Original Article

Prevalence of Periodontal Pathogens in a Group of Participants from the Middle East and North Africa Geographic Region with Minimal Periodontal Disease

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ABSTRACT

Aim and Objectives: To assess the prevalence of the three putative periodontal pathogens such as *Aggregatibacter actinomycetemcomitans* (*A. a*), *Porphyromonas gingivalis* (*P. g*), *and Prevotella intermedia* (*P. i*) in a group of Arab participants from the Middle East and North Africa (MENA) region who had minimal periodontal disease and no history of periodontal treatment and further to analyze the association among these three microorganisms.

Materials and Methods: Eighty-four participants of varied demographics and oral/dental care habits were screened for this study. Twenty-one participants who eventually gave their consent to fully participate in the study provided a balanced representation of the screened universe.

Results: Seventy-eight microbiological samples were taken from the deepest pockets. Twenty-three percent of all samples were found to be *A. a* positive and so were 79% for *P. g* and 82% for *P. i*. A highly remarkable association was observed between the presence of *P. i* along with *P. g* ($P < 0.00l$; Fisher's exact test). Of all samples, merely six cases were *P. i* positive but not *P. g* and eight cases were vice versa. There was a statistically significant association between *A. a* and *P. g* ($P = 0.016$). No significant relationship was detected between *P. i* and *A. a*.

Conclusions: This qualitative study shows very high frequency of the three periodontal pathogens (*A. a, P. g,* and *P. i*) in a group of Arab nationals with minimal periodontal disease. The lack of oral hygiene, minimal use of antimicrobial drugs and antiseptics, and the absence of dental care may partly explain the high prevalence of periodontal pathogens.

KEYWORDS: *Aggregatibacter actinomycetemcomitans,* Middle East and North Africa participants, minimal disease, *Porphyromonas gingivalis, Prevotella intermedia*

INTRODUCTION

Periodontitis is an infectious disease that leads to periodontal destruction and early tooth loss. However, not all bacteria seem to have the same pathogenicity. Only a few bacteria have been associated with disease progression such as *Aggregatibacter actinomycetemcomitans* (*A. a*), *Porphyromonas gingivalis* (*P. g*), *Prevotella intermedia* (*P. i*), *Eikenella, Campylobacter, Fusobacterium, Tannerella,* and *Treponema*.[1]

In the past 20 years, only a few bacteria, such as *A. a, P. g,* and *P. i, have* attracted particular attention.

Studies involving mainly North American and European participants showed an association of these microorganisms with deep periodontal pockets and less frequently in periodontally healthy participants of these countries.[2]

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Access this article online	
Quick Response Code:	**Website:** www.jispcd.org
	DOI: 10.4103/jispcd.JISPCD_126_17

The microbiota associated with periodontitis shows important geographical particularity.[1] For example, contrary to the association between deep pockets and the presence of *A. a, P. g,* and *P. i* in North American and European participants, high prevalence of these microorganisms was found in adult Kenyans without pockets whose depth not more than 3 mm.[2] Similar results were reported about subgingival microflora in immigrants descending from developing countries in Switzerland.[3] Where 87.5% of sites were not in excess of 3 mm and the percentage of sites bleeding on probing was 62. There is a difference between the subgingival flora of deep periodontal pocket in periodontitis patients and that of shallow pockets in nonperiodontitis participants.[4,5] The difference in the subgingival microbiota between deep and shallow pockets has also been shown within the same patient.[6]

Influenced by the ethnic racial characteristics of the population[1,7] and the scarcity of literature dedicated to participants from the Middle East and North Africa (MENA) region, the objective of the present study was to determine the prevalence of the three periodontal pathogens such as *A. a, P. g,* and *P. i* in a group of MENA participants who had minimal periodontal disease and no history of periodontal treatment as well as to analyze the association among these three microorganisms.

