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Tobacco smoking and periodontal microflora in a Saudi Arabian population

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Abstract

Objectives: To explore the subgingival periodontal microflora in a Saudi Arabian population with a special focus on its relationship with various smoking habits.

Material and Methods: A total of 198 individuals in the age range 17–60 years were included in the study. 29% were water-pipe smokers, 18% cigarette smokers, 13% smokers of both water pipe and cigarettes (mixed smokers) and 40% non-smokers. For each individual, a subgingival plaque sample from the deepest site in each quadrant was obtained. The checkerboard DNA–DNA hybridization technology was used to determine the presence of *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Tannerella forsythensis*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Treponoma denticola*, *Peptostreptococcus micros*, *Campylobacter rectus*, *Eikenella corrodens*, *Selenomonas noxia* and *Streptococcus intermedius*. Two cut-off levels for detection were used, score 1(10^5 bacteria) and score 3 (10^6 bacteria).

Results: The prevalence of individuals positive for the different microorganisms at score 1 cut-off varied from 7% to 95%. At score 3 cut-off the prevalence varied from 0% to 30%. The depth of sample site was a key factor for detection. When the depth of sample site was taken into account, no statistically significant differences were observed between cigarette smokers, water-pipe smokers, and non-smokers with regard to occurrence of the microorganisms studied.

Conclusions: No major differences were observed between cigarette smokers, water-pipe smokers, and non-smokers regarding the occurrence of the periodontal microorganisms studied suggesting that this portion of the subgingival periodontal microflora is independent of tobacco smoking.

Key words: cigarettes; periodontal microflora; Saudi Arabia; tobacco smoking; water pipe

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The evidence that the occurrence and severity of periodontal disease are greater in smokers than non-smokers is well documented (Bergström & Flodérus-Myrhed 1983, Feldman et al. 1983, Ismail et al. 1983, Preber & Bergström 1986, Haber & Kent 1992, Bergström et al. 2000, Bergström 2003, 2004 Khader et al. 2003). The progression of periodontal disease is suggested to be the result of a disturbance of the balance between the host response and some subgingival periodontal microorganisms such as *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia* (Slots et al. 1986, Socransky & Haffajee 1992).

It has been suggested that smoking may influence the composition of the subgingival periodontal microflora and favour the colonization of some microorganisms (Zambon et al. 1996, Umeda et al. 1998, Kamma et al. 1999, Shiloah et al. 2000, Van Winkelhoff et al. 2001). The relationship between smoking and subgingival periodontal microflora has been the object of several investigations with somewhat inconsistent results. The observations of most studies suggest that there is no association between smoking and the composition of the periodontal microflora (Preber et al. 1992, 1995, Stoltenberg et al. 1993, Boström et al. 1998, 1999, 2001, Renvert et al. 1998, Eggert et al. 2001). According to some other studies, however, smokers were observed to harbor higher levels of some periodontal microorganisms than non-smokers (Zambon et al. 1996, Umeda et al. 1998, Kamma et al. 1999, Shiloah et al. 2000, Van Winkelhoff et al. 2001). The inconsistent results may be attributed to several factors such as the severity of periodontal disease or characteristics of the study popu-

lation, e.g., early onset disease (Kamma et al. 1999, Shiloah et al. 2000) or adult periodontitis patients (Preber et al. 1992).

Water-filtered tobacco smoking or water-pipe smoking (also known as hubble–bubble, argila, hookah, sheesha) has old traditions and is widely encountered in the Middle East. Most studies about smoking and periodontal health or disease concern cigarette smoking. Studies on the oral health effects of water-pipe smoking are scarce. In a recent study, water-pipe smokers were found to run an increased risk for postoperative events following tooth extraction when compared to non-smokers (Al-Belasy 2004). Whether or not water-pipe smoking is harmful to the periodontal health in a manner similar to cigarette smoking is as yet unknown. We have recently studied the influence of water-pipe smoking on the gingival health in a Saudi Arabian population and observed less gingival bleeding in water-pipe smokers as well as cigarette smokers compared to non-smokers (Natto et al. 2004).

