Acta Odontologica Scandinavica, 2010; 68: 199–206

informa
healthcare

ORIGINAL ARTICLE

A microbiological study in relation to the presence of caries and calculus

GUNNAR DAHLÉN[1], KAROLINA KONRADSSON[1], SOPHIA ERIKSSON[1], RAWEE TEANPAISAN[2], SUPATCHARIN PIWAT[2] & ANETTE CARLÉN[1]

[1]*Department of Oral Microbiology, Institute of Odontology, Sahlgrenska Academy at Göteborg University, Göteborg, Sweden, and* [2]*Department of Stomatology, Faculty of Dentistry, Prince of Songkla University, Haf Yai, Thailand*

Abstract

Objective. To examine whether oral microflora in individuals with caries differs from that of individuals with calculus in Thai adolescents with poor oral hygiene. ***Material and methods.*** One hundred 13-year-old schoolchildren from Southern Thailand were examined for the presence of caries, calculus and plaque and saliva pH was also determined. Saliva samples were analysed by culture and approximal supragingival plaque samples were analysed with the DNA–DNA hybridization method ('checkerboard'). ***Results.*** Among the 100 children, mean DMFS was 3.43 [standard deviation (SD) 2.82] and 90% had calculus. The correlation between DMFS and the calculus index was 0.064. A total of 36 children were caries-free and 56 had calculus including teeth other than those in the lower anterior region (calculus score ≥3). A total of 23 caries-free children with a high calculus score (≥3) were compared with 22 children with the highest DMFS [mean 11.19 (SD 5.58)] and a calculus score < 3. No significant difference was obtained for saliva and plaque pH. The salivary levels of mutans Streptococci and Lactobacilli were low. Significantly more children in the caries group had high levels of glycolytic *Prevotella nigrescens* and *Filifactor alocis* in plaque compared with the calculus group. Calculus cases had a significantly higher total viable count in saliva. ***Conclusions.*** There was no inverse correlation between the presence of caries and calculus at a population level and a high calculus score only marginally reduced the individual likelihood of having caries. Several glycolytic bacteria were related to caries, while no specific bacteria could be related to calculus formation. Calculus seemed to be more closely related to poor oral hygiene.

Key Words: *Calculus, caries, oral microbiota*

Introduction

Caries and calculus formation are chemically contrasting processes. Calculus is characterized by mineralization: an increase in pH results in precipitation of calcium salts on the tooth surface [1]. A carious lesion is characterized by enamel demineralization following repeated falls in pH to below the critical pH [2]. An exchange of calcium and phosphate between the tooth surface and saliva and/or plaque occurs during both mineralization and demineralization.

Microorganisms play a key role in the exchange of ions and regulation of pH in the dental biofilm (plaque). Acid production in plaque and the subsequent decrease in pH is due to microbial fermentation of carbohydrates, particularly mono- and disaccharides (sugars). Caries is initiated and sustained by the acidogenic bacteria in plaque [3]. The microflora of cariogenic plaque is also related to adaptation of the microorganisms in order to survive in a more acidic environment [4]. A number of Gram-positive bacteria, e.g. Streptococci and Lactobacilli, are both aciduric and acidogenic and are favoured by low plaque pH. Mutans Streptococci and Lactobacilli have long been associated with the carious process, and compete with bacteria associated with a healthy non-carious environment.

Subgingival flora is characterized by proteolytic activity by predominantly anaerobic Gram-negative microorganisms [5]. The gingival pocket environment is characterized by inflammation and increased exudate flow into the pocket, originating from serum. This is low in carbohydrates, containing mostly proteins and no sugars. Saccharolytic microorganisms thus have no advantage and, according to the ecological plaque hypothesis, will be outnumbered by

Correspondence: Gunnar Dahlén, Department of Oral Microbiology, Institute of Odontology, Sahlgrenska Academy at Göteborg University, Box 450, SE 405 30 Göteborg, Sweden. E-mail: dahlen@odontologi.gu.se

(Received 3 August 2009; accepted 3 March 2010)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.3109/00016351003745514

proteolytic bacteria [6]. The environment is typically neutral or even slightly alkaline, as indicated by ammonia produced by ureolysis [7]. This alkaline environment favours calculus formation [8]. Calculus is associated with gingivitis and periodontitis and, in theory, caries-active individuals should not develop as much calculus as caries-free individuals, and *vice versa*. Some studies have sought clinically to confirm this inverse relationship between caries and calculus formation in young people. However, the literature offers no studies that have identified bacterial markers for these metabolic processes concomitantly in the same individuals. The purpose of this study was to search for such markers in the plaque and salivary microflora in a group of 13-year-old Thai children who had a high caries rate and heavy calculus formation and who received little dental care.

