, Tanzania in the period 1997–2003. One school recruits its children from affluent families and the other school is attended by the children of poor families. The children attended grade 1 at the first examination and had a mean age of 7.6 years (SD = 0.4); when the study was completed, the mean age was 13.3 years. Two-hundred and eighteen children examined in 1997 were re-examined in 1999; 147 of the children were examined in 1997 as well as in 2003, and 122 children were examined in all three years. An age- and sex-based body mass index (BMI) was computed to determine the nutritional status of each child. Each year, the same standard methods were used to determine the count of lactobacilli and mutans streptococci, stimulated flow rate and buffering capacity of saliva, and caries. Risk ratios were computed with generalized linear models using the tooth as the unit of analysis. *Results:* Mean annual caries increment was 0.27 in the period 1997–1999 and 0.80 in the period 1999–2003 with most children developing no caries at all. Malnutrition at baseline in 1997 was insignificantly predictive for the development of caries, whereas a low stimulated flow rate of saliva (≤0.7 ml/min) and a high count of lactobacilli (≥100 000/ml) at baseline in 1997 were significantly associated. However, the generalized linear models for the two time periods 1997–1999 and 1999–2003 presented a confusing picture with different risk ratios and without consistency of the associations between the exposure variables and the development of caries. In accordance with this finding, the consistency of the exposure variables over time for the individuals was very low. *Conclusions:* The results were inconclusive and left us with more questions than answers. The findings do, however, support the view that our methods for predicting caries are inappropriate or nonexistent.

Key words: cohort; children; caries risk factors; microbiology; saliva; malnutrition

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Submitted 24 March 2006;
accepted 11 August 2006

In a recent paper based on cross-sectional data we presented the association between dental caries in the primary dentition and malnutrition and various risk factors among Tanzanian schoolchildren (1). We had found a significant association between caries and malnutrition, lactobacilli and mutans streptococci. A recent review concluded that primary dentition caries has been associated with early childhood malnutrition, but that an effect on permanent dentition caries was not yet established. Psoter et al. (2) suggested that enamel hypoplasia, salivary glandular hypofunction and saliva

doi: 10.1111/j.1600-0528.2006.00365.x

compositional changes may be the mechanisms through which malnutrition is associated with caries. They also propose that longitudinal studies controlling for known confounders and focusing on biological mechanisms will add to our scientific knowledge base (2). The design of our previous study was cross-sectional and hence the direction of the effect could not be established (1). Since then, we have followed cohorts of Tanzanian children and recorded the amount of new caries lesions in the permanent dentition. The conditions most often used for clinical purposes for the prediction of caries have so far been counts of lactobacilli and mutans streptococci, flow rate of unstimulated and stimulated saliva, and buffering capacity of saliva. In the preparatory stages of this study, we therefore decided to explore the potential influence of lactobacilli, mutans streptococci, stimulated flow rate of saliva, and buffering capacity of saliva in connection with the potential influence of malnutrition. Since the implementation of our study, several papers have questioned whether it is possible to predict caries, using for example counts of lactobacilli, mutans streptococci, buffering capacity and flow rate of saliva [for review see Ref. (3)]. Other studies have shown that the levels of lactobacilli and mutans streptococci are inconsistent over time and that there are large intra-individual variations in adults (4, 5). Again (1) we included socioeconomic status as a potential risk factor on the incidence of caries in the permanent dentition. The present study is a cohort study in a child population characterized by little or no access to restorative and preventive dental care (6), and consequently, the investigation of the natural history of dental caries. We aimed to study the association between malnutrition and other risk factors or indicators with permanent dentition caries in a cohort of Tanzanian schoolchildren.

Materials and methods

Study design

A prospective cohort study where the participants were examined in September/October 1997, in July 1999 and in July 2003 using the same methods at each examination.

Children and schools

All children in grade 1 from Upanga and Mwananyamala primary schools in Dar es Salaam were eligible for inclusion in the study, but only children with known birth dates were actually enrolled in the study. However, as birthdates are available from the antenatal clinic (ANC) cards submitted during school registration and ANC attendance for pregnant women in Tanzania is approximately 100%, the percentage of children enrolled was very high (>99%) for both schools. Upanga primary school is located in an affluent area in the centre of Dar es Salaam; it has a high school fee and recruits children from an area populated by families with a high social position. In contrast, the Mwananyamala primary school is situated in an underprivileged suburban area; there is no school fee and the pupils are recruited from poor and underprivileged families residing in the neighbouring area. At baseline mean age was 7.6 years (SD = 0.4) and when the study was completed, the mean age was 13.3 years. We examined the children in 1997, 1999 and 2003, and identified cohorts of children within these groups of children (Fig. 1). Our main focus was on cohort 1; i.e. the cohort of 145 children examined in 1997 as well as in 2003. The other cohorts were used to evaluate the results obtained from cohort 1 and to examine variations in exposure over time. There were no significant differences in retention rates between the two schools, as school attendance is highly enforced by the government and legal action is normally instituted against parents whose children abscond from school. Any loss to follow-up was mainly because of children being transferred to another school, usually as a result of the relocation of the parents/guardians.

