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The microbiological profiles of saliva, supragingival and subgingival plaque and dental caries in adults with and without type 2 diabetes mellitus

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Introduction: The relationships between suspected bacteria in saliva, yeasts in oral rinse, and supragingival and subgingival plaque versus root surface and coronal caries in adults with type 2 diabetes mellitus and a non-diabetic group were explored.
Methods: One-hundred and five patients with type 2 diabetes and 103 non-diabetic subjects were recruited; their periodontal status, plaque index and magnitude of root surface and coronal caries were assessed. Saliva and an oral rinse were cultured for mutans streptococci, lactobacilli and yeasts. Toothbrush samples of supragingival plaque and curette samples of subgingival plaque were assessed for 17 bacterial species using the checkerboard DNA–DNA hybridization method.
Results: Type 2 diabetes patients had significantly more severe periodontitis, a higher plaque index and a higher prevalence and magnitude of root surface caries than non-diabetic subjects. Significantly more diabetic subjects had higher levels of *Treponema denticola*, *Prevotella nigrescens*, *Streptococcus sanguinis*, *Streptococcus oralis* and *Streptococcus intermedius* in their supragingival plaque than non-diabetic subjects. No significant difference was found for the organisms in saliva, oral rinse and subgingival plaque between the two groups. After adjustment for diabetic status, root surface caries was associated with an increased count of mutans streptococci, lactobacilli and yeasts in saliva and of *Streptococcus mutans* in supragingival plaque samples. Coronal caries was only associated with lactobacilli and yeasts in saliva.
Conclusion: The number of cariogenic organisms in saliva and oral rinse estimated by culture demonstrated a stronger association with both root surface and coronal caries compared to those 17 species assessed with the checkerboard method in supragingival and subgingival plaque.

Key words: coronal caries; microbiology, oral; root surface caries; type 2 diabetes mellitus

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Our previous study was conducted in adults to explore the effect of type 2 diabetes mellitus on root surface and coronal caries (12). A significantly higher prevalence of root surface caries in patients with type 2 diabetes than in non-diabetic subjects was reported. Poor periodontal condition and exposed root surfaces were also highly prevalent in the type 2 diabetic group (12). Increased saliva glucose level has been correlated with the glucose level in the blood of diabetic patients (2, 13, 16, 35). Similarly, the gingival crevicular fluid has been correlated with a high glucose concentration in the serum of diabetic subjects (8). Higher glucose levels have been detected in the gingival crevicular fluid from sites of

periodontal disease than from healthy sites (4). Elevated glucose levels in saliva and gingival crevicular fluid could induce an increase in the number of saccharolytic bacteria associated with dental caries in the saliva, and in the supragingival and subgingival plaque of diabetic patients. The high concentration of organic acids produced by the bacteria in dental plaque as a result of their metabolism of glucose may cause tooth surface demineralization and dental caries.

Root surface caries, like coronal caries, usually develops as a result of the interaction of bacterial plaque, dietary carbohydrates and an acid-susceptible tooth. However, the microbiology of root surface caries has been studied to a much lesser extent. In the general population, the cariogenic streptococci in dental plaque, such as *Streptococcus mutans* and *Streptococcus sobrinus*, as well as certain species of *Actinomyces* and *Lactobacillus* have been identified (37). In diabetic subjects, only *S. mutans* and *Lactobacillus* species have been reported in saliva (5, 21, 32, 35). A recent *in vitro* study suggested that *Candida albicans* could be a risk factor for root surface caries (22), which may be of particular interest in diabetic subjects. Thus, a broader microbiological survey of the dental plaque and saliva, including the various species known to be involved in coronal and root surface caries, in diabetic patients would be of interest. The checkerboard DNA–DNA hybridization developed by Socransky et al. (31) facilitates rapid processing of large numbers of plaque samples with a wide range of species, does not require bacterial viability and is particularly applicable in epidemiological studies (24). The aim of this study was to explore the relationships between suspected bacteria in saliva, yeasts in oral rinse, and supragingival and subgingival plaque versus root surface caries and coronal caries in adults with type 2 diabetes mellitus in comparison with a non-diabetic group using checkerboard and conventional culture methods.