Material and methods

The National Ethical Committee of the Ministry of Public Health in Thailand approved the study protocol and informed consent was obtained from parents.

Subjects

The study was carried out in the Songkhla Wittayakom School of the Songkhla Province in Southern Thailand. From 12 selected school classes containing a total of 576 13-year-old children, 100 were selected by teachers. They made up the study group that was examined at least 1 h after breakfast or lunch. There were three calibrated examiners (K. K., S. E. and S. P.). If an examiner was in doubt about a registration, a consensus was reached between all three. The children were requested not to brush their teeth on the examination day.

Clinical recordings

Clinical recordings included the presence of manifest caries, missing teeth, the presence of calculus, measurements of plaque and saliva pH and obtaining saliva and two supragingival plaque samples for microbiological analysis. Clinical examinations were conducted in daylight, using a dental mirror and probe. Individual status was recorded and the number of teeth present and caries experience (DMFS) calculated. However, the number of fillings was low and did not mirror the actual caries situation (activity) and the contribution of the F component to DMFS was negligible. Permanent teeth missing due to caries were first molars and were counted as three surfaces in the DMFS index. Caries in any retained deciduous teeth was registered separately.

Calculus was registered according to a specific index on six areas: right and left upper and lower premolar–molar region and upper and lower frontal region. When calculus was abundant, a score of 2 was assigned; 1 denoted sparse and 0 denoted no calculus in these six regions. The maximal score for an individual was thus 12. A clearly visible continuous band of supragingival calculus on several teeth or alternatively a combination of clearly supra- and subgingival calculus on one or several teeth was defined as abundant calculus. A thin band of supragingival calculus on one tooth in the region or alternatively non-continuous supra- or subgingival calculus on one or several teeth was defined as sparse calculus.

Microbiological sampling and transport

Paraffin-stimulated saliva was collected from each subject and 1 ml was transferred to a bottle using the transport medium Viability Medium, Gothenburg, Anaerobic (VMGA IIS) [9,10]. Approximal supragingival plaque samples were taken with a curette from the distal surfaces of teeth 16 and 36. In the case of absence of these teeth, the plaque sample was taken from the second molar or premolar. The plaque sample was transferred to an Eppendorf tube containing 150 µl of TE (10 mm Tris HCl, 1 mm EDTA, pH 7.6) buffer and all samples were treated immediately with 0.1 M NaOH. The samples were stored in a refrigerator until dispatch to the laboratory in Gothenburg. The maximum transfer time was 1 week. Plaque pH was measured with a pH indicator strip according to the method of Carlén et al. (unpublished work). Briefly, this is based on plastic indicator strips (Spezialindikator, Merck, Darmstadt, Germany; pH range 4.0–7.0) with a pointed end to allow insertion between the selected teeth for 10 s; colour changes are compared with a reference. The precision of the method was comparable with that of microelectrodes. pH was measured on the distal surface of teeth 26 and 46. When teeth were missing, pH was measured at the second molar or premolar.

Culture

The saliva samples were diluted in VMG I (Viability Medium, Gotheuburg) [9] into a tenfold dilution series and, from each dilution, 0.1 ml was spread evenly on Mitis–Salivarius agar with bacitracin according to the method of Gold et al. [11] for selective culture of mutans *Streptococci* and on Rogosa agar (Difco Rogosal SL agar; Becton, Dickinson and Co., Sparks, MD) for selective culture of *Lactobacilli*. The plates were incubated for 5 days at 37°C in 10% CO_2. Mutans *Streptococci* and *Lactobacilli* were counted. In addition, one Brucella (BBL, Cockeysville,

MD) blood agar plate supplemented with 1.5% haemolyzed human erythrocytes and 5×10^5 mg/l menadione was incubated anaerobically in jars for calculations of Fusobacteria and black-pigmented anaerobic rods. Fusobacteria (e.g. *Fusobacterium nucleatum*) were identified as greyish nacracious colonies and filamentous Gram-negative rods at Gram staining. Black-pigmented colonies with Gram-negative rods were identified based on their fluorescence under UV light either as *Porphyromons gingivalis* (non-fluorescent) or *Prevotella intermedia* (brick red fluorescence) [12].