The composition of the periodontal microflora in water-pipe smokers as well as the relationship between water-pipe smoking and the periodontal microflora have not been investigated to date. The present investigation, therefore, was undertaken with the object to explore the occurrence of 12 selected periodontal microorganisms in a Saudi Arabian population with a special focus on the relationship between these organisms and various smoking habits.

Material and Methods

Study population

Saudi residents in Jeddah, Saudi Arabia were invited to participate in the study by means of announcements in newspapers. The clinical examination and the microbiological sampling were carried out at King Faisal Specialty Hospital, Research Center, Jeddah, Saudi Arabia. Out of 375 individuals who appeared for screening, 198 (124 males and 74 females) in the age range 17–60 years volunteered to participate in the present study. The study population and the selection criteria have been presented elsewhere (Natto et al. 2004). Each participant was informed individually about the purpose of the study and signed an informed consent form. The clinical examiner interviewed each individual as to his/her smoking habits according to a standardized questionnaire. Following this questionnaire individuals were classified as water-pipe smokers (29%), cigarette smokers (18%), smokers of both water pipe and cigarettes, labelled mixed smokers (13%), and non-smokers, i.e. individuals who had never smoked (40%). The distribution of the study sample according to age, gender and smoking is demonstrated in Table 1. The study was approved by the local ethical committee of King Faisal Specialist Hospital and Research Center, Jeddah, in accordance with the Helsinki Declaration of 1975 and as revised in 1983.

Clinical recordings and bacterial sampling

The probing depth of un-treated periodontal pockets of all teeth in each individual was measured to the nearest mm with a Hillming probe at mesial, distal, buccal and lingual sites per tooth. The site with the deepest probing depth in each quadrant for each individual was selected to obtain a subgingival plaque sample. Thus four so-called sampled sites per individual served as representative sites for the collection of microbiological material. Prior to sampling, supragingival plaque and calculus were removed and the sampled tooth was dried and isolated by sterile cotton rolls. Then a paper point (ISO 50) was inserted into the periodontal pocket or the gingival sulcus and left in place for 30 s. After removal the four paper points of each individual were placed in dry Eppendorf tube. Sample sites with a probing depth of less than 5 mm were defined as shallow sites, 5–6 mm as medium sites, and sites greater than 6 mm as deep sites.

Laboratory procedures

All samples were transported within 1 month to The Laboratory of Oral Microbiology, Göteborg University, Gothenburg, Sweden for the detection of *P. gingivalis*, *P. intermedia*, *Prevotella nigrescens, Tannerella forsythensis* (*Bacteroides forsythus*), *A. actinomycetemcomitans*, *Fusobacterium nucleatum*, *Treponema denticola*, *Peptostreptococcus micros*, *Campylobacter rectus*, *Eikenella corrodens*, *Selenomonas noxia* and *Streptococcus intermedius* using the checkerboard DNA–DNA hybridization technique (Socransky et al. 1994, Papapanou et al. 1997b).

The samples were transferred to 100 µl TE buffer (10 mM TrisHCL, 1 mM EDTA, pH 7.6) and 100 µl 0.5 M NaOH were added and the suspensions were boiled for 5 min. After cooling, 800 µl 5 M ammonium acetate were added to each tube and the sample was further processed according to standardized procedures. The hybrids formed between the bacterial DNA and the probes were detected by application of an anti-digoxigenin antibody conjugated with alkaline phosphatase and incubation with a chemiluminiscent substrate (Roche Diagnostic, Mannheim, Germany). Evaluation of the chemiluminiscent signal was performed at a LumiImager™ Workstation (Boeringer-Mannheim), by comparing the obtained signals with those of pooled standard samples containing 10^6 or 10^5 of each of the 12 studied microorganisms as described by Papapanou et al. (1997b). The obtained chemiluminiscent units were transformed into a scale of scores from 0 to 5 according to Papapanou et al. (1997b), and related to the low and high standard, respectively. In addition, the specificity of each bacterial probe was tested against the 12 species consisting the panel. A 10% overlap was noticed between *P. intermedia* and *P. nigrescens*. Four samples of all individuals (792 samples) were microbiologically analysed. The median of the four sites was selected to represent each individual. The occurrence of individuals positive for each of the investigated microorganisms was described at two different cut-off levels, score 1 and

