original article

Periodontal pathogenic bacteria among high school children in Saudi Arabia

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BACKGROUND: The periodontal tissues are continuously exposed to specific bacterial components that have the ability to alter many local functions. Normal endogenous infections in healthy mouths cause disease when their numbers increase significantly.

OBJECTIVE: Determine the percentage of different periodontal pathogenic bacteria and their association with periodontal status.

DESIGN: Cross-sectional, analytical.

SETTINGS: School children of both genders in Saudi Arabia.

PATIENTS AND METHODS: Clinical examination consisted of measurement of the gingival and periodontal supporting tissue including attachment loss, probing pocket depth and furcation involvement following the National Health and Nutrition Examination Survey (NHANES) and taking samples of the subgingival bacterial flora.

MAIN OUTCOME MEASURES: The percentage of periodontal pathogenic bacteria and its association with periodontal status in Saudi Arabia.

SAMPLE SIZE: Bacterial samples were collected from 277 subjects.

RESULTS: *Aggregatibacter actinomycetemcomitans* was present in 21.7% of the subjects, *Porphyromonas gingivalis* in 21.3%; *Tannerella forsythia* in 10.1%; *Treponema denticola* in 34.7% and *Prevotella intermedia* in 12.3%. The red complex bacteria were found in 2.9% of the subjects.

CONCLUSIONS: The percentages of bacteria varied but only *T denticola* was significantly associated with periodontal breakdown. In addition, the presence of more than 2 of the 5 species tested were significantly associated with tissue damage.

LIMITATIONS: Cannot be generalized to all of Saudi Arabia. Larger controlled studies are needed.

CONFLICT OF INTEREST: None.

Periodontal diseases are abundant among children, adolescents, and adults.[1] They involve any inherited or acquired disorders of the tooth supporting structures (gingiva, cementum, periodontal ligament, and alveolar bone).[2] The periodontal tissues are constantly exposed to certain bacterial components that have the capability to modulate local functions.[3] Natural inflammatory processes protect the host and limit the pathogenic effect of biofilms, thus regulating some tissue destruction as a collateral effect of the defense.[4] Host-bacterial interaction theory is accepted as the current etiological basis for the disease.[5] According to the American Academy of Periodontology, "A bacterial infection alone is insufficient to result in periodontal disease. The host response plays a critical role in the tissue destruction seen in periodontitis and everyone is not equally susceptible to periodontal disease".[6] The concept of periodontal disease pathogenesis was introduced by Page in 1990,[7] who summarized the multifactorial nature of the disease, stating that while bacteria are crucial in causing the disease, they alone are insufficient. Host and environmental factors strongly influence the onset, severity of the disease and the rate of progression as well as the response to treatment.[8]

Of the bacterial species currently known to inhabit the oral cavity, many possess the ability to destroy periodontal structures even when present in small quantities.[9] As part of their pathogenic potential, these bacterial species are able to colonize the subgingival area, produce virulence factors that directly (enzymes and toxins) or indirectly (antigens and activators) lead to initiation of a destructive inflammatory reaction in the individual and injury of periodontal tissues.[10,11] The host immune response is activated through microbial infection which leads to production of specific antibodies, cytokines and other humoral factors to defend host tissues from inflammatory agents, microbial penetration and injury. Subgingival plaque present in deep periodontal pockets is dominated by gram-negative anaerobic rods and spirochetes.[12] Strong evidence has implicated *Porphyromonas gingivalis*[13] and *Aggregatibacter actinomycetemcomitans*[14] in the pathogenesis of adult periodontitis. In addition, *Tannerella forsythia*,[15] *Prevotella intermedia*,[16] *Peptostreptococcus micros*,[17] and *Fusobacterium nucleatum*[18] have been strongly associated with the initiation, severity and progression of periodontal diseases. There is strong evidence that these bacteria are associated and/or responsible for periodontal destruction.[19]

Motile rods, spirochetes and *P intermedia* are elevated proportionately in teenaged children with gingivitis and linked with clinical features such as bleeding on probing and the gingival index.[20] In a group of healthy children, 2 to 18 years of age, investigators have found that 60% and 75% of the children had detectable levels of *P gingivalis*, *A actinomycetemcomitans* and *T forsythia* in their plaque. These bacterial species, which cause gingivitis in children, are endemic in healthy mouths, and cause disease when their numbers increase significantly.[21] The current study aimed to examine the percentage of different periodontal pathogenic bacteria and their association with periodontal status based on cross-sectionally collected dental plaque samples from high school children in Saudi Arabia.