Material and methods

Study population

Data were obtained from 105 patients with type 2 diabetes and 103 non-diabetic subjects in Southern Thailand. The procedure and criteria for the subjects' selection have been described elsewhere (12). The design and methods of the study were approved by the Medical Ethics Committee of the Prince of Songkla University, Thailand.

Clinical examinations

The dental caries status of all teeth (except the third molars) was determined using a plain mouth mirror and a standard probe. Dental caries was recorded separately for coronal and root surfaces. Coronal caries was assessed according to the World Health Organization criteria (36). Root surface caries was examined according to Katz criteria (14). The decayed and filled teeth were expressed as coronal and root surface caries. Each subject was further defined according to the presence or absence of root surface caries and coronal caries. The buccal surfaces of teeth 16, 11, 26 and 31, and the lingual surfaces of teeth 36 and 46 were examined for plaque index (11). Probing depth and attachment levels were assessed on all teeth except the third molars and on six surfaces of each tooth: the mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual and distolingual. For each individual, periodontal condition was characterized by its severity and extent. Severity was described for all the individual sites as a whole and was categorized on the basis of clinical attachment loss (CAL) as follows: slight = 1–2 mm CAL, moderate = 3–4 mm CAL, and severe ≥5 mm CAL. Extent was characterized as localized if ≤30% of the sites were affected and generalized if >30% of the sites were affected with ≥1 mm CAL (10).

Saliva samples and culture technique

All subjects were examined for the number of mutans streptococci and lactobacilli colonies using the spatula method described by Kohler and Bratthall (15). The prevalence of yeasts in the mouths of all subjects was estimated by an oral rinse technique (27). After removal of any dentures, each subject was instructed to rinse the mouth thoroughly with 10 ml sterile phosphate-buffered saline (0.1 M, pH 7.2) for 60 s. The rinse was expectorated into a universal container that was then centrifuged at 1600 ***g*** for 15 min. The supernatant was discarded and the pellet was resuspended in 1 ml sterile phosphate-buffered saline to obtain a concentrated oral rinse. Only 0.1 ml of the concentrated oral rinse was spread on a Sabouraud dextrose agar plate. The plates were incubated aerobically at 37°C for 48 h. The number of yeasts colonies on each oral rinse plate was counted, and the number of colony-forming units (CFU) per ml oral rinse was derived. All yeast isolates were identified by chlamydospores and germ-tube production, sugar assimilation and fermentation patterns.

Supragingival and subgingival plaque samples

A toothbrush method was used to take supragingival plaque from all subjects (23). Supragingival plaque on buccal and lingual surfaces of all remaining teeth was collected using a newly opened toothbrush. The plaque adhering to the brush was vigorously removed into a tube of 10 ml sterile distilled water. The plaque deposits were harvested by centrifugation at 1600 ***g*** for 20 min. Individual cell pellets were transferred into an Eppendorf tube containing 150 µl sterile TE buffer (10 mM Tris–HCl, 1.0 mM ethylenediaminetetraacetic acid, pH 7.6), 150 µl of 0.5 M NaOH solution was added and the tube was vortexed. The samples were kept at −20°C until processing.

A tooth site with a periodontal pocket depth ≥4 mm was selected in each subject classified as having periodontal disease and cotton rolls were used to isolate the sampling area from saliva. Supragingival plaque was removed and the supragingival area was cleaned with a piece of dry cotton roll. The subgingival plaque sample was obtained using a sterile Gracey curette and transferred into an Eppendorf tube containing 1 ml absolute ethanol and stored at −20°C. Before further processing, the subgingival plaque sample was centrifuged. The plaque pellet was dried and transferred into an Eppendorf tube containing 150 µl sterile TE buffer (10 mM Tris–HCl, 1.0 mM ethylenediaminetetraacetic acid, pH 7.6), 150 µl of 0.5 M NaOH solution was added and the tube was vortexed.