Table 1. Study population according to gender and smoking

Smoking habit	Age mean (95% CI)	Male *n* (%)	Female *n* (%)	Total *n* (%)
Water-pipe smokers	38.6 (36.1; 41.0)	42 (72)	16 (28)	58 (100)
Cigarette smokers	37.3 (34.3; 40.2)	28 (80)	7 (20)	35 (100)
Mixed smokers	33.0 (29.5; 36.6)	15 (60)	10 (40)	25 (100)
Non-smokers	40.0 (35.3; 40.6)	39 (49)	41 (51)	80 (100)
Total	37.4 (35.9; 38.9)	124 (63)	74 (37)	198 (100)

score 3, respectively. Score 1 cut-off was selected to contrast colonized *versus* non-colonized individuals and score 3 cut-off to contrast heavily colonized versus non-colonized and less heavily colonized individuals.

Statistics

The chi-squared test was applied for testing the significance of prevalence differences between groups. Multiple logistic regression was performed to estimate the relative risk associated with the microorganisms, one at time, as the response variable, transformed into a 0/1 variable. At score 1 cut-off, 0 denoted score 0 and 1 scores 1–5. At score 3 cut-off, 0 denoted scores 0–2 and 1 scores 3–5. When testing the statistical significance with regard to the microorganisms studied, the null-hypothesis was rejected at $p<0.01$ because of multiple comparisons and p-values $0.01<p<0.05$ were considered to indicate a trend. The STATISTICA 6.1 software program (StatSoft Scandinavia AB, Uppsala, Sweden) was used for the calculations.

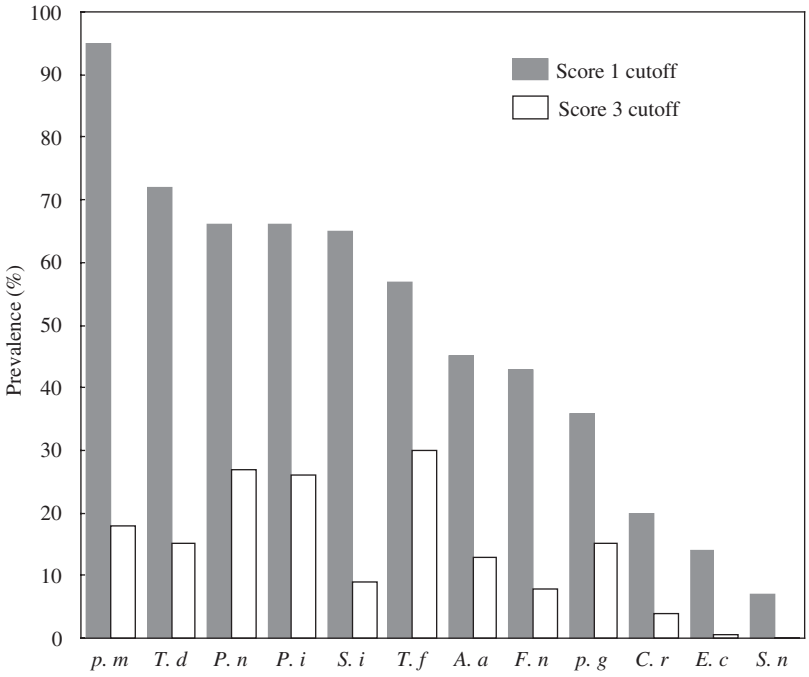

Fig. 1. Prevalence of individuals positive for the 12 studied periodontal microorganisms according to cut-off scores 1 and 3, respectively. *P. gingivalis* (*P.g*), *P. intermedia* (*P.i*), *P. nigrescens* (*P.n*), *T. forsythensis* (*T.f*), *A. actinomycetemcomitans* (*A.a*), *F. nucleatum* (*F.n*), *T. denticola* (*T.d*), *P. micros* (*P.m*), *C. rectus* (*C.r*), *E. corrodens* (*E.c*), *S. noxia* (*S.n*) and *S. intermedius* (*S.i*).