PATIENTS AND METHODS

The current study was part of an ongoing larger national cross-sectional descriptive study that is appraising the pattern of gingival and periodontal diseases among high school children in Saudi Arabia. In the large study, we examined a random sample of 2435 school children grades 10 to 12 (15-18 years old) of both genders in September 2012 until January 2016 (41 months) The study focused mainly on larger cities but will also include some smaller cities in more rural areas. The selected cities were: Riyadh, Jeddah, Dammam, Abha and Tabuk. A multistage clustered sampling design was followed to guarantee an adequate representation of all children in the country within the specified school grades.

Two hundred and seventy-seven subjects were included in the current study from a total sample of 2435 children. They were divided into children diagnosed with periodontitis (209 subjects) and periodontally healthy control (69 subjects) from the total sample of 2435 children. The study protocol was approved by the Institutional Review Board, Faculty of Dentistry, King Abdulaziz University (no. 073-09-12). Informed consent was provided by the parents of children to be included in the study. No children were admitted to the study without their parents' approval. Subject name, gender, age, address, and contact information were recorded. At an examination visit, the examiners reviewed the medical history with the subjects and recorded the information. A dental history questionnaire was completed by each subject and revised with the examiner. All examinations were performed on permanent teeth only; primary teeth (if present) and partially erupted teeth were excluded from the examination.

The periodontal examination consisted of measurement of the gingival and periodontal supporting tissue including gingivitis, attachment loss, probing pocket depth and furcation involvement; and the assessment of gingival bleeding, and dental plaque. Measurements

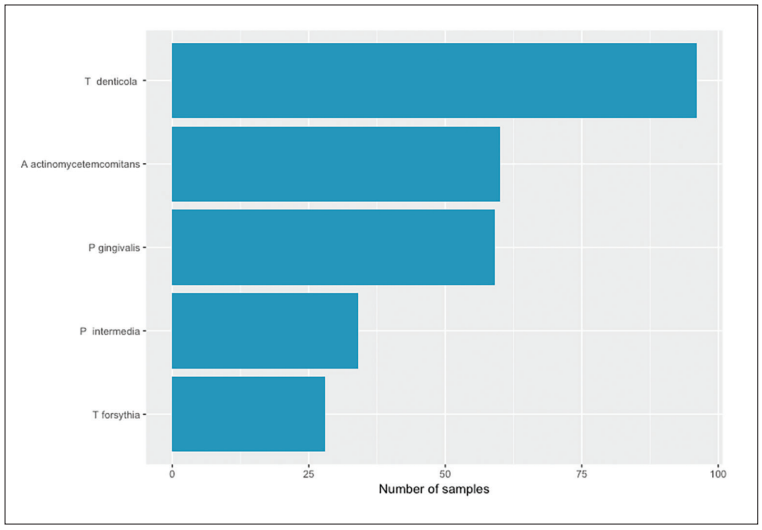

Figure 1. Number of samples by type of bacteria.

Table 1. Characteristics of the study samples relative to the presence of *A actinomycetemcomitans*.

Variables	*A actinomycetemcomitans*		*P* value
	Yes	No	
Total (n)	60 (21.7)	217 (78.3)	N/A
Age (years)	17.0 (0.9)	17.3 (1.0)	.032
Gender			
Male	16 (15.8)	85 (84.2)	.075
Female	44 (25.0)	132 (75.0)	
Nationality			
Non-Saudi	12 (19.0)	51 (81.0)	.567
Saudi	48 (22.4)	166 (77.6)	
Smoker			
Yes	2 (11.8)	15 (88.2)	.307
No	58 (22.3)	202 (77.7)	
Plaque Index	1.36 (0.8)	1.52 (0.8)	.161
Gingival Index	1.30 (0.8)	1.22 (0.8)	.505
PD (mm)	0.65 (0.2)	0.68 (0.2)	.370
PD ≥4 mm	7.45 (9.6)	7.46 (9.2)	.996
CAL (mm)	0.17 (0.3)	0.18 (0.3)	.921
CAL ≥1m	9.36 (20.4)	10.42 (23.4)	.758