Year	No. of children	Cohort 1	Cohort 2	Cohort 3	Cohort 4
1997	296	145	122	218	
1999	229		122	218	125
2003	273	145	122		125

Fig. 1. The number of children examined and four identified cohorts.

Stimulated saliva secretion rate and buffer capacity

The pupils were asked to chew a piece of paraffin wax for 5 min, during which they spat in a small measuring cylinder with a funnel. All saliva samples were collected between 09:00 and 13:00 hours. Stimulated saliva secretion rate was later dichotomized using a cut-off point at 0.7 ml/min, a rate at this level or below indicating an increased caries

risk (7). Buffering capacity of saliva was estimated using Dentobuff (Orion Diagnostika A/S, Hellebæk, Denmark) and, based on the results, the children were categorized into three groups: low, medium or high buffer capacity of saliva. All pH values and buffering capacity of the saliva samples were calculated within 30 min of saliva collection.

Mutans streptococci, lactobacilli and yeast counts in saliva

Dentocult SM and Dentocult LB were used to measure growth density of mutans streptococci and lactobacilli, respectively (Orion Diagnostika A/S). The scoring system for Dentocult SM (mutans streptococci) ranges from 0 to 3, reflecting the amount of colony-forming units on the strip with cut-off points at $<10^4$ CFU/ml (class 0), 10^4–10^5 CFU/ml (class 1), 10^5–10^6 CFU/ml (class 2) and $>10^6$ CFU/ml (class 3). Dentocult LB (lactobacilli) has five classes with cut-off points at 0, 10^3, 10^4, 10^5 and 10^6 CFU/ml (Orion Diagnostika A/S). Dichotomization of bacterial counts was performed before data analysis according to recommended cut-off points and grouped children into high- and low-risk individuals (8). High-risk children were those with a lactobacilli count ≥ 100 000/ml or mutans streptococci count ≥ 1 000 000/ml.

Dental caries examination

Using the WHO criteria, each pupil was examined to register the number of decayed, missing and filled permanent surfaces (DMFS) (9). New caries lesions in the permanent dentition were calculated on the individual level as well as on the tooth level. The examinations were performed by trained and calibrated examiners. Ten per cent of the children were re-examined to assess reliability; the kappa statistic was close to 90% for intra-examiner agreement and varied between 70% and 99% for inter-examiner agreement. All examinations, including the calibrations, were performed under natural light with the child seated on an office chair.

Anthropometric data for determination of nutritional status

Data on height and weight were transformed to standard deviation *z*-scores of body mass index (BMI) for each child according to the child's age and sex using growth reference curves for children (10). The analyses were carried out using Stata version 9.1 (11, 12). A child was categorized as being malnourished if the *z*-score was ≤−1.96 standard deviations.

Data processing and statistical analysis

Data were keyed in twice and validated using EpiData (13). A validated file was exported to Stata (12) and then checked for errors and inconsistencies. The validated data file on which the analyses and data managements were based can be downloaded from http://www.odont.au.dk/s&p/default.htm in Stata format. Data were analysed using the individual as well as the tooth as the unit of analysis. As the development of caries on different teeth in a subject's mouth is not independent events, robust variance estimates were obtained for the calculation of confidence intervals and *P*-values, when the unit of analysis was the tooth (12, 14). The overall strategy of analysis was as recommended by Kirkwood & Sterne (15). Crude risk ratios were calculated in two-by-two tables as a measure of the association between the development of new caries lesions and the exposure variables and potential confounders at the baseline from 1997. This part of the analysis gave the first indication of the associations between the outcome and the exposure variables. It also allowed us to look for effect modification between growth density of mutans streptococci and lactobacilli and buffering capacity. Generalized linear modelling (the binomial family and the log link) was used to compute risk ratios, sometimes termed as relative risks (12, 16). Computational problems may occur for this type of statistical analysis when an outcome is not rare; if for example the risk in the unexposed group is 0.5, the maximum possible value of the risk ratios is 2.0. We checked also for effect modification in the multivariable analysis and evaluated if the numerical standardized BMI *z*-scores could be modelled as a linear continuous variable. As a result of the minimal numbers of variables, we decided not to aim for a more parsimonious model. The consistency of the exposures in each child over time was evaluated using the information gained about the exposures at each examination. Ninety-five per cent confidence intervals were calculated, and accordingly a *P*-value ≤0.05 was considered to be statistically significant.