MATERIALS AND METHODS

PATIENTS

Eighty-four (59 males and 25 females) MENA participants, of low-to-middle socioeconomic groups aged between 24 and 49 years, were screened for participation in this study. Nine different nationalities were represented: Lebanon, Syria, Jordan, Palestine, Iraq, Algeria, Tunisia, Morocco, and Egypt. Eventually, 21 participants (16 males and 5 females) extended informed consent to participate in the study; those were out of 29 participants who fulfilled the following inclusion criteria:
- Presence of at least twenty teeth
- Minimum age of 18 years, with no history of dental treatment except fillings and extractions
- Presence of minimal periodontal disease, defined by a mean probing depth <3.5 mm and presence of <5% of pockets deeper than 5 mm (Al Yahfoufi *et al.*, JCP1995).[8]

The exclusion criteria were systemic diseases (diabetes mellitus, cancer, HIV, metabolic disease, radiation, or immunosuppressive therapy), pregnancy or lactation, and systemic antibiotics within the previous 6 months.

PLAQUE SAMPLING AND CLINICAL MEASUREMENTS

The plaque index (PI), Silness and Loe,[9] was scored on four surfaces of every tooth. Then, subgingival microbiological samples were taken with paper points from the deepest pocket in each quadrant. Supragingival plaque was removed with sterile cotton pellets before sampling. The area was isolated and dried; one sterile paper point (Absorbent points 2715 fine, Johnson and Johnson, NJ, USA) was inserted into the bottom of a pocket for a period of 10 s. After sampling, probing of the pocket depth and recession were measured and bleeding on probing was determined on four surfaces of each tooth. One single investigator (ZA) collected all clinical indices and samples. A standard periodontal probe (GF-w, Hu-Freddy, 11, USA) was used for periodontal probing.

DNA PROBE ANALYSIS OF BACTERIA

DNA probes were utilized for specific identification and quantification of *A. a, P. g,* and *P. i* (Omni Gene, MA, USA; ANAWA Laboratories, Switzerland). The probes were used as described by French *et al.*[10] In summary, the processing of plaque specimens for DNA probe analysis required disruption of the bacterial cells and the denaturing of the DNA molecules into two separate strands. This was implemented by suspending the plaque in a high pH solution. The samples were then equilibrated with loading buffer, applied to nitrocellulose filters, rinsed in 0.5 M NaCl, air-dried, and baked at 80°C for 2 h. After prehybridization, the filters were hybridized with the corresponding P^{32}-labeled DNA probe for 4 h at 65°C and washed for 5 min once at moderate temperature with WASH I and at 65°C twice for 15 min with WASH II.

The filters were then air-dried and exposed to Kodak film for 48 h at −70°C using a specialized screen. The developed X-ray images were filmed with a video camera and processed using image analysis software. Each of the three filters for the microorganisms under study contained 10^5, 10^4, and 10^3 control cells in triplicate, which were used as references for a standard curve. Each participant of the sample was measured against the reference curve to obtain a numerical value representing the number of specific bacteria available in the samples.

RESULTS

CLINICAL PARAMETERS

Out of 84 participants reviewed for partaking in this study, 25 individuals were excluded because they were assessed as healthy in periodontal terms, another 38 participants were left out because 11 were diagnosed with periodontitis, twenty as gingivitis, and seven for lacking one or more of the other qualifying criteria. Basic profile of the above breakdown is presented in Table 1.

The demographics of the participants and their exposure to antibiotics in the past 6 years, frequency of oral care, and a number of sites in each participant are exhibited in Table 2.

The clinical parameters are provided in Graph 1. The average PI was 1.17, the mean pocket depth was 2.70 mm, the mean recession measured 0.30 mm, and the percentage of sites bleeding upon probing was 62.

The frequency distribution of pocket depth is displayed in Graph 2. A probing pocket depth of 3 mm was recorded in 87.5% of all sites, whereas 12% of all surfaces had a probing pocket depth ranging from 4 to 5 mm. Only 12 out of 2466 sites permitted probe penetration deeper than 5 mm, these deep pockets were found in six participants.

MICROBIOLOGICAL ANALYSIS FOR THE THREE MARKER ORGANISMS

A. a was detected in six participants, *P. g* in 19 patients, but all participants showed positive DNA probe results for *P. i*.

Nearly 23% of all samples were *A. a* positive and so were 79% for *P. g* and 82% for *P. i* [Graph 3].