Results

Prevalence

The prevalence of individuals positive for the periodontal microflora studied at score 1 and score 3 cut-offs is illustrated in Fig. 1. *P. micros* and *T. denticola* were the most frequently detected microorganisms at score 1 cut-off (in more than 70% of individuals). In addition, *P. nigrescens*, *P. intermedia*, *S. intermedius* and *T. forsythensis* were detected in more than 50% of the individuals, while *C. rectus*, *E. corrodens* and *S. noxia* were infrequently detected (in less than 20% of individuals). The prevalence decreased throughout at score 3 cut-off (Fig. 1). Only *T. forsythensis, P. nigrescens* and *P. intermedia* were detected in more than 25% of the individuals. *S. intermedius*, *F. nucleatum*, *C. rectus* and *E. corrodens* were detected in less than 10% of individuals, while *S. noxia* was not detected at all.

The prevalence did not significantly differ with age with the exceptions of *C. rectus* at score 1 cut-off and *T. forsythensis* at score 3 cut-off for which organisms a significantly higher prevalence was found in age group 41–60 years as compared to age group 17–30 years ($p<0.01$). The prevalence did not significantly differ between genders neither at score 1 nor score 3 cut-off.

The prevalence of individuals positive for the various periodontal micro-

Table 2. Prevalence of individuals positive for the periodontal microorganisms, according to depth of sampled sites at score 1 and 3 cut-off

Microorganism	Score 1 cut-off					Score 3 cut-off				
	Shallow sites <5 mm ($n=72$) %	Medium sites 5–6 mm ($n=90$) %	Deep sites >6 mm ($n=36$) %	χ^2	p	Shallow sites <5 mm ($n=72$) %	Medium sites 5–6 mm ($n=90$) %	Deep sites >6 mm ($n=36$) %	χ^2	p
P. micros	94	97	92	1.4	NS	18	14	28	3.1	NS
T. denticola	65	68	94	11.3	0.003	13	6	44	30.9	0.000
P. nigrescens	94	70	61	1.2	NS	22	22	47	9.4	0.009
P. intermedia	67	70	53	3.4	NS	24	22	42	5.4	NS
S. intermedius	67	69	50	4.2	NS	6	3	28	20.9	0.000
T. forsythensis	49	58	69	4.3	NS	31	21	53	12.2	0.002
A. actinomycetemcomitans	40	37	70	16.3	0.003	4	14	28	12.0	0.003
F. nucleatum	44	38	56	3.4	NS	13	6	3	4.2	NS
P. gingivalis	33	37	42	0.7	NS	9	13	28	6.5	0.039
C. rectus	14	23	25	2.8	NS	6	1	8	4.1	NS
E. corrodens	22	10	8	6.1	NS	0	0	3	4.5	NS
S. noxia	7	8	3	1.1	NS	–	–	–	–	–