DNA–DNA hybridization ('checkerboard' methodology)

The presence and level of 12 bacterial species generally associated with periodontitis were estimated with the DNA–DNA hybridization method as described by Socransky et al. [13] and modified by Dahlén and Leonhardt [14]. It is ideal for field studies due to its lower sensitivity to longer transportation [15,16]. The samples were boiled for 5 min, neutralized and applied horizontally in rows on a nylon membrane (Roche, Mannheim, Germany) with a Minislot applicator (Immunogenetics, Cambridge, MA) and immobilized in UV light. In a Miniblotter apparatus, 12 deoxygenin-marked (Roche, Mannheim, Germany) whole genomic probes were applied in rows vertically on the membrane. After 2 h of prehybridization, the DNA probes were allowed to hybridize overnight at 42°C. After thorough washing at 65°C, the hybridization products were treated with phosphate-conjugated antidioxygenin antibodies and the signals were visualized in a LumiImager workstation (Boehringer Mannheim). The evaluation of the number of bacterial cells in the sample was carried out by comparing the signals with signals of pooled standards corresponding to 10^5 and 10^6 cells of each species. The signals were coded on a scale from 0 to 5. Special attention was given to score 3, which corresponded to the standard $> 10^5$ and $< 10^6$, indicating high levels of the bacterial cells. The following bacterial species were recorded: *P. gingivalis*, *P. intermedia*, *Prevotella nigrescens*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans*, *F. nucleatum*, *Parvimonas micra* (previously *Peptostreptococcus micros*), *Campylobacter rectus*, *Porphyromonas endodontalis*, *Filifactor alocis* and *Prevotella tannerae* [14].

Group selection

After recording the calculus and caries scores of the children, the 23 children with the highest calculus index (≥3) were selected from among the caries-free children and were compared with the 22 children with the highest caries scores (DMFS ≥6). These two groups formed the calculus and caries groups in subsequent comparisons.

Statistics

Statistically significant differences in the frequency distributions of the calculus and caries groups for clinical and microbiological variables were analysed using the chi-square test. The correlation coefficient between calculus scores and DMFS was calculated. The non-parametric rank sum test was used for the calculation of statistical differences in bacterial counts between the calculus and caries groups. Statistical significance was set at $P < 0.05$.

Results

Fifty-four girls and 46 boys participated in the study; their mean DMFS was 3.48 [standard deviation (SD) 5.01]. Only six fillings were recorded. The relationship between caries and calculus among the entire 100 children and the various subgroups is shown in Tables I and II. Thirty-six children were caries-free and 10 had no calculus (Table I). Fifty-six children had a calculus score of ≥3, indicating calculus on more teeth than the lower anterior teeth. Fifty-eight children had caries and calculus concomitantly. The mean DMFS was not significantly different between the trichotomized calculus groups and the probability of having caries among those with abundant calculus (> 5) was only marginally lower than in the other two calculus groups (Table II). No inverse correlation was found between caries experience and the presence of calculus in the whole group (correlation coefficient

Table I. Frequency distribution of the 100 13-year-old Thai children according to the presence of caries (DMFS) and calculus score.

		No. of children in calculus groups			
Caries groups	No. of children	Calculus score 0	Calculus score 1–2	Calculus score 3–5	Calculus score > 5
Caries-free (DMFS = 0)	36	4	9	18	5
Low caries (DMFS 1–5)	42	4	18	17	3
High caries (DMFS > 5)	22	2	7	9	4
Total	100	10	34	44	12

Table II. Trichotomized calculus scores in 100 13-year-old Thai children versus caries and the probability of having caries according to the calculus score.

Calculus score	Caries group		Caries-free group	Likelihood of having caries (%)
	Mean DMFS (SD)	No. of children	No. of children	
0–2	4.80 (5.06)	31	13	70
3–5	6.02 (4.77)	26	18	59
>5	5.62 (4.47)	7	5	58
Total	5.53 (5.22)	64	36	64

0.064). The overall mean pH of saliva was 7.09 (SD 0.39) and of plaque 6.55 (SD 0.28) and 6.63 (SD 0.30), respectively on the two molar sites.