The association and the amount of the three organisms in the 78 samples are shown in Graph 4a-4c. The majority

of *P.g*-positive samples were also positive for *P. i* [Graph 4a]; only 8% of total samples were positive for *P. g* but not for *P. i* and only 6% were *P. i* but not *P. g* positive. Statistically, the association between these two species was remarkable (*P* < 0.00l; Fisher's exact test). Graph 4b shows the relation of *P. g* to *A. a*. This association was also statistically significant (*P* = 0.016); all *A. a* positive samples were also *P. g* positive. No significant relationship was found between *P. i* and *A. a* [Graph 4c].

DISCUSSION

The Arab world consists of 22 countries across North Africa and the Middle East with a population of over 365 million people. Most published surveys describing periodontal diseases in the Arab world have been carried out in children and adolescents. Aiming to expand the presumably scant discourse on adults, this study has shown a high prevalence of three periodontal pathogens in participants with a mean age of 29.61 years old, low to lower-middle socioeconomic levels, with inadequate personal oral hygiene and no history of dental treatments, except extraction and fillings, with mean pocket depth of 2.7 mm, and minimal periodontal disease. It may be deduced that the oral microflora of these patients had developed over years without any external factors. Inadequate personal oral hygiene and tobacco factor have been proposed as risk factors for periodontal diseases, particularly in low socioeconomic groups.[11] Dahlén *et al*.[4] compared the presence of periodontopathogens in healthy and deceased Kenyan participants with

Table 1: Breakdown of the screened universe in terms of inclusion/exclusion condition

Group	Number of participants	Age	Male	Female	Relevance to study
Minimal disease	21	22-48	16	5	Yes
Healthy	25	22-36	12	13	No
Periodontitis	11	30-40	6	5	No
Gingivitis	20	18-40	10	17	No
Lacking criteria	7	20-45	5	2	No

Table 2: Demographics, exposure to antibiotics and oral care

Sex	Age	Education	Income bracket	Origin	3-min brushing duration	Antiseptics	Antibiotics last 6 years	Number of sites
Male	23	Basic	400-450	Syria	1×day <	Yes	No	120
Male	30	High school	1000-1200	Morocco	5×week <	No	No	128
Male	25	Basic	1000-1200	Lebanon	1×day >	No	No	112
Male	22	High school	400-450	Lebanon	1×day <	No	Yes	120
Female	31	Basic	400-450	Lebanon	2×day <	Yes	Yes	116
Female	48	Voccational	400-450	Lebanon	2×day <	No	No	100
Male	29	Basic	1000-1200	Syria	2×day <	No	No	108
Female	28	Basic	400-450	Palestine	1×day <	No	No	80
Male	30	High school	1000-1200	Syria	4×week <	Yes	No	128
Male	35	High school	400-450	Jordan	I×day <	No	No	128
Female	26	Basic	400-450	Tunisia	2×day <	No	No	128
Male	34	High school	1000-1200	Egypt	2×day <	No	No	124
Male	29	Basic	1000-1200	Iraq	1×day >	Yes	Yes	96
Male	31	High school	1000-1200	Iraq	2×day <	Yes	No	124
Male	29	Vocational	400-450	Syria	1×day <	No	No	128
Male	26	Vocational	1000-1200	Iraq	Never	No	No	128
Male	30	Vocational	400-450	Lebanon	3×week <	No	No	128
Male	36	Vocational	1000-1200	Tunisia	1×day >	No	Yes	128
Female	21	Vocational	1000-1200	Morocco	3×day <	No	No	94
Male	25	High school	1000-1200	Lebanon	I×day <	No	No	128
Male	28	High school	1000-1200	Syria	2×week <	Yes	No	120
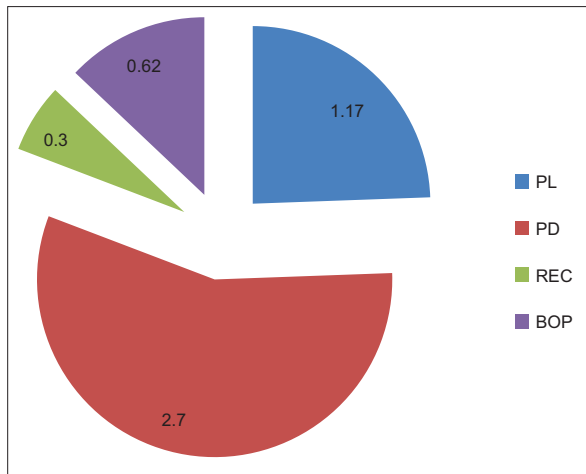
Graph 1: Overall mean values of clinical measurements of participating participants
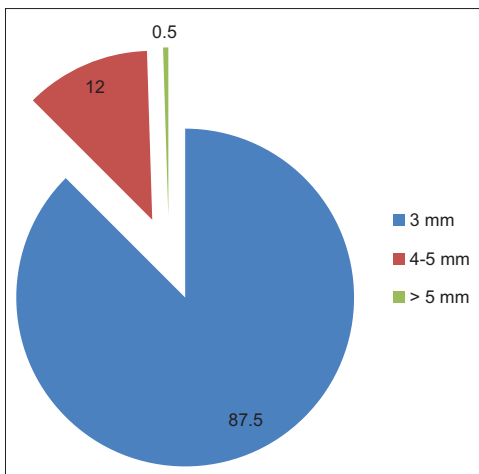
Graph 2: Frequency distribution of pocket depth
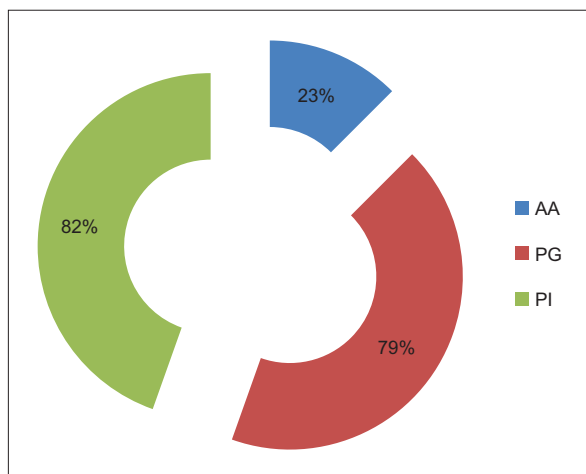
Graph 3: Frequency of *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis*, and *Prevotella intermedia* in 78 samples