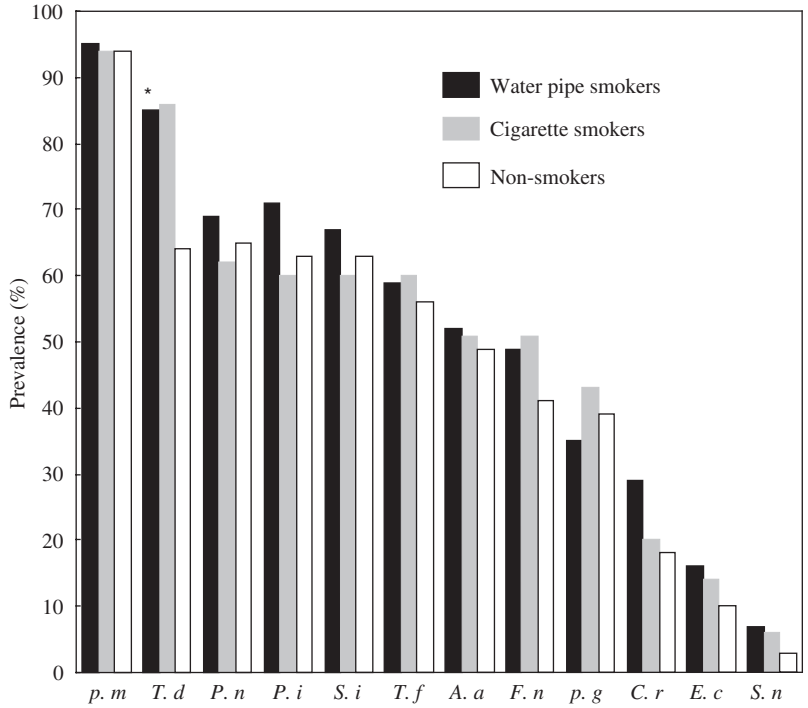

Fig. 2. Prevalence of individuals positive for the 12 studied periodontal microorganisms according to smoking. Score 1 cut-off. See Fig. 1 for abbreviations. $^{*}p<0.01$.

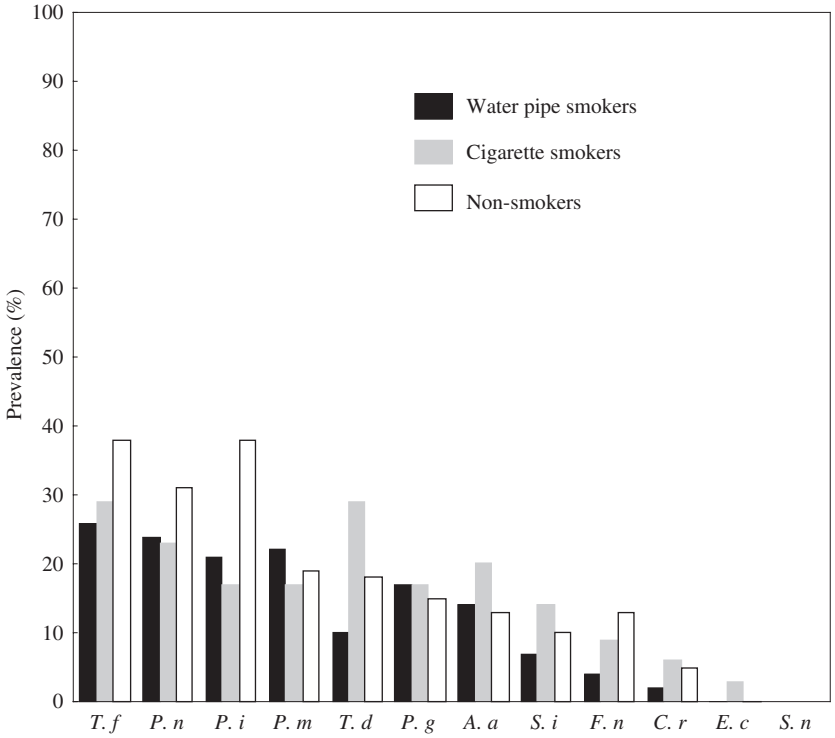

Fig. 3. Prevalence of individuals positive for the 12 studied periodontal microorganisms according to smoking. Score 3 cut-off. See Fig. 1 for abbreviations.

organisms with regard to depth of sample site at score 1 and score 3 cut-offs is demonstrated in Table 2. At score 1 cut-off, the prevalence was significantly higher in individuals with deep sites compared to individuals with shallow or medium sites for *T. denticola* and *A. actinomycetemcomitans* ($p<0.01$). Using score 3 cut-off, the prevalence was significantly higher in individuals with deep sites compared to individuals with shallow or medium sites for *T. denticola*, *P. nigrescens*, *S. intermedius*, *T. forsythensis* and *A. actinomycetemcomitans* ($p<0.01$).