One calculus group (n = 23) and one caries group (n = 22) were identified using a cut-off calculus score of ≥3 among the caries-free group, in contrast with the high caries group with a DMFS of ≥5. By definition, the mean DMFS was thus significantly different ($P < 0.001$) between these two groups [DMFS 0 versus 11.19 (SD 5.58)]. The individuals in the caries group had 65% less calculus than those in the calculus group. No pH values differed significantly between the groups. The mean pH in plaque on 26d and 46d was 6.50 (SD 0.21) and 6.60 (SD 0.33) in the calculus group, compared with 6.60 (SD 0.27) and 6.60 (SD 0.28) in the caries group. Saliva pH was 7.08 (SD 0.45) and 7.06 (SD 0.42) for the two groups, respectively.

The microbial levels in saliva for all children and for the children in the calculus and caries groups are presented in Table III. Mutans Streptococci were detected in 23 children (23%), generally at low levels [highest value 14,000 colony-forming units (CFU)/ml], while Lactobacilli were more frequent (65%), but also at low levels (highest value 16,000 CFU/ml). *P. gingivalis* was found by culture in only three subjects, while *P. intermedia* (95%) and *F. nucleatum* (95%) were found in almost all subjects (Table III). These levels were significantly higher than those for mutans Streptococci and Lactobacilli (*P. intermedia* 20.7×10^5 CFU/ml and *F. nucleatum* 3.99×10^5 CFU/ml). Although found at low levels, the Lactobacilli and mutans Streptococci were significantly more prevalent in the caries group ($P < 0.05$) compared with the calculus group [mean values 0.02 (SD 0.09)$\times 10^5$ and 0.14 (SD 0.19)$\times 10^5$ CFU/ml in the calculus and caries groups, respectively for mutans Streptococci and 0.05 (SD 0.04)$\times 10^5$ and 0.17 (SD 0.21)$\times 10^5$ CFU/ml for Lactobacilli]. Periodontitis-associated bacteria in saliva were not significantly different between the groups. In contrast, the calculus group had significantly ($P < 0.01$) higher total numbers of bacteria (total viable count) compared with the caries group (6.6×10^8 versus 4.91×10^6 CFU/ml saliva).

Table IV shows the prevalence of periodontitis-associated species disclosed with the 'checkerboard' method in approximal supragingival plaque for all 100 children. A high detection frequency (>90%) of bacteria was noticed at both subject and site levels for *F. nucleatum*, *P. intermedia*, *P. nigrescens* and *P. tannerae*. These bacterial species also showed the highest prevalence among all children at a score of ≥3, in contrast to *P. gingivalis*, *A. actinomycetemcomitans*, *C. recta* and *T. forsythia*, which were found only sporadically at high levels (Table IV).

The weakly glycolytic bacteria *P. tannerae* was the most frequently found bacteria in both the calculus and caries groups and also showed the highest mean scores among the tested species (Table V). A high

Table III. Microbial levels in saliva for the total group and for the calculus and caries groups.

Bacterial species	All children (N = 100)			Calculus group (N = 23)		Caries group (N = 22)	
	Prevalence (%)	Mean (SD) $\times 10^{5\#}$	Range $\times 10^5$ (positive samples)	Prevalence (%)	Mean in positive samples (SD)$\times 10^{5\#}$	Prevalence (%)	Mean in positive samples (SD)$\times 10^{5\#}$
P. gingivalis	3	3.6 (1.0)	0.8–8.0	0	nd	1	nd
P. intermedia	95	20.7 (96.6)	0.1–710	20	6.2 (13.4)	19	14.0 (25.9)
F. nucleatum	95	3.99 (9.41)	0.008–48	22	3.4 (8.4)	18	1.9 (3.2)
Mutans *Streptococci*	23[a]	0.008 (0.014)	0.0001–0.14	2	0.02 (0.09)	10⋆	0.14 (0.19)
Lactobacilli	65	0.012 (0.033)	0.0001–0.16	8	0.05 (0.04)	17⋆	0.17 (0.21)
Total viable counts/ml saliva	100	6170 (4560)	1.7–22,000	23	6600 (5570)	22	49.1 (36.4)⋆⋆

[a]One sample had overgrowth of Gram-negative enterics.
⋆$P < 0.05$; ⋆⋆$P < 0.01$ between the calculus and caries groups; #CFU/ml.
nd = no data.