insufficient oral hygiene, *P. g* was frequently found not only in dental sites and the tongue of diseased patients but also from periodontally healthy individuals. This

pointed out that deep periodontal pockets are not the only probable ecological environment for development and advancement of anaerobic bacteria.

The distribution of bacterial species related to periodontitis presents potential particularities associated with ethnic groups and also geographic locations. Albandar showed 11.5% of participants in a young age Iraqi group (children of 14 years old), with one or more sites, to be having bone loss, the researchers indicated that early periodontitis is prevalent in the Iraqi teenagers compared to Norwegians and Danish children.[12] In Indonesia, the presence of *A. a* was associated with periodontal disease progression.[13] Contrarily in China, these putative periodontal pathogens have been shown to have a wide prevalence in young Chinese without significant periodontal disease.[14] Herrera *et al*.[7] studied the microbiota of patients with chronic periodontitis in Colombia, Chile, and Spain. Patients from Colombia revealed greater severity of periodontitis, significantly higher total bacterial colony counts, and increased levels of *P. g* and Gram-negative enteric rods. Chilean patients showed a high prevalence of *Parvimonas micra,* and *Eikenella corrodens,* and relatively low percentages of *A. a, P. i, T. forsythia,* and *Capnocytophaga* species. Spanish patients exhibited increased levels of *P. i* and did not yield Gram-negative enteric rods. The study suggested that differences exist in the periodontopathic microbiota of participants in these three countries.

Mombelli *et al*.[15] estimated that more than 25 samples would be required in some participants to show in a reliable manner the presence of *P. g*. In the present study, 18 of the 21 participants were *P. g* positive in at least two out of only four samples. Selection of the deepest pocket in each quadrant was a relatively practical approach for the detection of *P. g* with a small number of samples. Using that technique in this study, *P. g* was found in 100% of more than 50% of patients since every single sample was positive in those participants. Three samples out of four were positive in five more participants. Those detections of *P. g* in the present investigation were in line with Gaetti-Jardim *et al*.[16] who studied the prevalence of periodontal pathogens in ethnic group from native Brazilian participants with periodontitis.