Ethics

The Ethics Committee of the Muhimbili University College of Health Sciences and the Dar es Salaam City Medical Officer approved the study. Informed consent was obtained from one or both parents and they were informed that their child could leave the study at any time. However, no parents refused to

participate allowing us to examine all children who were present during the examination sessions of the trial.

Results

Only two children had one filling done and two children had three fillings made during the 6 years the study lasted. All remaining treatments were tooth extractions. At baseline mean DMFS was 0.33, 0.37 and 0.32 for cohorts 1, 2, and 3, respectively. In cohort 1, i.e. the 145 children examined in 1997 as well as in 2003, mean DMFS increased to 3.77 during the 6 years. Mean annual caries increment for the 122 children examined in all 3 years was 0.27 in the period 1997–1999 and 0.80 in the period 1999–2003. A similar pattern was seen in the other cohorts. The distributions were much skewed as most children developed no caries at all or only one decayed surface each year. Twenty-six (21.3%) of the children examined all 3 years developed new caries lesions in both time periods.

Crude risk ratios are shown in Table 1. Apparently, no effect modification was seen. When the individual was the unit of analysis it was difficult to fit multivariable models as only a few variables were allowed if the models were to converge. Therefore, none of these constrained models are shown in Table 1. In contrast, there were no problems fitting the models when the unit of analysis was the tooth. The risk ratio and confidence interval for malnutrition indicate a possible association with new caries lesions during the period 1997–2003 using the recordings from 1997 as exposures, but only a low stimulated flow rate of saliva (≤0.7 ml/min) and a high count of lactobacilli (≥100 000/ml) were significantly associated (Table 1). It was justified to model nutritional level as a linear continuous variable in the multivariable analysis, but doing so hardly changed the risk ratios for the other exposure variables. As there was information from 1999 as well, risk ratios were computed also for the two time periods 1997–1999 and 1999–2003 (Table 2). These models present a rather confusing picture without any uniformity in

Table 1. Crude and adjusted risk ratios (RR) with 95% confidence intervals (CI) for new caries in the time period 1997–2003 ($n = 145$) using generalized linear modelling

	Individual level				Tooth level					
Exposure	Cases	Noncases	Crude RR	*P*-value	Cases	Noncases	Crude RR	Adjusted RR	95% CI	*P*-value
Malnourished										
No (reference)	71	56			209	3292				
Yes	12	5	1.26	0.25	40	432	1.42	1.55	0.92–2.62	0.10
Lactobacilli										
<100 000/ml (reference)	56	49			151	2744				
≥100 000/ml	26	11	1.32	0.07	96	926	1.80	1.73	1.03–2.92	0.04
Mutans streptococci										
<1,000 000/ml (reference)	62	54			184	3021				
≥1,000 000/ml	20	7	1.39	0.05	63	677	1.48	1.24	0.69–2.20	0.47
Stimulated saliva flow rate										
>0.7 ml/min (reference)	78	62			227	3634				
≤0.7 ml/min	2	0	1.79	0.21	16	40	4.86	3.67	1.71–7.86	0.00
Buffering capacity										
≥6.0 (reference)	51	48			129	2623				
4.5–5.5	18	9	1.29	0.16	67	662	1.96	1.83	0.91–3.65	0.09
≤4.0	9	5	1.25	0.37	40	340	2.25	1.67	1.00–2.79	0.05
School										
Mwananyamala (reference)	47	42			144	2322				
Upanga	36	20	1.21	0.17	105	1430	1.17	0.70	0.39–1.28	0.25
Sex										
Girl (reference)	50	36			139	2246				
Boy	33	26	0.96	0.79	110	1506	1.17	1.08	0.70–1.68	0.73

For some cases not all information were available.

Table 2. Adjusted risk ratios (RR) with 95% confidence intervals (CI) for new caries during the two time periods 1997–1999 ($n = 218$) and 1999–2003 ($n = 125$) using generalized linear modelling using the tooth as the unit of analysis and exposures as recorded at each baseline