Table IV. Bacterial prevalence (%) at subject and site levels in approximal supragingival samples as analysed with the checkerboard method.

Bacterial species	Subject level ($N = 100$)	Site level ($N = 200$)	Subject level score 3–5 ($N = 100$)	Site level score 3–5 ($N = 200$)
A. actinomycetemcomitans	37	24.5	3	1.5
C. recta	36	26.0	2	1.0
F. alocis	72	81.0	46	30.5
F. nucleatum	97	98.5	23	13.5
P. endodontalis	55	59.5	18	10.5
P. gingivalis	37	24.5	5	4.0
P. intermedia	90	93.5	58	54.0
P. micra	79	84.0	21	12.0
P. nigrescens	90	94.0	45	34.0
P. tannerae	98	99.0	83	64.0
T. forsythia	61	24.5	3	1.5

detection frequency of the weakly glycolytic *P. nigrescens* and *F. alocis* was also found in the caries group and was significantly higher ($P < 0.05$) than in the calculus group. The glycolytic *A. actinomycetemcomitans*, *F. nucleatum* and *P. micra* were found to a higher extent in the caries group compared with the calculus group, but these differences were not statistically significant. The non-glycolytic (asaccharolytic) species *P. gingivalis*, *T. forsythia* and *C. rectus* were only found sporadically and at low levels.

Discussion

The present study failed to show a general inverse relation between the presence of caries and the presence of calculus in a group of 100 13-year-old Thais. However, when 23 caries-free individuals with the highest calculus score were compared with 22 subjects with the highest caries experience (DMFS), the latter group generally had a lower calculus score. These two groups were contrasted for microbiological analyses. The calculus group had significantly more bacteria (total viable counts) in saliva compared with the caries group. Although low numbers of mutans Streptococci and Lactobacilli were present, these bacteria were significantly more frequent in the caries group than in the calculus group. An interesting finding was that the weakly glycolytic *P. nigrescens* and *F. alocis* species, which are usually associated with periodontal disease, were significantly more frequent in the caries group compared with the calculus group.

Many clinical studies have been performed on the aetiology and epidemiology of caries and calculus

Table V. Prevalence of bacterial species (checkerboard score > 3) and mean level in approximal supragingival samples from individuals in the calculus and caries groups.

	Calculus group ($N = 44$)		Caries group ($N = 48$)	
Bacterial species	Detection frequency score > 3; *n* (%)	Mean score	Detection frequency score > 3; *n* (%)	Mean score
A. actinomycetemcomitans	5 (11)	1.39	8 (17)	1.45
C. recta	2 (5)	0.36	1 (2)	0.31
F. alocis	7 (16)	1.23	16 (33)*	1.69
F. nucleatum	4 (9)	1.59	9 (19)	1.81
P. endodontalis	6 (14)	1.16	3 (6)	0.88
P. gingivalis	2 (5)	0.48	1 (2)	0.36
P. intermedia	13 (30)	1.8	15 (31)	1.81
P. micra	2 (5)	0.98	6 (13)	1.38
P. nigrescens	10 (23)	1.52	22 (46)*	2.14
P. tannerae	28 (64)	2.5	27 (61)	2.67
T. forsythia	1 (2)	0.36	0 (0)	0.31

*$P < 0.05$ versus the calculus group.

processes. While early studies by Stones et al. [17] and by Little et al. [18] did not find an inverse relation between caries and calculus formation, later reports support the hypothesis of an inverse relation at the population level [19–22]. No inverse relation in the whole group of 100 children was found in the present study. This was explained by the fact that almost all children (90%) had calculus, while 36% were caries-free. Many children (58%) had caries and calculus concomitantly. The high prevalence of calculus seems to be a general characteristic of the South East Asian population [23,24]. In the latter study, in two groups of 11–13 year-olds in Northern Thailand, one with caries and one with calculus, it was concluded that these two processes were due to independent variables [24]. Notably, 53% had calculus in that study, compared to 90% in the present study. The groups in the study of Pattanaporn and Navia [24] did not differ with respect to saliva secretion rate, buffer capacity and pH. Similarly, no difference in plaque pH was found between the groups in the present study. Although in the population sample an inverse relation between caries/calculus was not noted, we found that selected subjects with a high proportion of caries had 65% less calculus than those who were caries-free. Crossner and Holm [25] found an inverse relation between caries and supragingival calculus in primary teeth but not in the permanent dentition, which is possibly explicable by the age dependency of caries and the ability to form calculus. It is possible that caries or calculus formation may occur concomitantly in the same mouth due to fluctuation in the biochemical processes. An inverse relation may therefore be more pronounced at site level, as illustrated clinically by the fact that no carious lesions were found in the lower incisors, where calculus occurred when it was present. To overcome some of the site dependence of caries as well as calculus, we contrasted children with a more general ability to form calculus (calculus score of ≥3) and to develop caries, with the aim of comparing microflora and pH at a subject level.