In the literature, deeper pockets have a greater epithelial surface area to which red complex species such as *P. g* and *T. denticola* may attach.[17] Microbial associations in human dental plaque have been analyzed in many studies,[18-20] and the significance of interspecies relationships has been described.[20] In the case of adversary association, one species may compete with another for nutrients or to occupy sites necessary for the attachment for that other species. In the case of favorable
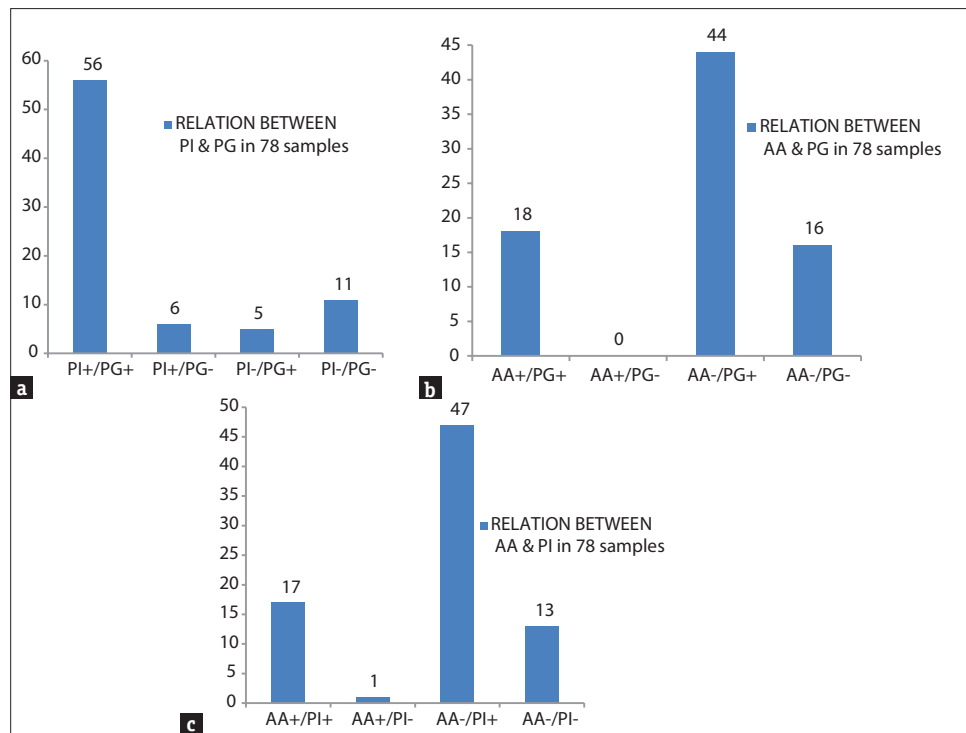
Graph 4: (a) Presence of *Porphyromonas gingivalis* in relation to the presence of *Prevotella intermedia*. (b) Presence of *Aggregatibacter actinomycetemcomitans* in relation to *Porphyromonas* gingivalis. (c) Presence of *Aggregatibacter actinomycetemcomitans* in relation to *Prevotella intermedia*

association, one species may support another species by generating growth factors or changing the environment. In this study, there was an observation of a highly significant association between *P. g* and *P. i.* In 56 sites, both species were found together whereas *P. g* was found individually in five sites whereas *P. i* was detected in six sites. There was also a significant association between *P. g* and *A. a,* as reported in other studies.[19,20]

In Europe and North America, the presence of such putative periodontal pathogens is increasingly used as a periodontal diagnostic test. As this study has indicated, in Arab and African groups, the presence of these bacteria appears to have a different clinical value.

Conclusions

This pilot qualitative study indicates a very high frequency of these three putative periodontal pathogens (*A. a, P. g, and P. i*) in a group from different Arab countries with minimal periodontal disease. The lack of adequate oral hygiene, the minimal use of antimicrobial drugs, antiseptics, and the absence of dental care may explain a part of the high prevalence of periodontal pathogens.

From the literature available, it is clear that there is a critical underrepresentation of data from the MENA region. Consequently, given this region's significance in terms of its sizable population and vast geography and the perceived high rate of susceptibility to the prevalence of periodontal pathogens, there is a need for further epidemiological and microbiological studies from this region.

Conflicts of interest
There are no conflicts of interest.