Processing of bacterial plaque samples

Supragingival and subgingival plaque samples were analysed using the checkerboard DNA–DNA hybridization method (25, 31). Digoxigenin-labeled, whole genomic DNA probes prepared using a labeling kit (Roche, Mannheim, Germany) were used to examine the occurrence of 17 bacterial species in the plaque samples. Whole genomic DNA probes were prepared from the following species: *Porphyromonas gingivalis* (strain FDC381), *Prevotella intermedia* (ATCC 25611), *Prevotella nigrescens* (ATCC 33563), *Tannerella forsythia* (ATCC 43037), *Actinobacillus actinomycetemcomitans* (FDCY4), *Fusobacterium nucleatum* (ATCC 10953), *Treponema denticola* (OMGS 3271), *Campylobacter rectus*

(ATCC 33238), *Eikenella corrodens* (ATCC 23834), *Selenomonas noxia* (OMGS 3118), *Streptococcus intermedius* (ATCC 27335), *Streptococcus sanguinis* (ATCC 10556), *Streptococcus oralis* (AT CC 35037), *S. mutans* (ATCC 25175), *Veillonella parvula* (ATCC 10790), *Actinomyces naeslundii* genospecies 2 (ATCC 15987) and *Lactobacillus acidophilus* (ATCC 4356).

The plaque samples were boiled for 5 min, neutralized with 0.8 ml 5 M ammonium acetate, transferred onto nylon membranes using a blotting device (Minislot device, Immunetics, Cambridge, MA) and fixed to the membrane by ultraviolet light followed by baking at 120°C for 20 min. The membranes were prehybridized at 42°C for 2 h then the DNA probes were allowed to hybridize overnight with the sample DNA using a Miniblotter device (Immunetics) at 42°C. After a series of stringency washes at 70°C, hybrids were detected by applying an anti-digoxigenin antibody conjugated with alkaline phosphatase and incubating the membrane with an appropriate chemiluminescent substrate (CSPD, Roche). Evaluation of the number of bacteria in the samples was performed by comparing the obtained signals with the ones generated by pooled standard samples containing 10^6 and 10^5 of each the species.

Statistical analysis

Data was computerized using Epidata version 2.1 (The EpiData Association, Odense, Denmark) and then analysed using Stata version 7 (Stata Corporation, College Station, TX). Yeasts were categorized into no yeasts and ≥1 CFU/ml of oral rinse. Counts of mutans streptococci were categorized as ≤20 and >20 CFU/1.5 cm^2. Counts of lactobacilli were categorized as ≤10 and >10 CFU/1.5 cm^2. The cut-off point for the number of bacteria in plaque was either $>10^5$ or $\geq 10^6$ cells, depending on the distribution of the magnitude of the microbial species. The chi-squared test was used to compare the percentage of each category of each species between patients with type 2 diabetes and non-diabetic subjects. Hypothesis testing related to the bacteria species was based on a Bonferroni correction (29). The significance value was set to $1 - (1 - 0.05)^{1/17}$ or 0.003, instead of 0.05, and the highly significant value was $1 - (1 - 0.01)^{1/17}$ or 0.0005. Student's *t*-test was used to compare the mean fasting plasma glucose concentration (mg/dl) between the two groups of subjects. The Mantel–Haenszel odds ratio was used for analysing the relationship between the number of microbes and the presence of coronal and root surface caries stratified by diabetic status. Odds ratios were also adjusted for variables that were significantly associated with dental caries in the same population (12), including diabetic status, wearing of removable dentures and smoking habit.

Results

Among the type 2 diabetes patients, the mean duration of diabetes was 8.7 years (SD 5.7). The mean glycosylated hemoglobin or HbA1c value in the group was 8.5% (SD 2.1) and ranged from 5.6 to 15.8%. Forty-three per cent of the patients had at least one diabetic complication (macroangiopathy, microangiopathy, retinopathy, neuropathy or nephropathy). Eighty-five per cent of the patients were receiving oral anti-diabetic treatment or dietary controls only, 7.5% had insulin treatment and 7.5% were treated with a combination of insulin and oral anti-diabetic agents.