In planning the present study, we intended to compare caries-free children with the highest calculus index with a similar number of children with no or low calculus. However, many of these low-calculus-score children also had little caries (DMFS 0 or 1). It is possible that the children with little calculus belonged to a group with better oral hygiene, rather than belonging to a group whose oral ecology was less capable of calculus formation. We decided therefore to contrast the 'no caries, high calculus' children with those who had the highest DMFS to evaluate possible differences in the microflora between the two groups and to see whether they represented different ecologies. We found that the calculus group had significantly more bacteria in their saliva than the caries group. This suggests that a large amount of calculus may not represent a different ecology. Rather, it may be the result of poor oral hygiene, leading to more plaque and calculus and subsequently to greater retention of bacteria and to higher counts in saliva.

No study has hitherto addressed the question of whether caries and calculus formation represent different biochemical processes with different ecologies, as reflected by differing microbiological compositions of saliva and/or plaque? We know that caries is associated with acidogenic and aciduric species, predominantly Gram-positive saccharolytic bacteria such as mutans Streptococci and Lactobacilli. The microflora specifically related to calculus formation have been less well studied [1]. Since both supra- and subgingival calculus occur with gingivitis and periodontitis, the microbial spectrum with predominantly anaerobic and mostly Gram-negative species is believed to be the same and independent of the amount of supragingival calculus.

Mutans Streptococci and Lactobacilli levels were low but the detection frequency was significantly higher in the caries group. This confirms the general finding that mutans Streptococci and Lactobacilli are associated with the presence of caries [26–28], even if the counts in this study were surprisingly low. Higher levels were reported from other populations [29,30] and from other Thai populations [31,32]. The latter studies used slide tests, which may have overestimated the number of mutans Streptococci and Lactobacilli, whereas underestimation may have occurred in the present work using selective Mitis–Salivarius and Rogosa agar plates. Another explanation for our findings might be the long transportation time. However, this seems unlikely since, in the same samples, high levels of the more fastidious and anaerobic bacteria like *P. intermedia* and *F. nucleatum* were found. These findings indicate that besides mutans Streptococci and Lactobacilli, other more proteolytic and less glycolytic bacteria than the hitherto identified saccharolytic non-mutans *Streptococci* and *Actinomyces* spp. [3,33] are of importance for caries development and progression. Periodontitis-associated bacteria such as *P. gingivalis* were apparently not detected in the saliva of our participants.

The plaque analysis using the checkerboard technique confirms existing knowledge that non-glycolytic bacteria (e.g. *P. gingivalis*, *P. endodontalis*, *T. forsythia* and *C. rectus*) increase with age [34] and they were not more frequent in the calculus group. It would be interesting to examine whether this observation holds in calculus-prone older individuals before periodontitis develops. The glycolytic *F. alocis* and *P. nigrescens* species were significantly more often found in high levels (score ≥3) in the caries group plaque. The bacteria in the panel are covariates and there is a risk of overemphasizing weak and single significances due to multiple comparisons. Hence the statistical significance for *F. alocis* and *P. nigrescens* should be

interpreted with caution. On the other hand, these glycolytic bacterial species are acidophilic, although they usually donot ferment sucrose. They metabolize other sugars such as glucose and fructose and may also adapt, survive and even grow in a more acidic environment [35,36]. Notably, type 2 diabetics showed significantly more *P. nigrescens* and non-mutans *Streptococci* in the supragingival plaque than non-diabetics [37], indicating that glycolytic activity may play a significant role in the plaque ecology in some populations. Other glycolytic and often anaerobic and Gram-negative bacteria may have the same characteristics although, in this study, only a tendency towards more frequent detection in the caries group compared to the calculus group was seen for *P. intermedia*, *A. actinomycetemcomitans*, *F. nucleatum* and *P. micra*.

