Journal of Oral Science, Vol. 46, No. 3, 157-161, 2004

Original

Rate of cultivable subgingival periodontopathogenic bacteria in chronic periodontitis

Mohammad Hossein Salari[§] and Zainab Kadkhoda[†]

[§]Department of Pathobiology, School of Public Health and Institute of Public Health Research and
[†]Department of Periodontology, School of Dentistry,
Tehran University of Medical Sciences, Tehran, Iran

(Received 14 November 2003 and accepted 7 July 2004)

Abstract: Although microbiological studies have identified more than 400 bacterial species in periodontal pockets, only a limited number have been implicated as periodontal pathogens. The purpose of this study was to investigate the incidence of cultivable subgingival periodontopathogenic bacteria in chronic periodontitis. Bacterial samples were collected with sterile paper points from the deepest periodontal pockets ((5 mm) of 203 patients: 92 males and 111 females, aged 35 - 55 years. The samples were cultured under anaerobic and capnophilic conditions using selective and non-selective media. Isolates were characterized to species level by conventional biochemical tests and a commercial rapid test system. The isolates were *Actinobacillus actinomycetemcomitans* (26.8%), *Porphyromonas gingivalis* (21.9%), *Capnocytophaga sputigena* (16.7%), *Eikenella corrodens* (13.2%), *Prevotella intermedia* (10.5%), *Prevotella disiens* (3.1%), *Peptostreptococcus micros* (2.9%), *Capnocytophaga gingivalis* (2.2%), *Prevotella corporis* (1.8%), *Peptostreptococcus magnus* (1.3%) and *Fusobacterium nucleatum* (0.4%). No periodontopathogenic bacterial growth was observed in 14 of the samples (6.2%). The number of samples associated with monobacterial growth and polybacterial growth were 74.9% and 18.2% respectively. It is concluded that the bacterial composition associated with a number of patients' samples is quite complex, and that some of cultivable anaerobic and capnophilic bacteria act as periodontal pathogens in chronic periodontitis. (J. Oral Sci. 46, 157-161, 2004)

Keywords: rate; cultivable; periodontopathogenic bacterium; chronic periodontitis.

Correspondence to Dr. M.H. Salari, Department of Pathobiology, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, 14155-6446. Tehran, Iran
Tel: +98-216402640
Fax: +98-216462267
E-mail: msalari2000@yahoo.com

Introduction

Chronic periodontitis may be defined as a mixed infection affecting individual or multiple sites within the oral cavity and leading to the loss of the supporting periodontal tissues. This disease is generally chronic in nature and can persist in the absence of treatment (1-4). The bacterial etiology of chronic periodontal disease is complex, with a variety of organisms responsible for the initiation and progression of disease. Although over 400 different bacterial species have been detected in the oral cavity, only a limited number have been implicated as periodontal pathogens. Many of these organisms may also be present in periodontally healthy individuals and can exist in communal harmony with the host (5).

The microorganisms of dental plaque have been shown to be capable of initiating the mechanisms of destruction of the periodontal tissues, while their effective control has been shown to be the most appropriate means of arresting the progression of periodontal disease. Certain groups of Gram-negative bacteria have been found consistently in periodontal lesions (6,7). Among them, *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Capnocytophaga* species and spirochetes have been associated with chronic or refractory periodontitis (8-10). However, some anaerobic Gram-positive microorganisms such as *Peptostreptococcus*

micros and certain *Eubacterium* species have only recently been implicated in destructive periodontal disease (10,11).

The purpose of this study was to investigate the incidence of cultivable subgingival periodontopathogenic bacteria in chronic periodontitis.

Materials and Methods

The study is based on data obtained from a series of subgingival microbial samples collected at the Department of Periodontology, School of Dentistry, Tehran University of Medical Sciences over a 2-year period. A total of 203 patients underwent microbiological examination: 92 males and 111 females, aged 35 - 55 years, who had been referred to the Department of Periodontology for diagnosis and treatment of periodontitis. Prior to the clinical examination, subgingival microbial samples were collected from the deepest pockets ((5 mm). Patients then underwent treatment and follow-up until recovery and resolution of their pockets occurred. The sampling area was isolated with cotton rolls, carefully cleaned with sterile cotton pellets, and then air-dried. For single sites, two sterile paper points (Antaeos, Munich, Germany) were inserted to the bottom of the pocket for a 20-s period and then transferred into a reduced transport fluid medium with 25% glucose (12). Being a liquid, this transport medium offered the advantage of diluting the microorganisms, while at the same time maintaining the viability of the wide variety of capnophile and obligate anaerobes. In addition, this medium allowed the samples to be frozen for further examination. For pooled samples, at least one paper point per site from up to four sites was collected. The samples were processed within 24 h. For isolation of anaerobes, the samples were plated on non-selective Brucella agar plates (Becton Dickinson, Heidelberg, Germany) enriched with 0.5% hemolysed blood and 5 mg/L menadione. Kanamycin-vancomycin laked blood (KVLB, Becton Dickinson, Heidelberg, Germany) agar plates were used for selective recovery of obligately anaerobic Gram-negative rods. Columbia agar with 5% sheep blood and standard chocolate agar were used for the cultivation of capnophilic microorganisms. Trypticase soy agar plates supplemented with horse serum, bacitracin and vancomycin (TSBV) were used for selective recovery of *Actinobacillus actinomycetemcomitans* (13).