Smoking

The prevalence of individuals positive for the studied microorganisms according to smoking habit (excluding mixed smokers) is illustrated in Figs 2 and 3 at cut-off scores 1 and 3, respectively. There were no statistically significant differences between smoking groups for any one of the organisms with the exception of *T. denticola* at score 1 cut-off where the prevalence was significantly higher in water-pipe smokers compared to non-smokes (chi-squared = 7.2, $p<0.01$) and a trend towards higher prevalence in cigarette smokers compared to non-smokers (chi-squared = 3.4, $p<0.05$).

The prevalence according to smoking habit (excluding mixed smokers) and depth of sample site (dichotomized into <6 mm and $\geqslant 6$ mm) using score 1 cut-off is demonstrated in Table 3. There was no statistically significant difference between smoking groups at shallow (<6 mm) sample sites for any one of the microorganisms studied. Virtually the same result was obtained for deep sites ($\geqslant 6$ mm) except for a trend towards a higher prevalence in water-pipe smokers and cigarette smokers compared to non-smokers for *T. denticola* (chi-squared = 8.3, $p<0.05$). In contrast, a trend towards a higher prevalence in non-smokers was observed for *P. nigrescens* (chi-squared = 6.6, $p<0.05$). The same held true using score 3 cut-off (data not shown).

Multivariate logistic regression analysis was performed with the periodontal microorganisms, one at time, as the response variable, dichotomized, and smoking (yes/no) and depth of sample site as independent variables. The analysis at score 1 cut-off indicated an increased risk of detecting *T. forsythensis* associated with deep sample sites (OR = 1.7, $p<0.01$). Smoking was not associated with an increased risk of detecting the microorganisms studied (Table 4). At score 3 cut-off, an increased risk of detecting *T. forsythensis*, *T. denticola* and *P. gingivalis* was associated with deep sample sites (OR = 1.9, 2.0 and 2.4, respectively,

Table 3. The prevalence of individuals positive for the 12 studied periodontal microorganisms according to smoking habit and depth of sample site at score 1 cut-off

Microorganism	Depth of sample site < 6 mm					Depth of sample site ⩾ 6 mm				
	WPS $n = 24$ %	CS $n = 15$ %	NS $n = 70$ %	χ^2	p	WPS $n = 34$ %	CS $n = 20$ %	NS $n = 10$ %	χ^2	p
P. micros	92	100	93	1.2	NS	97	90	100	2.0	NS
T. denticola	75	73	64	1.2	NS	91	95	60	8.3	0.015
P. nigrescens	67	93	63	5.3	NS	71	40	80	6.6	0.037
P. intermedia	79	80	61	3.7	NS	65	45	70	2.6	NS
S. intermedius	79	80	61	3.7	NS	59	45	70	1.9	NS
T. forsythensis	42	53	53	1.0	NS	71	65	80	0.7	NS
A. actinomycetemcomitans	42	20	50	4.6	NS	59	75	40	3.6	NS
F. nucleatum	42	53	44	0.5	NS	47	50	20	2.7	NS
P. gingivalis	17	40	39	4.1	NS	47	45	40	0.2	NS
C. rectus	17	6	20	3.6	NS	38	35	0	5.5	NS
E. corrodens	29	20	11	4.2	NS	6	10	0	1.2	NS
S. noxia	0	13	3	5.0	NS	12	0	0	3.8	NS

n, number of individuals; WPS, water-pipe smokers; CS, cigarette smokers; NS, non-smokers.