In conclusion, this study found no inverse correlation between the presence of caries and calculus in a group of 100 children and a high calculus score only marginally reduced the likelihood of having caries at the individual level. Caries-free calculus-formers had higher total viable counts in saliva than those with a high caries prevalence. Although low amounts of mutans Streptococci and Lactobacilli were present, they were significantly more frequent in the caries group than the calculus group. Similarly, high levels of Gram-negative, glycolytic *P. nigrescens* and *F. alocis* were more frequent in the caries group compared with the calculus group. This study on 13-year-old Thais with a high rate of calculus and/or caries identified Gram-negative bacterial species as markers for caries in addition to mutans Streptococci and Lactobacilli. No specific microorganism was associated with calculus formation. Calculus seemed to be more related to a higher level of bacteria in saliva, possibly linked to poor oral hygiene.

Acknowledgements

We thank the Prince of Songkla University, Hat Yai, and the Songkhla Wittayakom Secondary School, Songkhla Province for their appreciated cooperation with the recruitment of schoolchildren for this study. We also thank Mrs Lisbeth Bengtsson for excellent technical assistance. This study was supported by grants from the Swedish patient revenue fund and Swedish Research Links, Swedish Research Council, Stockholm, Sweden (348-2007-6650).

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Jin Y, Yip HK. Supragingival calculus: Formation and control. Crit Rev Oral Biol Med 2002;13:426–41.

[2] Baelum V, Fejerskov O. Caries diagnosis: "a mental resting place on the way to intervention"? In: Fejerskov O, Kidd EAM, editors. Dental caries: the disease and its clinical management. Oxford, UK: Blackwell Munksgaard; 2003. p. 101–10.

[3] Takahshi N, Nyvad B. Caries ecology revisited: Microbial dynamics and the caries process. Caries Res 2008;42:409–18.

[4] Welin-Neilands J, Svensäter G. Acid tolerance of biofilm cells of Streptococcus mutans. Appl Environ Microbiol 2007;73:5633–8.

[5] Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontol 2000 2005;38:135–87.

[6] Marsh PD. Are dental diseases examples of ecological catastrophes? Microbiology 2003;149:279–94.

[7] Sissons CH, Wong L, Shu M. Factors affecting the resting pH of in vitro human microcosm dental plaque and Streptococcus mutans biofilms. Arch Oral Biol 1998;43:93–102.

[8] White DJ. Dental calculus: recent insights into occurrence, formation, prevention, removal and oral health effects of supragingival and subgingival deposits. Eur J Oral Sci 1997; 105:508–22.

[9] Möller ÅJR. Microbiological examination of root canals and periapical tissues of human teeth. Methodological studies. Odontol Tidskr 1966;74:1–380.

[10] Dahlén G, Pipattanagovit P, Rosling B, Möller AJ. A comparison of two transport media for saliva and subgingival samples. Oral Microbiol Immunol 1993;8:375–82.

[11] Gold OG, Jordan HV, van Houte J. A selective medium for *Streptococcus mutans*. Arch Oral Biol 1973;18:1357–64.

[12] Slots J, Reynolds HS. Long-wave UV-light fluorescence for identification of black-pigmented *Bacteroides*. J Clin Periodontol 1982;16:1148–51.

[13] Socransky SS, Haffajee AD, Smith C, Martin L, Haffajee JA, Uzel NG, et al. Use of checkerboard DNA-DNA hybridization to study complex microbial ecosystems. Oral Microbiol Immunol 2004;19:352–62.

[14] Dahlén G, Leonhardt Å. A new checkerboard panel for testing bacterial markers of periodontitis. Oral Microbiol Immunol 2006;21:6–11.

[15] Papapanou PN, Madianos P, Dahlén G, Sandros J. Checkerboard versus culture: A comparison between two methods for identification of subgingival microbiota. J Eur J Oral Sci 1997;105:389–96.

[16] Papapanou PN, Teanpaisan R, Obiechina NS, Pithpornchaiyakul W, Pongpaisal S, Pisuithanakan S, et al. Periodontal microbiota and clinical periodontal status in a rural sample in southern Thailand. Eur J Oral Sci 2002; 110:345–52.