Brucella agar plates and KVLB agar plates were incubated for 7 days at 36(C under anaerobic conditions (BBL GasPak System, Becton Dickinson Microbiology Systems, Cockeysville, MD, USA). The TSBV, Columbia agar and chocolate agar plates were incubated at 36(C under capnophilic conditions (BBL Campy Pak Plus, Becton Dickinson Microbiology System, Cockeysville, MD, USA). After 7 days of incubation, colonies with differing characteristics were subjected to various tests. One to three colonies of each selected type were isolated and purified for further identification based on cell morphology, Gram stain reaction, and biochemical tests and enzymatic activities including catalase, oxidase, indole hydrolysis, esculin hydrolysis, gelatin hydrolysis, urea hydrolysis, and fermentation of glucose and lactose (BD Crystal ANR ID Kit, Becton Dickinson, Heidelberg, Germany).

Gram-positive anaerobic cocci formed chains and star-shaped colonies adhering strongly to TSBV agar, being catalase-, galactose-, maltose- and xylose-positive (9), and were identified as peptostreptococci and *Actinobacillus actinomycetemcomitans* respectively.

Results

The incidence of isolated bacteria according to sex and age of patients is shown in Tables 1 and 2. Anaerobic and capnophilic gram-negative rods were the most frequently detected bacteria in the samples. The incidence of isolated bacteria was *Actinobacillus actinomycetemcomitans* (26.8%), *Porphyromonas gingivalis* (21.9%), *Capnocytophaga sputigena* (16.7%), *Eikenella corrodens* (13.2%), *Prevotella intermedia* (10.5%), *Prevotella disiens* (3.1%), *Capnocytophaga gingivalis* (2.2%), *Peptostreptococcus micros* (2.9%), *Prevotella corporis* (1.8%), *Peptostreptococcus magnus* (1.3%), and *Fusobacterium nucleatum* (0.4%). The number of samples associated with monobacterial growth (74.9%) and polybacterial growth (18.2%) are shown in Table 3. Periodontopathogenic bacterial growth was not observed in 14 of the samples (6.9%) (Table 3). The results of statistical analysis using the chi square test show no significant differences between sex and age group of patients. There is a significant difference in the incidence of isolated *Eikenella corrodens* according to the age group of patients ($df = 1, \chi^2 = 5.7, P < 0.02$) (Table 2). Our findings also showed a high prevalence of anaerobic bacteria in the patients with the deepest pockets.

Discussion

Results from this evaluation confirm the complexity of the bacterial composition associated with a number of patients' samples. Our findings showed that *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Capnocytophaga sputigena*, and *Eikenella corrodens* occurred in the highest proportions in the patients. These results are in accordance with the studies by Murray et al. and Riggio et al.(9,14). In Iran, the incidence of these bacteria in periodontally healthy subjects has been reported to be 4.1% *Actinobacillus actinomycetemcomitans*, 10.3%

Table 1 The incidence of isolated capnophilic and anaerobic bacteria according to sex

Bacterium	Sex						χ^2	P Value
	Male (n = 98)		Female (n = 130)		Total (n = 228)			
	n	%	n	%	n	%		
Capnophiles:								
Actinobacillus actinomycetemcomitans	25	25.5	36	27.7	61	26.8	0.13	< 0.80
Eikenella corrodens	17	17.3	13	10	30	13.2	2.63	< 0.20
Capnocytophaga sputigena	13	13.3	25	19.2	38	16.7	1.43	< 0.30
Capnocytophaga gingivalis	2	2	3	2.3	5	2.2	0.08	< 0.80
Anaerobes:								
Porphyromonas gingivalis	24	24.5	26	20	50	21.9	0.65	< 0.50
Prevotella corporis	2	2	2	1.5	4	1.8	0.08	< 0.80
Prevotella intermedia	7	7.1	17	13	24	10.5	2.08	< 0.20
Prevotella disiens	3	3.1	4	3.1	7	3.1	0.00	> 0.99
Peptostreptococcus magnus	2	2	1	0.8	3	1.3	1.00	< 0.50
Peptostreptococcus micros	3	3.1	2	1.5	5	2.9	0.61	< 0.50
Fusobacterium nucleatum	_	_	1	0.8	1	0.4	0.75	< 0.40

