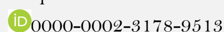
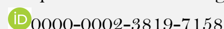
Pesquisa Brasileira em Odontopediatria e Clínica Integrada 2019, 19:e4679
DOI: http://doi.org/10.4034/PBOCI.2019.191.73
ISSN 1519-0501

ORIGINAL ARTICLE

Prevalence and Molecular Analysis of Novel Newly Identified Periodontal Pathogens in Subgingival Plaque Samples of Saudi Patients with Chronic Periodontitis

Kiran Kumar Ganji[1], Bilal Ahmad Tantry[2]

[1]Department of Preventive Dentistry, College of Dentistry, Jouf University, Al Jouf, Kingdom of Saudi Arabia.
0000-0002-3178-9513
[2]Department of Pathology, Sub Division Microbiology, College of Medicine, Jouf University, Al Jouf, Kingdom of Saudi Arabia.
0000-0002-3819-7158

Author to whom correspondence should be addressed: Dr. Kiran Kumar Ganji, H.no. 9271, Faissalyia, Sakaka, 72721, Al Jouf Province, Kingdom of Saudi Arabia. Phone: +96 6540640338. E-mail: kiranperio@gmail.com.

Academic Editors: Alessandro Leite Cavalcanti and Wilton Wilney Nascimento Padilha

Received: 11 February 2019 / Accepted: 05 April 2019 / Published: 11 April 2019

Abstract

Objective: To evaluate the prevalence of novel newly identified periodontal pathogenic strains in subgingival plaque samples and relate it with bleeding on probing, probing pocket depth and age. **Material and Methods:** 268 chronic periodontitis patients with a mean age of 46.0 ± 6.0 years were included. The following microorganisms were evaluated: Campylobacter gracilis (Cg), Fusobacterium nucleatum (Fn), Porphyromonas gingivalis (Pg), and Tannerella forsythia (Tf). Full mouth examination was registered; the probing pocket depth and clinical attachment level were assessed at six sites per tooth. Dental subgingival plaque samples were taken in the deepest pocket per arch in the maxilla and mandible. DNA analysis was performed using DNA-strip technology. Pearson's correlation coefficient was used for statistical analysis. **Results:** Porphyromonas gingivalis and Tannerella forsythia were detected at high level of 80% and 82% respectively. F nucleatum revealed a rate of 94%. Bacterial load significantly increased by increasing mean probing depth of the examined sites. Pearson's coefficient was the highest for Pg (r=0.62, p=0.0001) and the lowest for Cg (r=0.08, p=0.04). The bacterial load significantly increased by increasing the number of bleeding sites; Pearson's coefficient varied from r= 0.14 for Pg (p=0.01) to r=0.39 for Tf (p=0.001). **Conclusion:** It was confirmed the presence of main putative periodontal pathogens detectable in Saudi periodontal subjects, also providing a comprehensive view for correlation of these putative periodontal pathogens with the increase in probing pocket depth to the presence and load of all the bacterial species.

Keywords: Chronic Periodontitis; Periodontal Pocket; Porphyromonas gingivalis.

Association of Support to Oral Health Research - APESB

Introduction

Periodontitis is initiated by microorganisms that grow on subgingival tooth surfaces, modified with a host immune response. Many clinical studies have been conducted worldwide providing evidence of associations between bacterial species and chronic periodontitis [1]. There is moderate evidence in the literature to substantiate and support the association of 17 newly identified species or phylotypes from the phyla Bacteroidetes, Candidatus, Saccharibacteria, Firmicutes, Proteobacteria, Spirochaetes and Synergistetes. The phylum Candidiatus Saccharibacteria and the Archaea domain also seem to have an association with disease [1]. These archives point out the need for the importance of previously unidentified species in the etiology of periodontitis and might guide future investigations on the actual role of these suspected new periodontal pathogens in the onset and progression of periodontal disease.

A recent molecular survey performed via pyrosequencing of 16S rRNA gene amplicons provided a much broader picture of overall differences in relative abundances of B700 subgingival species-level taxa, confirming the association of Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia with periodontitis, and revealing new species, among them Filifactoralocis, strongly associated with disease [2].

Recently studies using several molecular approaches of periodontal pathogens including next generation sequencing techniques were published in the periodontal literature [2-6]. The global data provided by these studies for more than a decade suggested the existence of new periodontal pathogens. Associating data from different countries/population has become apparent that there are substantial differences in the composition of the subgingival microbiota [7-10]. Owing to this, the study of the subgingival microbiota in a particular country befits relevant information not only to understand its implication in the pathogenesis of periodontal disease but also to identify its possible impact on treatment outcomes.