Table 1 shows that patients with type 2 diabetes and non-diabetic subjects were balanced for age and sex. The prevalence and mean of root surface caries (for decayed and filled teeth) were significantly higher in patients with type 2 diabetes than in non-diabetic subjects while these parameters were similar for coronal caries in the two groups. The plaque index and the percentage of severe and generalized periodontitis were significantly higher in type 2 diabetics than in the control group. Samples of supragingival plaque were obtained from all subjects while subgingival plaque samples could only be obtained from 73 type 2 diabetes patients and 59 non-diabetic subjects who had at least one periodontal pocket with a depth of 4 mm or more.

The levels of mutans streptococci and lactobacilli in saliva and of yeasts in oral rinse samples were not significantly different in diabetics and non-diabetics (Table 2). *C. albicans* was identified in 78% and 64% of yeast isolates from type 2 diabetic patients and non-diabetic subjects, respectively. While the prevalence of yeasts was not associated with type 2 diabetes mellitus, wearing dentures had an independent effect on yeast infestation (odds ratio = 2.7, 95% confidence interval = 1.4–5.2).

In supragingival plaque samples, bacteria associated with type 2 diabetes mellitus were *T. denticola*, *S. sanguinis*, *P. nigrescens*, *S. intermedius* and *S. oralis*. None of the bacterial species tested in the subgingival plaque samples demonstrated an association with type 2 diabetes mellitus (Table 3).

After adjustment for diabetic status, a significant association was found between root surface caries and microorganisms for high levels of salivary mutans streptococci, salivary lactobacilli, presence of yeasts in oral rinse samples and for *S. mutans* in supragingival plaque (Table 4). Furthermore, salivary mutans streptococci and yeasts were significantly associated with coronal caries. For subgingival plaque samples, no bacterial species was found to be associated with root surface caries or

Table 1. A comparison of subjects' characteristics, caries and periodontal status (percentage or mean ± SD) and numbers of supragingival and subgingival plaque samples between patients with type 2 diabetes mellitus and non-diabetic subjects

Characteristics	Diabetic ($N = 105$)	Non-diabetic ($N = 103$)	*P* value[1]
Age (years)	54.3 ± 8.7	53.3 ± 7.6	0.38
Sex (% females)	50.5	50.5	0.99
Fasting plasma glucose (mg/dl)	154.8 ± 47.3	91.1 ± 8.1	<0.001
Current or ex-smoker	33.3	24.3	<0.05
Removable denture wearer	32.4	17.5	<0.05
Prevalence of coronal caries	83.8	72.8	0.06
Prevalence of root surface caries	40.0	18.5	0.001
Root surface caries (decayed or filled teeth)	1.0 ± 0.2	0.4 ± 0.1	<0.001
Coronal caries (decayed or filled teeth)	3.8 ± 0.2	3.3 ± 0.3	0.25
Plaque index	1.4 ± 0.1	1.2 ± 0.1	<0.05
Clinical attachment level (mm)	2.5 ± 2.1	1.7 ± 1.8	<0.001
Generalized periodontitis	98.1	87.4	<0.01
Severe periodontitis	84.8	72.8	<0.05
Number of subgingival plaque samples	73	59	
Number of supragingival plaque samples	105	103	

[1]Chi-squared test if the variable is categorical, Student's *t*-test if the variable is continuous with normal distribution and Mann–Whitney test if the variable is continuous but with a skewed distribution.

Table 2. A comparison of prevalence of mutans streptococci and lactobacilli in saliva and yeasts in oral rinse by culture between type 2 diabetes patients and non-diabetic subjects

Bacteria/yeasts[1]	Diabetics ($N = 105$) n (%)	Non-diabetics ($N = 103$) n (%)	P-value[2]
Mutans streptococci >20 CFU/1.5 cm^2	22 (21.0)	17 (16.5)	0.41
Lactobacilli >10 CFU/1.5 cm^2	26 (24.8)	16 (15.5)	0.10
Yeasts ≥1 CFU/ml of oral rinse	37 (35.2)	31 (30.1)	0.43

[1]CFU, colony-forming units; [2]chi-squared test.

coronal caries. After adjustment for diabetic status, wearing of removable dentures and smoking, the significant association between coronal caries and a high level of bacteria/yeasts in saliva disappeared. A high level of *P. intermedia* in supragingival plaque was significantly negatively associated with root surface and coronal caries.