Table 2 The incidence of isolated capnophilic and anaerobic bacteria according to age

Bacterium	Age (years)						χ^2	*P* Value
	35 - 45 (n = 123)		46 - 55 (n = 105)		Total (n = 228)			
	n	%	n	%	n	%		
Capnophiles:								
Actinobacillus actinomycetemcomitans	29	23.6	32	30.4	61	26.8	1.35	< 0.30
Eikenella corrodens	22	17.9	8	7.9	30	13.2	5.17	< 0.02
Capnocytophaga sputigena	15	12.2	23	21.9	38	16.7	3.82	< 0.10
Capnocytophaga gingivalis	3	2.4	2	1.9	5	2.2	0.07	< 0.80
Anaerobes:								
Porphyromonas gingivalis	27	22	23	21.9	50	21.9	0.00	> 0.99
Prevotella corporis	2	1.6	2	1.9	4	1.8	0.04	< 0.90
Prevotella intermedia	15	12.2	9	8.6	24	10.5	0.04	< 0.90
Prevotella disiens	4	3.3	3	2.9	7	3.1	0.02	< 0.90
Peptostreptococcus magnus	1	0.8	2	1.9	3	1.3	0.11	< 0.80
Peptostreptococcus micros	4	3.3	1	0.9	5	2.9	1.39	< 0.30
Fusobacterium nucleatum	1	0.8	_	_	1	0.4	0.85	< 0.40

Eikenella corrodens, 15.9% *Capnocytophaga species*, 11% *Porphyromonas gingivalis*, 7.6% *Prevotella intermedia*, 3.4% *Prevotella melaninogenica*, 3% *Peptostreptococcus magnus* and 5% *Peptostreptococcus micros* (15). On the basis of our findings and the above-mentioned study, the incidence of these bacteria, especially *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* was higher in patients with chronic periodontitis than in healthy subjects.

Porphyromonas gingivalis is one of the most strongly active organisms associated with chronic periodontitis and also seems to play a role in the pathogenesis of other forms of periodontitis (16). Other studies have found a higher prevalence of this microorganism, suggesting that the differences reported are due to the differing methods applied (17). There is also extensive evidence associating *Actinobacillus actinomycetemcomitans* with chronic periodontitis (9).

Mandell repoted that *Eikenella corrodens* and *Actinobacillus actinomycetemcomitans* have been found together in some lesions of periodontitis (18). Prevotella species and *Capnocytophaga* species were also identified in some patients' samples. This was in accordance with a study (19) in which *Prevotella intermedia* was shown to be the most prevalent species in a group of young adults suffering from periodontitis. von Troil-Linden et al. (20) found that *Prevotella intermedia* was significantly elevated in saliva samples from subjects with periodontitis compared to samples from subjects with initial or no periodontitis. Murray named only *Capnocytophaga sputigena* as a prominent bacteria in subgingival samples from patients

Table 3 The number of samples associated with monobacterial growth and polybacterial growth

Bacterium Genus Species	Sample (n = 203)		Isolated bacteria (n = 228)	
	n	%	n	%
Monobacteria (n = 152, 74.9%)				
Actinobacillus actinomycetemcomitans	36	17.7	36	15.7
Capnocytophaga sputigena	20	9.9	20	8.8
Eikenella corrodens	18	8.9	18	7.9
Capnocytophaga gingivalis	5	2.5	5	2.2
Porphyromonas gingivalis	36	17.7	36	15.7
Prevotella intermedia	21	10.3	21	9.2
Prevotella disiens	5	2.5	5	2.2
Prevotella corporis	4	2	4	1.8
Peptostreptococcus magnus	3	1.5	3	1.3
Peptostreptococcus micros	3	1.5	3	1.3
Fusobacterium nucleatum	1	0.5	1	0.4
Polybacteria (n = 37, 18.2%)				
Actinobacillus actinomycetemcomitans *Eikenella corrodens*	2	1	4	1.8
Actinobacillus actinomycetemcomitans *Capnocytophaga sputigena*	12	5.9	24	10.5
Actinobacillus actinomycetemcomitans *Porphyromonas gingivalis*	8	3.9	16	7
Actinobacillus actinomycetemcomitans *Prevotella intermedia*	3	1.5	6	2.6
Eikenella corrodens *Capnocytophaga sputigena*	6	3	12	5.3
Eikenella corrodens *Porphyromonas gingivalis*	4	2	8	3.5
Porphyromonas gingivalis *Peptostreptococcus micros* *Prevotella disiens*	2	1	6	2.6
No growth (n = 14, 6.9%)	14	6.9%	_	_

with chronic periodontitis (9). *Peptostreptococcus micros* is considered to be a pathogen in the etiology of mixed anaerobic infections, including periodontitis (7,21). It is isolated more often and in increased percentages from patients with periodontitis, especially in disease-active subjects (22,23). Our results are in accordance with previous observations (24) suggesting that *Peptostreptococcus species* may be associated with periodontitis.