Data are number (percentage) or mean (standard deviation). PD: Pocket depth, CAL: Clinical attachment loss Note: Numbers do not add up in some cells due to missing data

were based on criteria set by the US National Health and Nutrition Examination Survey (NHANES) IV where Random Half-Mouth was used.[22]

In periodontally healthy children (69 subjects), samples were collected from two randomly selected subgingival sites. In children with periodontal diseases, samples were collected from the two deepest pockets. For sample collection, the tooth was dried and supragingival plaque was removed from sampling sites; a subgingival plaque sample was collected using a sterile curette in a single vertical stroke. Each sample was immediately placed in a sterile centrifuge tube containing 0.5 mL ethylenediamine tetraacetic acid (EDTA) buffer. The samples were sent to King Abdulaziz University Hospital microbiology lab for analysis. An improved multiplex nested PCR technique was used to allow sensitive detection of all five periodontal pathogens.[23,24]

The data obtained was analyzed using IBM SPSS version 22. A simple descriptive statistic was used to define the characteristics of the study variables as counts and percentages for the categorical and nominal variables while continuous variables are presented by mean and standard deviation. To establish a relationship between categorical variables, we used the chi-square test. When comparing two group means and more than two groups, an independent *t* test or one-way ANOVA were used. The conventional *P* value of <.05 was the criteria to reject the null hypothesis.

RESULTS

The 277 subjects ranged in age from 15 to 19 years with a mean (standard deviation) of 17.3 (1.0) years. Two hundred sixty-one (94%) were Saudi, the remainder were other Arab nationalities. More than half of the subjects were males (54.6%). Bacterial samples were obtained from the 277 subjects. *A actinomycetemcomitans* was present in 21.7%, *P gingivalis* in 21.3%; *T forsythia* in 10.1%; *Treponema denticola* in 34.7%; and *P intermedia* in 12.3% (**Figure 1**). The red complex bacteria were found in 2.9% of the subjects.[25] About 57.4% of the participants had at least one type of bacteria, whereas 2 or more bacteria were found in 19.1%. Only 9.0% of the participants had 3 or more bacteria, and 4 or more bacteria were found in only 1.8%.

Age had a significant relationship with *A actinomycetemcomitans* (**Table 1**). **Table 2** shows characteristics of samples relative to the presence of *P gingivalis*. Pocket depth ≥ 4 mm had a significant relationship with the presence of *T denticola* (**Table 3**). None of the characteristics of the sample relative to *T forsythia* and *P intermedia* were significantly related to these bacteria. None of the characteristics of the sample relative to the

Table 2. Characteristics of the study samples relative to the presence of *P gingivalis*.

Variables	*P gingivalis*		*P* value
	Yes	No	
Total (n)	59 (21.3)	218 (78.7)	N/A
Age (years)	17.35 (0.9	17.25 (1.0	.440
Gender			
Male	21 (20.8)	80 (79.2)	.876
Female	38 (21.6)	138 (48.4)	
Nationality			
Non-Saudi	14 (22.2)	49 (77.8)	.839
Saudi	45 (21.0)	169 (79.0)	
Smoker			
Yes	3 (17.6)	14 (82.4)	.704
No	56 (21.5)	204 (78.5)	
Plaque Index	1.49 (0.9)	1.49 (0.8)	.997
Gingival Index	1.29 (0.8)	1.22 (0.8)	.592
Plaque diameter (mm)	0.69 (0.2)	0.67 (0.2)	.627
PD ≥4mm	8.78 (9.6)	7.10 (9.2)	.219
CAL (mm)	0.17 (0.2)	0.18 (0.3)	.832
CAL ≥1m	9.73 (20.3)	10.31 (23.4)	.867

Data are number (percentage) or mean (standard deviation). Note: Numbers do not add up in some cells due to missing data.

Table 3. Characteristics of the study samples relative to the presence of *T denticola*.