Table 4. Multiple logistic regression with the 12 studied microorganisms, one at a time, as the dependent variable and smoking and depth of sample site as independent variables. Score 1 cut-off

Dependent variable	Smoking			Depth of sample sites		
	OR	95% CI	p	OR	95% CI	p
P. micros	1.02	0.24; 4.30	NS	1.18	0.54; 2.57	NS
T. denticola	2.33	1.04; 5.25	0.039	1.49	0.93; 2.38	NS
P. nigrescens	1.27	0.61; 2.63	NS	0.83	0.58; 1.21	NS
P. intermedia	1.60	0.77; 3.34	NS	0.74	0.51; 1.08	NS
S. intermedius	1.53	0.73; 3.19	NS	0.70	0.48; 1.02	NS
T. forsythensis	0.72	0.35; 1.45	NS	1.66	1.14; 2.41	0.008
A. actinomycetemcomitans	0.77	0.39; 1.55	NS	1.50	1.04; 2.16	0.027
F. nucleatum	1.41	0.71; 2.82	NS	0.90	0.63; 1.28	NS
P. gingivalis	0.70	0.34; 1.44	NS	1.39	0.96; 2.01	NS
C. rectus	1.14	0.48; 2.69	NS	1.47	0.97; 2.24	NS
E. corrodens	2.87	1.05; 7.85	0.039	0.46	0.25; 0.84	0.015
S. noxia	2.49	0.41; 15.1	NS	1.09	0.50; 2.39	NS

OR, odds ratio; 95% CI, 95% confidence interval.

Table 5. Multiple logistic regression with 7 studied microorganisms, one at a time, as the dependent variable and smoking and depth of sample sites as independent variables. Score 3 cut-off

Dependent variable	Smoking			Depth of sample sites		
	OR	95% CI	p	OR	95% CI	p
T. forsythensis	0.30	0.13; 0.70	0.005	1.86	1.21; 2.84	0.004
P. nigrescens	0.49	0.22; 1.09	NS	1.41	0.94; 2.13	NS
P. intermedia	0.25	0.11; 0.60	0.002	1.56	1.00; 2.42	0.046
P. micros	0.92	0.38; 2.19	NS	1.23	0.79; 1.91	NS
T. denticola	0.49	0.18; 1.31	NS	1.97	1.21; 3.23	0.007
P. gingivalis	0.47	0.17; 1.35	NS	2.40	1.42; 4.06	0.001
A. actinomycetemcomitans	0.93	0.34; 2.56	NS	1.46	0.89; 2.40	NS

OR, odds ratio; 95% CI, 95% confidence interval.

$p < 0.01$). Furthermore, the risk of being positive for *T. forsythensis* and *P. intermedia* was significantly decreased in smokers (OR = 0.3 and 0.3, respectively, $p < 0.01$, Table 5).

Discussion

The present investigation provides the first information about the subgingival periodontal microflora in a Saudi Arabian population. The overall prevalence of the microorganisms studied largely corresponds to what has been reported in different other demographic populations (Ali et al. 1997, Papapanou et al. 1997b, 2000, Timmerman et al. 1998, Craig et al. 2001, 2003, Dowsett et al. 2002). The present and previous results suggest that the periodontal microflora is similar in individuals from different geographic locations but still some differences may exist that could be dependent on the sensitivity and specifity of the detection method or the characteristics of the population studied. Presently, we used the checkerboard DNA–DNA hybridization technique that is well suited for field studies (Papapanou et al. 2000, 2002). Crossreactions occurring between whole genomic probes and related microorganisms have been shown (Wong et al. 1996). The specificity was checked for the species in the test panel of 12 strains and cross hybridization was noticed between *P. intermedia* and *P. nigrescens*. It is still possible that crossreactions were occurring between the probes and other microorganism not included in the panel, however, this disturbing factor would have similar effect on all samples and might not interfere with the overall result. The prevalence using cut-off score 1 may lead to an overestimation due to occasionally interfering background. It occurred specifically for some microorganism e.g. *P. nigrescens*, and to a lesser degree *P. micros* and *T. denticola*. This was explained by the presence of proteins tightly bound to the membrane (or the DNA) and could not be diminished by the washing steps. It makes it difficult to read the membranes using the computer program, which cannot distinguish between the background and true signals of hybridization. We therefore read the membranes also by the naked eye to avoid the risk of overestimation of these three microorganisms in the present study. Therefore, beside cut-off score 1, cut-off score 3 was used in the analysis to ensure an unambiguous bacterial level of detection.