Discussion

Type 2 diabetes patients and non-diabetic subjects in our study showed no difference in the prevalence of microorganisms in their saliva as assessed by culture techniques. On the other hand, supragingival plaque samples from the diabetic patients gave higher counts for five of the 17 species of bacteria examined with the checkerboard technique. The present study failed to demonstrate an association between caries and most organisms tested in plaque using the checkerboard method, except for an association between *S. mutans* in supragingival plaque and root surface caries. Three organisms assessed by culture, salivary mutans streptococci, salivary lactobacilli and yeasts in oral rinse, were associated with root surface caries; salivary lactobacilli and yeasts in oral rinse were also associated with coronal caries. However, after adjustment for diabetic status, wearing of removable dentures and smoking, the associations between salivary lactobacilli and yeasts and coronal caries became non-significant.

The prevalence of commonly suspected cariogenic organisms, mutans streptococci, lactobacilli and yeasts, in saliva and oral rinse cultures was not significantly different between the two groups of subjects. Other studies have also reported that counts of these bacteria in adults with type 2 diabetes mellitus are not significantly higher compared with healthy controls (5, 21). However, oral colonization with yeasts, especially *Candida* spp., has been reported as being higher in diabetic patients compared to non-diabetic individuals (9, 33). A high blood glucose level may enhance candidal adhesion to epithelial cells *in vivo* because it is known that *Candida* spp. cultured in a medium supplemented with glucose show enhanced adhesion to epithelial cells *in vitro* (6). Local oral factors, such as the presence of dentures, have been shown to have more influence on both the amount of oral yeasts and the species of oral yeasts present, than the systemic effect of diabetes mellitus (17). This is consistent with our finding that the wearing of dentures, not the diabetic status, had a significant impact on oral yeast carriage; presumably, because the fitting surfaces of dentures support the colonization of yeasts.

In supragingival plaque, there were high levels of most bacterial species and *T. denticola*, *P. nigrescens*, *S. sanguinis*, *S. intermedius* and *S. oralis* reached statistical significance even after Bonferroni corrections for multiple comparisons. The very high prevalence of most bacteria at high levels could also raise biological and clinical concerns in both diabetic and non-diabetic subjects. The supragingival plaque sample was taken by the toothbrush method, which is apparently not well suited to individuals with poor oral hygiene and an abundance of plaque. This certainly overloaded the sample with bacteria and differences at levels $>10^6$ were difficult to distinguish in the checkerboard methodology. Dilution of samples should be considered in the future for a more adequate comparison.

Changes within the subgingival microflora in diabetic patients compared to non-diabetic subjects have been reported (18, 28, 34). The increased glucose content in serum, and subsequently in gingival crevicular fluid, could hypothetically stimulate bacterial growth selectively, facilitating the higher proportion of fermenting bacteria of the facultatively gram-positive species such as streptococci, lactobacilli and *Actinomyces*. Thorstensson

Table 3. Distribution of bacterial species detected by checkerboard method in toothbrush supragingival and curette subgingival plaque samples in comparison between type 2 diabetes patients and non-diabetic subjects