The data from our investigation suggest that there is heterogeneity in the subgingival periodontopathogenic bacteria among subjects. However, as many bacteria in the oral cavity cannot be cultured, it is likely that these still uncharacterized bacteria could play a role in the initiation and progression of periodontal disease.

Acknowledgments

The authors wish to thank Dr. K. Ghazi Saeedi, R. Hafezi, N. Iranparast and F. Falah for their kind assistance in this study.

References

1. Armitage GC (1999) Development of a classification of system for periodontal diseases and conditions. Ann Periodontol 4, 1-6
2. Page RC, Schroeder HE (1976) Pathogenesis of inflammatory periodontal disease. A summary of current work. J Lab Invest 34, 235-249
3. Page RC (1991) The role of inflammatory mediators in the pathogenesis of periodontal disease. J Periodontal Res 26, 230-242
4. Ranney RR (1991) Immunologic mechanisms of pathogenesis in periodontal disease: an assessment. J Periodontal Res 26, 243-254
5. Moore WEC, Moore LVH (1994) The bacteria of periodontal disease. Periodontol 2000 5, 66-77
6. Genco RJ, Zambon JJ, Christersson LA (1988) The origin of periodontal infections. Adv Dent Res 2, 245-259
7. Haffajee AD, Socransky SS (1994) Microbial etiological agents of destructive periodontal diseases. Periodontol 2000 5, 78-111
8. Dzink JL, Socransky SS, Haffajee AD (1988) The predominant cultivable microbiota of active and inactive lesions of destructive periodontal disease. J Clin Periodontol 15, 316-323
9. Mutter R (1999) Actinobacillus, Capnocytophaga, Eikenella, Kingella, and other fastidious or rarely encountered gram-negative rods. In Manual of clinical microbiology. 7th ed, Murray PR ed, ASM Press, Washington DC, 561-571
10. Slots J (1977) Microflora in the healthy gingival sulcus in man. Scand J Dent Res 85, 247-254
11. Uematsu H, Hoshino E (1992) Predominant obligate anaerobes in human periodontal pockets. J Periodontal Res 27, 15-19
12. Syed SA, Loeshe WJ (1972) Survival of human dental plaque flora in various transport media. Appl Microbiol 24, 638-644
13. Slots J (1982) Selective medium for isolation of *Actinobacillus actinomycetemcomitans*. J Clin Microbiol 15, 606-609
14. Riggio MP, Macfarlane TW, Mackenzie D, Lennon A, Smith AJ, kinane D (1996) Comparison of polymerase chain reaction and culture methods for detection of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in subgingival plaque samples. J Periodontal Res 31, 496-501
15. Salari MH, Oulia P, Khoshreza M, Kadkhoda Z (1998) Investigation of periodontitis patients, bacteria and comparison with control group. Iranian J Publ Health 27, 63-72
16. van Winkelhoff AJ, Van Steenbergen TJ, de Graaff J (1988) The role of black-pigmented Bacteroides in human oral infections. J Clin Periodontol 15, 145-155
17. Griffen AL, Becker MR, Lyons SR, Moeschberger ML, Leys EJ (1998) Prevalence of *Porphyromonas gingivalis* and periodontal health status. J Clin Microbiol 36, 3239 -3242
18. Mandell RL (1984) A longitudinal microbiological investigation of *Actinobacillus actinomycetemcomitans* and *Eikenella corrodens* in juvenile periodontitis. Infect Immun. 45, 778-780
19. van Steenbergen TJ, van der Velden U, Abbas F, de Graaff J (1991) Microflora and bacterial DNA restriction enzyme analysis in young adults with periodontitis. J Periodontol 62, 235-241
20. von Troil-Linden B, Saarela M, Matto J, Alaluusua S, Jousimies-Somer H, Asikainen S (1996) Source of suspected periodontal pathogens re-emerging after periodontal treatment. J. Clin Periodontol 23, 601-607
21. Murdoch DA, Mitchelmore IJ, Tabaqchali S (1988) Isolation of *peptostreptococcus micros* from polymicrobial abscesses. Lancet 1, 594
22. Moore WEC, Moore LVH, Ranney RR, Smibert RM, Burmeister JA, Schenkein HA (1991) The microflora of periodontal sites showing active destructive progression. J Clin Periodontol 18, 729-739
23. Rams TE, Feik D, Listgarten MA, Slots J (1992) *Peptostreptococcus micros* in human periodontitis.

Oral Microbiol Immunol. 7, 1-6
24. Tanner A, Kent R, Maiden MF, Taubman MA (1996) Clinical, microbiological and immunological profile of healthy, gingivitis and putative active periodontal subjects. J Periodontal Res 31, 195-204