In the present study, 50% of individuals were positive for *T. forsythensis*, whereas in a Chinese and a Thai population all individuals were positive for *T. forsythensis* (Papapanou et al. 1997a, 2002).

The occurrence of *P. intermedia* at a low detection level in the present population is similar to that reported in a Swedish population (Papapanou et al.

1997b), whereas a comparably higher prevalence (up to 70%) has been reported in populations from other geographic locations (Ali et al. 1997, Dowsett et al. 2002).

The occurrence of *A. actinomycetemcomitans* and *P. gingivalis* was higher than that observed in a Swedish population (Papapanou et al. 1997b). The occurrence of *A. actinomycetemcomitans* and *P. gingivalis* was not related to age in the present study. Whereas using other detection methods, the occurrence of *A. actinomycetemcomitans* was related to the younger age group and *P. gingivalis* was related to the older age group (Savitt & Kent 1991, Hamlet et al. 2001). However, presently, only *T. forsythensis* in heavily colonized individuals (at cut-off score 3) was more prevalent in the older subjects (age group 41–65 years). In addition, no association between gender and the occurrence of the studied microorganisms was observed which agrees with what has been reported by Schenkein et al. (1993) but disagrees with the results of Umeda et al. (1998) suggesting that male gender was a risk factor for *P. intermedia*.

Probing depth of the sampled sites was the most important risk factor associated with the microorganisms studied. Individuals with deep sites were at a significantly higher risk especially for *T. denticola, A. actinomycetemcomitans, P. nigrescens, S. intermedius*, and *T. forsythensis* than individuals with shallow or medium sites.

Smoking

The main object of the present study was to investigate whether or not cigarette and/or water-pipe smoking might be related to the occurrence of specific microorganisms. The results demonstrated that, in principle, cigarette smokers, water-pipe smokers and non-smokers exhibited the same microflora with reference to the 12 microorganisms investigated, suggesting that tobacco smoking has little influence on the composition of the subgingival periodontal microflora. This observation is in agreement with a number of previous studies that have investigated the relationship between cigarette smoking and the periodontal microflora using the same or other detection techniques (Preber et al. 1992, Stoltenberg et al. 1993, Renvert et al. 1998, Boström et al. 2001, Eggert et al. 2001). However, some studies suggest that smokers would be more frequently positive for, e.g. *A. actinomycetemcomitans*, *T. forsythensis*, *P. gingivalis*, *P. micros* or *T. denticola* than non-smokers (Zambon et al. 1996, Umeda et al. 1998, Kamma et al. 1999, Van Winkelhoff et al. 2001, Haffajee and Socransky 2001).

The present observations indicated that cigarette smokers and water-pipe smokers might be more frequently positive for *T. denticola*, although only at score 1 cut-off and deep sampled sites. However, when pocket depth of the sample site was accounted for in the logistic regression models, the association of *T. denticola* with smoking was attenuated and the significance disappeared. It seems more likely that possible differences in the prevalence of the microorganisms are related to differences in the pocket depth between smokers and non-smokers and not to the smoking effect itself. It should be noted that in the present study 84% of individuals with deep sites were smokers.

The present study is the first one to investigate the association between water-pipe smoking and the periodontal microflora. The results indicate that there was no special influence of water-pipe smoking on the microorganisms studied and that there is no reason to believe that water-pipe smokers would differ from either cigarette smokers or non-smokers regarding this portion of the subgingival periodontal microflora.

In summary, the present investigation has explored the occurrence of the periodontal microflora associated with periodontal disease in a Saudi Arabian population. In this population, a high prevalence was observed for *P. micros*, *T. denticola*, *P. nigrescens*, *P. intermedia*, *S. intermedius* and *T. forsythensis* at a low level of detection. When the level of detection was raised, the prevalence declined consistently. No major differences were observed between cigarette smokers, water-pipe smokers, and non-smokers particularly when the depth of the sample site was taken into account.

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