[17] Stones HH, Lawton FE, Bransby ER, Hartley HO. Original communications, dental caries and length of institutional residence. Relationship between dental caries and calculus, dental caries and the pH of saliva. Br Dent J 1950; LXXXIX:199–203.

[18] Little MF, Scott Wiley H, Dirksen TR. Concomitant calculus and caries. J Dent Res 1960;39:1151–62.

[19] Gedalia I, Yardeni J, Gershon I. Dental caries rate and fluoride content of dental calculus and saliva. J Am Dent Assoc 1963;66:525–8.

[20] Sutfin LV, Sweeney EA, Ascoli W. Calcifying dental plaque and reduced dental caries in permanent molars of children from two Guatemalan villages. J Dent Res 1970;49: 772–5.

[21] Duckworth RM, Huntington E. Evidence for putting the calculus: caries inverse relationship to work. Commun Dent Oral Epidemiol 2005;33:349–56.

[22] Duckworth RM, Huntington E. On the relationship between calculus and caries. Monogr Oral Sci 2006;19:1–28.

[23] Hölttä P, Alaluusua S, Saarela M, Asikainen S. Isolation frequency and serotype distribution of *mutans streptococci*

and *Actinobacillus actinomycetemcomitans*, and clinical periodontal status in Finnish and Vietnamese children. Scand J Dent Res 1994;102:113–19.

[24] Pattanaporn K, Navia JM. The relationship of dental calculus to caries, gingivitis, and selected salivary factors in 11- to 13-year old children in Chiang Mai, Thailand. J Periodontol 1998;69:955–61.

[25] Crossner CG, Holm AK. A descriptive and comparative study of oral health in 8-year old Swedish children. Acta Odont Scand 1975;33:135–42.

[26] Van Houte J. Role of micro-organisms in caries etiology. J Dent Res 1994;73:672–81.

[27] Beighton D. The complex oral microflora of high-risk individuals and groups and its role in the caries process. Commun Dent Oral Epidemiol 2005;33:248–55.

[28] Ge Y, Caufield PW, Fisch GS, Li Y. *Streptococcus mutans* and *Streptococcus sanguinis* colonization correlated with caries experience in children. Caries Res 2008;42:444–8.

[29] Zickert I, Emilson C-G, Krasse B. Streptococcus mutans, lactobacilli and dental health in 13–14-year-old Swedish children. Commun Dent Oral Epidemiol 1982;10: 77–81.

[30] Petti S, Hausen HW. Caries prediction by multiple salivary mutans streptococci counts in caries-free children with different levels of fluoride exposure, oral hygiene and sucrose intake. Caries Res 2000;34:380–7.

[31] Bratthall D, Serinarach R, Carlsson P, Lekfuangfu S. *Streptococcus mutans* and dental caries in urban and rural schoolchildren in Thailand. Commun Dent Oral Epidemiol 1986;14:274–6.

[32] Teanpaisan R, Kintarak S, Chuncharoen C, Akkayanont P. Mutans streptococci and dental caries in schoolchildren in Southern Thailand. Commun Dent Oral Epidemiol 1995;23:317–18.

[33] Sansone C, Van Houte J, Joshipura K, Kent R, Margolis HC. The association of mutans streptococci and non-mutans streptococci capable of acidogenesis at a low pH with dental caries on enamel and root surfaces. J Dent Res 1993;72:508–16.

[34] Darby I, Curtis M. Microbiology of periodontal disease in children and young adults. Periodontol 2000 2001;26:33–53.

[35] Takahashi N, Schachtele CF. Effect of pH on the growth and proteolytic activity of *Porphyromonas gingivalis* and *Bacteroides intermedius*. J Dent Res 1990;69:1266–9.

[36] Takahashi N, Saito K, Schachtele CF, Yamada T. Acid tolerance and acid-neutralizing activity of *Porphyromonas gingivalis*, *Prevotella intermedia* and *Fusobacterium nucleatum*. Oral Microbiol Immunol 1997;12:323–8.

[37] Hintao J, Teanpaisan R, Chongsuvivatwong V, Ratarasan C, Dahlén G. The microbiological profiles of saliva, supragingival and subgingival plaque and dental caries in adults with and without type 2 diabetes mellitus. Oral Microbiol Immunol 2007;22:175–81.