References

1. Feng X, Zhang L, Xu L, Meng H, Lu R, Chen Z, *et al.* Detection of eight periodontal microorganisms and distribution of *Porphyromonas gingivalis* fimA genotypes in Chinese patients with aggressive periodontitis. J Periodontol 2014;85:150-9.
2. Dahlén G, Manji F, Baelum V, Fejerskov O. Black-pigmented *Bacteroides* species and *Actinobacillus actinomycetemcomitans* in subgingival plaque of adult Kenyans. J Clin Periodontol 1989;16:305-10.
3. McNabb H, Mombelli A, Gmür R, Mathey-Dinç S, Lang NP. Periodontal pathogens in the shallow pockets of immigrants from developing countries. Oral Microbiol Immunol 1992;7:267-72.
4. Dahlén G, Manji F, Baelum V, Fejerskov O. Putative periodontopathogens in "diseased" and "non-diseased" persons exhibiting poor oral hygiene. J Clin Periodontol 1992;19:35-42.
5. Haffajee AD, Cugini MA, Tanner A, Pollack RP, Smith C, Kent RL Jr., *et al.* Subgingival microbiota in healthy, well-maintained elder and periodontitis subjects. J Clin Periodontol 1998;25:346-53.
6. Ximénez-Fyvie LA, Haffajee AD, Socransky SS. Comparison of

the microbiota of supra- and subgingival plaque in health and periodontitis. J Clin Periodontol 2000;27:648-57.

7. Herrera D, Contreras A, Gamonal J, Oteo A, Jaramillo A, Silva N, *et al.* Subgingival microbial profiles in chronic periodontitis patients from Chile, Colombia and Spain. J Clin Periodontol 2008;35:106-13.

8. Al Yahfoufi Z, Mombelli A, Wicki A, Lang NP. The effect of plaque control in subjects with shallow pockets and high prevalence of periodontal pathogens. J Clinical Periodontology 1995;22;78-84.

9. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121-35.

10. French CK, Savitt ED, Simon SL, Eklund SM, Chen MC, Klotz LC, *et al.* DNA probe detection of periodontal pathogens. Oral Microbiol Immunol 1986;1:58-62.

11. Kalam MA. Periodontal disease: Its risk factors among Bangladeshi populations. In: Bartold PM, editor. Risk Factors in Asian Pacific Populations. Brisbane: Asian Pacific Society of Periodontology; 1996. p. 16-21.

12. Albandar JM. Prevalence of incipient radiographic periodontal lesions in relation to ethnic background and dental care provisions in young adults. J Clin Periodontol 1989;16:625-9.

13. Taani Q. The periodontal status of Jordanian adolescents measured by CPITN. Int Dent J 1995;45:382-5.

14. Mombelli A, Gmür R, Frey J, Meyer J, Zee KY, Tam JO, *et al. Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in young Chinese adults. Oral Microbiol Immunol 1998;13:231-7.

15. Mombelli A, McNabb H, Lang NP. Black-pigmenting Gram-negative bacteria in periodontal disease. II. Screening strategies for detection of *P. gingivalis*. J Periodontal Res 1991;26:308-13.

16. Gaetti-Jardim E Jr., Pereira MF, Vieira EM, Schweitzer CM, Okamoto AC, Ávila-Campos MJ. Occurrence of periodontal pathogens in ethnic groups from a native Brazilian reservation. Arch Oral Biol 2015;60:959-65.

17. Kigure T, Saito A, Seida K, Yamada S, Ishihara K, Okuda K. Distribution of *Porphyromonas gingivalis* and *Treponema denticola* in human subgingival plaque at different periodontal pocket depths examined by immunohistochemical methods. J Periodontal Res 1995;30:332-41.

18. Ali RW, Lie T, Skaug N. Early effects of periodontal therapy on the detection frequency of four putative periodontal pathogens in adults. J Periodontol 1992;63:540-7.

19. Mombelli A, McNabb H, Lang NP. Black-pigmenting Gram-negative bacteria in periodontal disease. I. Topographic distribution in the human dentition. J Periodontal Res 1991;26:301-7.

20. Socransky SS, Haffajee AD, Dzink JL, Hillman JD. Association between microbial species in subgingival plaque samples. Oral Microbiol Immunol 1988;3:1-7.