	1997–1999				1999–2003			
Exposure	Cases	Noncases	RR (95% CI)	*P*-value	Cases	Noncases	RR (95% CI)	*P*-value
Malnourished								
No (reference)	76	3117			162	2836		
Yes	10	513	1.09 (0.58–2.03)	0.79	9	214	1.10 (0.58–2.07)	0.78
Lactobacilli								
<100 000/ml (reference)	47	2796			169	2884		
≥100 000/ml	39	789	2.63 (1.28–5.38)	0.01	20	343	1.04 (0.56–1.94)	0.90
Mutans streptococci								
<1,000 000/ml (reference)	67	3008			153	2623		
≥1 000 000/ml	19	598	1.12 (0.53–2.34)	0.77	36	604	1.06 (0.51–2.22)	0.88
Stimulated saliva flow rate								
>0.7 ml/min (reference)	78	3518			189	3227		
≤0.7 ml/min	8	67	8.56 (2.72–26.92)	0.00	2	26	1.18 (0.42–3.30)	0.76
Buffering capacity								
≥6.0 (reference)	41	2571			24	393		
4.5–5.5	30	578	3.29 (1.32–8.23)	0.01	91	1651	0.98 (0.45–2.16)	0.97
≤4.0	13	326	1.53 (0.63–3.73)	0.35	76	1209	1.19 (0.54–2.61)	0.66
School								
Mwananyamala (reference)	47	1806			113	1929		
Upanga	39	1838	0.51 (0.23–1.09)	0.08	78	1324	0.91 (0.52–1.59)	0.75
Sex								
Girl (reference)	57	2396			114	2014		
Boy	29	1248	0.77 (0.37–1.59)	0.48	77	1239	1.12 (0.66–1.88)	0.67

For some cases not all information were available.

Table 3. Variation in exposures over time among children examined in 1997 as well as 1999 ($n = 122$)

Exposure	Total in 1997	Only in 1997	Total in 1999	Only in 1999	Both 1997 and 1999
Malnourished	16	12	7	3	4
Lactobacilli ≥ 100 000/ml	30	26	13	8	4
Mutans streptococci ≥ 1 000 000/ml	23	21	23	21	2
Stimulated saliva flow rate ≤ 0.7 ml/min	2	2	1	1	0
Buffering capacity 4.5–5.5	23	1	62	48	10
Buffering capacity ≤ 4.0	10	0	45	26	6

Information missing for some cases.

the associations between the outcome and the exposure variables. In accordance with this, the consistency of the exposures for each child over time was very low (Table 3).

Discussion

The strengths of the present study are that we followed cohorts of children with little or no access to restorative or preventive dental care for 6 years using similar methods at each examination. Although we have no reason to believe so, the recording of caries may have varied over time. We strived to minimize this potential bias by asking the same local persons to supervise the calibration exercises and we used the same criteria during all examinations. The cohorts in the study were sampled from the children examined at the three examination sessions and took place without any knowledge of the disease or the exposure status. There is therefore no reason to believe any serious bias was introduced. We took advantage of the presence of different cohorts by using them to

evaluate our risk model, according to the principle that a model derived from an analytical study describing the associations between exposures and outcome, should be validated on other groups. In our study, we could not validate our risk model even in very similar groups.

Some exposures such as low stimulated saliva secretion rate and malnourishment were uncommon. Only two individuals in 1997, who also happened to be 'cases', had a low stimulated secretion rate. This fact would have made it impossible to calculate a confidence interval around the point estimate, had we used the individual as the unit of analysis. This computational problem was overcome when the unit of analysis was the tooth, but the few individuals exposed to a low stimulated saliva secretion rate are reflected in a wide confidence interval. Only 17 individuals were categorized as malnourished in 1997, and although this is 12% of the children, it should be noted that the fewer 'cases' we sample the higher our uncertainty will be.

Our study gave very puzzling results. The associations between new caries lesions and the risk factors for the period 1997–1999 were different from those of the period 1999–2003, despite the fact that the annual mean caries increment seemed to be three times higher in latter period. An assumption made in most cohort studies is that exposure is quite constant over time; this was clearly not the case in this study. Had we recorded exposures only at baseline in 1997, we could have concluded that a low stimulated flow rate of saliva and a high count of lactobacilli were significantly associated with the development of new caries lesions in this cohort of Tanzanian children, and that malnutrition might be predictive for permanent dentition caries. However, the different models and the inconsistency of exposure variables over time contradict this view. An issue that may have influenced our results is the fact that we collected stimulated saliva when unstimulated saliva may have been a better choice (17). The use of commercial tests to determine the microbial flora and the buffering capacity of saliva may also have had a negative influence on our results. We conclude that the included risk factors were poorly associated with caries incidence, and as in other studies, we also noted that the level of the microbial flora was subjected to large variations in the individual (3–5, 18). In coherence with recent findings and views, our results indicate a lack of appropriate methods for predicting caries and we find ourselves left with more questions than answers. One of the questions we would like to explore is the reliability over time of exposures traditionally associated with the development of caries. It seems justified to study their reliability on a short-term as well as on a long-term basis using laboratory, as well as commercial tests. Finally, we question whether other cohort studies would have shown similar disturbing results, if exposures were recorded at different times during the study period.

Acknowledgements

The present study received financial support from University of Aarhus Research Foundation grant no. E-1997-SUN-1-84.

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