Variables	*T denticola*		*P* value
	Yes	No	
Total n	96 (34.7)	181 (65.3)	N/A
Age (years)	17.18 (1.0)	17.29 (0.9)	.406
Gender			
Male	34 (33.7)	67 (66.3)	.792
Female	62 (35.2)	114 (64.8)	
Nationality			
Non-Saudi	21 (33.3)	42 (66.7)	.802
Saudi	75 (35.0)	139 (65.0)	
Smoker			
Yes	8 (47.1)	9 (52.9)	.267
No	88 (33.8)	172 (66.2)	
Plaque Index	1.48 (0.8)	1.50 (0.8)	.856
Gingival Index	1.27 (0.8)	1.22 (0.8)	.598
PD (mm)	0.69 (0.2)	0.67 (0.2)	.477
PD ≥4 mm	9.67 (11.5)	6.28 (7.7)	.004
CAL (mm)	0.21 (0.3)	0.16 (0.3)	.158
CAL ≥1m	12.18 (23.9)	9.08 (22.0)	.303

Data are number (percentage) or mean (standard deviation). Numbers of subjects. Note: Numbers do not add up in some cells due to missing data.

presence of up to two bacteria showed a significant association. The mean PD had a significant relationship with the presence of 3 or more and 4 or more bacteria. The Gingival Index, mean PD and percentage of PD ≥4mm had a significant relationship with the presence of 4 or more bacteria. None of the variables showed a significant association with the red complex bacteria.

DISCUSSION

We found *A actinomycetemcomitans* in 21.7% of the population and its presence was significantly associated with younger age. Lopez reported the prevalence of *A actinomycetemcomitans* to be between 6.25% and 12.5% in a population of adults.[26] *A actinomycetemcomitans* was also found in very young children (6-36 months) with a percentage of up to 30%. *A actinomycetemcomitans* is a gram-negative small non-motile rod that is strongly associated with destructive and aggressive, i.e. grade C, forms of periodontal disease.[27]

P gingivalis and *T forsythia* were found in 21.3% and 10.1% of the population, respectively. A study showed that the percentage of *P gingivalis* ranged from 3% for Caucasians to 17% for Indian subjects.[28] The latter population seems to be closer to the population in this study. The presence of these species was not associated with any of the characteristics examined in this population, which might indicate that they may be part of the normal flora in some individuals.

P gingivalis is a gram-negative black-pigmented anaerobic rod that is readily found in subgingival biofilms. It is widely recognized as a contributor to the development of periodontal infections and implicated as a major pathogen in adult periodontitis.[29-32] *P gingivalis* showed the highest load while *A actinomycetemcomitans* had the lowest load in an adult Italian population.[33] *T forsythia* (previously *Bacteroides forsythus*) is another gram negative non-motile rod which is found in periodontal sites with active destruction as well as with disease recurrence.[34]

The highest *prevalence* found in this study was

Table 4. Characteristics of the study samples relative to the presence of 3 or more bacteria.

Variables	3 or more bacteria		*P* value
	Yes	No	
Total (n)	25 (9.0)	252 (91.0)	N/A
Age (years)	16.95 (1.1)	17.28 (0.9)	.151
Gender			
Male	9 (8.9)	92 (91.1)	.960
Female	16 (9.1)	160 (90.9)	
Nationality			
Non-Saudi	5 (7.9)	58 (92.1)	.732
Saudi	20 (9.3)	194 (90.7)	
Smoker			
Yes	1 (5.9)	16 (94.1)	.641
No	24 (9.2)	236 (90.8)	
Plaque Index	1.26 (0.9)	1.51 (0.8	.143
Gingival Index	1.42 (0.9)	1.22 (0.8	.257
PD (mm)	0.77 (0.2)	0.67 (0.2)	.021
PD ≥4 mm	11.61 (11.4)	7.04 (9.0)	.062
CAL (mm)	0.25 (0.4)	0.17 (0.3)	.275
CAL ≥1m	14.99 (27.7)	9.67 (22.1)	.276

Note: Numbers do not add up in some cells due to missing data.

Table 5. Characteristics of the study samples relative to the presence of 4 or more bacteria.