Species	Cut-off point	Supragingival plaque ($N = 208$)		Subgingival plaque ($N = 132$)	
		Diabetic ($N = 105$) n (%)	Non-diabetic ($N = 103$) n (%)	Diabetic ($N = 73$) n (%)	Non-diabetic ($N = 59$) n (%)
P. gingivalis	$>10^5$	70 (66.7)	64 (62.1)	47 (64.4)	25 (42.4)
T. forsythia	$>10^5$	81 (77.1)	82 (79.6)	51 (69.9)	34 (57.6)
A. actinomycetemcomitans	$>10^5$	95 (90.5)	97 (93.2)	48 (65.8)	45 (76.3)
F. nucleatum	$>10^5$	103 (98.1)	96 (93.2)	68 (93.2)	48 (81.4)
T. denticola	$>10^5$	63 (60.0)	46 (44.7)*	53 (72.6)	36 (61.0)
C. rectus	$>10^5$	21 (20.0)	11 (10.7)	32 (43.8)	25 (42.4)
S. noxia	$>10^5$	79 (75.2)	78 (75.7)	40 (54.8)	29 (49.2)
V. parvula	$>10^5$	95 (90.5)	81 (78.6)	43 (58.9)	24 (40.7)
S. sanguinis	$\geq 10^6$	105 (100)	93 (90.3)*	11 (15.1)	14 (23.7)
P. intermedia	$\geq 10^6$	38 (36.2)	24 (23.3)	10 (13.7)	7 (11.9)
P. nigrescens	$\geq 10^6$	50 (47.6)	28 (27.2)*	9 (12.3)	7 (11.9)
E. corrodens	$\geq 10^6$	79 (75.2)	92 (89.3)	36 (49.3)	27 (45.8)
S. intermedius	$\geq 10^6$	102 (97.1)	83 (80.6)*	7 (9.6)	14 (23.7)
S. oralis	$\geq 10^6$	85 (81.0)	56 (54.4)*	5 (6.9)	4 (6.8)
S. mutans	$\geq 10^6$	81 (77.1)	70 (68.0)	28 (38.4)	11 (18.6)
A. naeslundii	$\geq 10^6$	105 (100)	100 (97.1)	56 (76.7)	39 (66.1)
L. acidophilus	$\geq 10^6$	14 (13.3)	6 (5.8)	7 (9.6)	1 (1.7)

*Statistical significance at P value < 0.003 followed Bonferroni correction.

Table 4. Odds ratios of root surface and coronal caries for high numbers of selected bacteria and presence of yeasts stratified by diabetic status

		Root surface caries				Coronal caries			
		Diabetic ($N = 105$)	Non-diabetic ($N = 103$)	All subjects ($N = 208$)		Diabetic ($N = 105$)	Non-diabetic ($N = 103$)	All subjects ($N = 208$)	
Samples	Bacteria/yeasts	OR (95% CI)	OR (95% CI)	OR (95% CI)[1]	OR (95% CI)[2]	OR (95% CI)	OR (95% CI)	OR (95% CI)[1]	OR (95% CI)[2]
Saliva	Mutans streptococci >20 CFU/1.5 cm^2[I]	2.7 (1.0–7.0)*	4.3 (1.4–13.5)*	3.3 (1.5–6.8)**	2.6 (1.2–5.7)*	5.0 (0.6–40.1)	1.9 (0.5–7.2)	2.7 (0.9–8.4)	1.9 (0.5–5.9)
	Lactobacilli >10 CFU/1.5 cm^2[II]	3.3 (1.3–8.2)*	2.4 (7.1–7.9)*	2.9 (1.4–6.2)**	2.4 (1.1–5.1)*	6.3 (0.8–50.5)	3.0 (0.6–14.1)	4.1 (1.2–14.4)*	3.1 (0.9–10.8)
	Yeasts ≥1 CFU/ml of oral rinse[III]	5.1 (2.2–12.2)*	3.3 (1.2–9.3)*	4.3 (2.1–8.7)***	3.8 (1.9–7.5)***	2.0 (0.6–6.5)	2.4 (0.8–7.2)	2.2 (1.0–5.0)*	2.2 (0.9–5.2)
Supragingival plaque	*T. denticola* $>10^5$ cells[IV]	0.4 (0.2–0.9)*	1.1 (0.4–3.1)	0.6 (0.3–1.7)	0.6 (0.1–1.4)	0.6 (0.2–1.8)	0.9 (0.4–2.2)	0.8 (0.4–1.5)	0.7 (0.3–1.4)
	C. rectus $>10^5$ cells[V]	0.5 (0.2–1.5)	1.0 (0.2–5.0)	0.6 (0.3–1.5)	0.6 (0.2–1.4)	0.8 (0.2–2.7)	0.3 (0.1–0.9)*	0.5 (0.2–1.1)	0.4 (0.2–1.0)
	P. intermedia $\geq 10^6$ cells[VI]	0.4 (0.2–0.9)*	0.9 (0.3–2.9)	0.5 (0.3–1.0)	0.4 (0.2–0.9)*	1.4 (0.5–4.5)	0.3 (0.1–0.7)**	0.6 (0.3–1.1)	0.4 (0.2–0.8)*
	S. mutans $\geq 10^6$ cells[VII]	3.2 (1.1–3.4)*	2.0 (0.6–6.2)	2.6 (1.1–5.8)*	2.2 (1.0–5.1)	2.9 (1.0–8.8)	1.6 (0.6–3.9)	2.0 (1.0–4.0)	1.8 (0.8–3.9)