Variables	4 or more bacteria		*P* value
	Yes	No	
Total (n)	5 (1.8)	272 (98.2)	N/A
Age (years)	17.00 (0.7)	17.26 (1.0)	.556
Gender			
Male	0	101 (100.0)	.087
Female	5 (2.8)	171 (97.2)	
Nationality			
Non-Saudi	0	63 (100.0)	.221
Saudi	5 (2.3)	209 (97.7)	
Smoker			
Yes	0 (0.0)	17 (100.0)	.564
No	5 (1.9)	255 (98.1)	
Plaque Index	1.78 (0.3)	1.48 (0.8)	.417
Gingival Index	2.00 (0.3)	1.22 (0.8)	.003[a]
PD (mm)	0.42 (0.2)	0.68 (0.2)	.008[b]
PD ≥4 mm	3.46 (1.4)	7.53 (9.4)	<.001[a]
CAL (mm)	0.12 (0.3)	0.18 (0.3	.675
CAL ≥1m	1.90 (4.2)	10.35 (22.9)	.411

[a]Welch's *t* test <.05 level, [b]Independent *t* test Note: Numbers do not add up in some cells due to missing data.

that of *T denticola*, which was detectable in 34.7% of the samples. The presence of this microorganism was significantly associated with higher percentage of PD ≥4 mm. *T denticola* is also part of the red complex, which is a major contributor to common adult forms of periodontitis.[34] It is a gram-negative motile anaerobe related to periodontal lesions. More commonly found in patients with severe periodontitis, rather than in patients with healthy periodontium or gingivitis. In both healthy and HIV-infected individuals in an adult Serbian population, *T forsythia* was the most and *T denticola* was the least frequent bacteria, which is opposite to our results. This can lead to primary conclusion that *T denticola* is more associated with younger individuals but more studies are needed.[35,36]

Another bacteria strongly associated with periodontal disease which was checked for in this sample is *P intermedia*.[16,34,37] The percentage of subjects with detectable *P intermedia* was 12.3%, but it was not associated with any other characteristics. Ellwood and coworkers reported a prevalence of 2% for subjects who were positive for *P intermedia*.[28] In a study comparing Italian and Dutch groups, all bacterial loads differed significantly.[38] *T denticola* and *P gingivalis* were significantly more prevalent in the Italian group when compared to Dutch group. This can suggest the importance of ethnicity and background in the bacterial profile related to periodontal disease.

More than half of the sample (57%) had 1 or more bacterial species detectable, while 19% had 2 or more bacterial species present. Neither was significantly related to any of the characteristics examined. On the other hand, 9% of the sample was positive for 3 or more bacterial species and this was positively associated with higher mean PD. Furthermore, 1.8% had 4 or more of the examined pathogenic bacteria. This was associated with higher gingival index, percentage of PD ≥4 mm and mean PD. Red complex bacteria was found in 2.9% of the sample, although not significantly related to any characteristic. However, the probability of having bacteria belonging to the red complex is 25% to 48% in the presence of bleeding

on probing and with a pocket depth >6 mm by 20% to 37% in adult Italian population.[39] This suggests that when more pathogenic bacteria are present there is an increased chance of periodontal breakdown. This appears to confirm the non-specific theory of bacterial colonization, which suggests that different combinations of indigenous bacteria, rather than just a single species, can produce the pathogenic potential necessary to cause progression from gingivitis to destructive periodontitis.[40]

The use of microbiological assays for detecting specific microbes as an adjunct in the diagnosis and management of periodontal disease has been strongly recommended. It is clear that plaque control and root planing is very important in the management of most forms of periodontal disease; however, identifying and monitoring of specific microorganisms represents a new method to the management of periodontal disease and provides a rationale for treatment of the specific infections of the periodontium. Future technologies may provide more efficient and improved tests.[41]

Within the limitations of the study, we can conclude that all bacterial species tested were present with variable prevalence, but only *A actinomycetemcomitans* and *T denticola* were significantly associated with periodontal breakdown. In addition, the presence of more than 2 of the 5 species tested was significantly associated with tissue damage. This study should be followed by further studies with larger sample sizes covering large and small cities in Saudi Arabia. Also, studies using real-time quantitative PCR for detecting whether the level of bacteria has an effect on the parameters studied can be compared to the results obtained in this study.[42]

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