Reference level: I = mutans streptococci ≤20 colony-forming units (CFU)/1.5 cm^2, II = lactobacili ≤10 CFU/1.5 cm^2, III = no yeasts, IV = *T. denticola* $\leq 10^5$ cells, V = *C. rectus* $\leq 10^5$ cells, VI = *P. intermedia* $<10^6$ cells, VII = *S. mutans* $<10^6$ cells.

[1]Mantel–Haenszel odds ratios adjusted for diabetic status.

[2]Odds ratios adjusted for diabetic status, wearing of removable denture and smoking.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

et al. (34) showed a high prevalence of *P. gingivalis* in both deep and shallow pockets of diabetic patients while *P. gingivalis* could be demonstrated in only the deep pockets of the non-diabetic controls. Sandholm et al. (28) also found more gram-negative anaerobes in diabetic than non-diabetic subjects. In patients with type 1 diabetes, Mashimo et al. (18) found a high proportion of *Capnocytophaga* spp. and 30% harbored *A. actinomycetemcomitans*. The higher numbers of *Capnocytophaga* spp. were recently confirmed by Ciantar et al. (3) using polymerase chain reaction identification of cultured samples. In these few studies, the analysis was limited to the presence of periodontal pathogens and the proportion of cariogenic bacteria was not tested. The type 2 diabetes patients in our study had significantly higher serum glucose levels and poor metabolic control compared to the non-diabetic subjects. In addition, they also had poorer oral hygiene, as indicated by the higher plaque index, as well as having more generalized and severe periodontitis than the non-diabetic group. However, we were not able to find any microbiological differences between the subgingival plaque of the diabetic patients and the non-diabetics. This could, in part, be explained by the relatively small number of teeth available with deep (>6 mm) pockets that might reduce the power to test the hypothesis. In future studies, site-specific samples from the root surfaces should be considered.

S. mutans, the most established cariogenic bacteria, has been reported to be associated with the early process of initial root surface caries but less with dentinal caries (7). Most of the root surface carious lesions in our study were at the initial stage whereas coronal lesions were deep dentine cavities. This may be the reason why salivary mutans streptococci and *S. mutans* in supragingival plaque were found to be associated with root surface caries but not with coronal caries in the present study.

Lactobacilli have previously been detectable less in the dental plaque than in saliva (19, 20) and we found that the detection frequency of lactobacilli was lower in supragingival plaque and least in subgingival plaque. The DNA–DNA hybridization technique detected only one species of *Lactobacillus*, *L. acidophilus* and consequently had a lower power to detect a possible association between both root surface and coronal caries and lactobacilli in plaque than the total salivary lactobacilli count. A significant odds ratio

for both root surface and coronal caries was found for lactobacilli in all subjects, supporting the strong association between lactobacilli and caries.

The present study demonstrated that yeasts, predominantly *C. albicans*, were associated with root surface caries. *Candida* spp. have been reported to be prevalent in root surface caries lesions (1, 30). A recent *in vitro* study suggested that *C. albicans* could be a risk factor for root surface caries of abutment teeth. (22). The cariogenicity of *Candida* has been explained in terms of its high acidogenic potential (26) and its ability to adhere to and dissolve hydroxylapatite (22).

After adjustment for diabetic status, wearing of removable dentures and smoking, the association of lactobacilli in saliva and of yeasts in oral rinse with coronal caries lost its statistical significance whereas the significant association of *P. intermedia* with the two types of caries emerged. This suggests that the multivariate models were not stable and is probably the result of the small sample size compared to the overloading explanatory parameters.

In conclusion, the number of cariogenic organisms in saliva and oral rinse estimated by culture demonstrated a stronger association with both root surface and coronal caries compared to those 17 species assessed with the checkerboard method in supragingival and subgingival plaque. When using the checkerboard method in patients with poor oral hygiene, site-specific samples should be considered.

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