Therefore, the aim of the present study was to evaluate the prevalence of novel newly identified periodontal pathogenic strains such as Campylobacter gracilis (Cg), Fusobacterium nucleatum (Fn), Porphyromonas gingivalis (Pg), and Tannerella forsythia (Tf) in subgingival plaque samples and also to relate the novel newly identified strains with severity of chronic periodontitis.

Material and Methods

Sample Collection

The samples were collected from oral cavity of 268 Saudi patients attending the OPD of dental clinics of Jouf University. Registered patients were selected based on inclusion criteria: diagnosis of chronic periodontal disease, presence of at least 16 teeth, no history of periodontal therapy in last 6 months, systemically health patients and no history of antibiotics and anti-inflammatory therapy.

Five periodontal involved teeth with periodontal probing depth (PPD) >4mm were selected and at least one element for each quadrant was examined. Before sampling the supragingival plaque

CC BY

was carefully removed using a gracey curette after isolation with cotton roll and air dry. For each site, a sterile paper cone was inserted into the bottom of the pocket and removed after 10 seconds. For each patient all paper cones were pooled in a sterile empty test tube and then transferred immediately to the microbiology laboratory for processing, culture and identification.

Isolation of Periodontal Pathogens

The periodontal pathogens were isolated using the brain heart infusion, blood agar and tryptic soy agar. A liquot of 100 µl of each sample was spread on culture. Samples were analyzed in triplicates. The plates were inverted and incubated for 48 hours at 37°C. Results were recorded as colony forming units (CFU). Morphologically different colonies were selected and purified for further investigations.

Phenotypic and Biochemical Characterization

The phenotypic characteristics of all isolates were determined and compared to phenotypic data of known organisms described in the Bergey's Manual of Systematic Bacteriology [11-14]. Colony morphology of bacterial isolates was evaluated from first picked colonies from the original plate.

Morphological Characteristics

Gram stain reaction, cell morphology and motility of bacterial isolates were examined as previously described [15]. The motility of the isolates was observed by hanging drop method.

Methyl Red Test

100 µl 18 hours old culture of each isolate was inoculated in the MR-VP medium. The incubated at 35°C for 48 hours.

Indole Formation

To tryptone broth 100 µl of 18 hours old culture was added. The tubes were incubated at 35°C for 48 hours. 1.0 ml of Kovács' reagent solution was added. The positive result was indicated by a bright red color within seconds after adding reagent.

Acid from carbohydrate utilization: Five different sugars including D-galactose, glucose, lactose, maltose and sucrose were used to test for acid production from carbohydrate utilization. For preparation of inoculum, pure culture of each bacterial isolate to be tested was grown in nutrient broth and incubated at 37°C for 48 hours. Acid production was indicated by the change in the color of the broth from red to yellow.

Hydrogen sulfide production: Each bacterium to be tested was stabbed deep into the lead acetate agar slant and also streaked on the surface of the slant, then incubated at 35°C for 48 hours

CC BY

until bacterial growth was observed. Brownish color formed on the surface and along the line of the stab indicated production of hydrogen sulfide.

In brief this method was used to determine the total bacterial load present in the sample and quantifies four major periodontal pathogens with a sensitivity of 100 cells per type of pathogen: Campylobacter gracilis (Cg), Fusobacterium nucleatum (Fn), Porphyromonas gingivalis (Pg), and Tannerella forsythia (Tf).

Statistical Analysis

Prevalence and standard errors of the rates were tabulated. Pearson's correlation coefficient was used to evaluate the correlation between the bacterial cell count and the deepest value of probing depth, number of sites with bleeding on probing. Each patient was identified by the five PPDs median value; the medians of each patient were synthesized using arithmetic mean. Multinomial logistic regression (method backward) was used to explain which of the following variables (age, gender, PPD, number of sites with BOP), was putatively associated with the presence of the microbial species.

Ethical Aspects

Ethical clearance was obtained from Local Committee of Bioethics, Jouf University wide reference number 5-22-2/40.

Results

Table 1 presents the demographic and clinical presentations of examined samples. Four periodontal pathogens were detected and at least one was found in each patient: Fn and Cg were the most and the least prevalent microorganisms, respectively (Table 2).

With regard to the total bacterial load Fn, Pg and Tf were the most abundant representing 80% of the total bacterial load of oral cavity and 66% of the total bacteria load of the 4 studied bacteria. Similar percentages were recorded for Pg (37%), Fn (24%) and Tf (19%). Bacterial load significantly increased by increasing mean probing depth of the examined sites. Pearson's coefficient was the highest for Pg ($r=0.62$; $p=0.0001$) and the lowest for Cg ($r=0.08$; $p=0.04$). The bacterial load significantly increased by increasing the number of bleeding sites; Pearson's coefficient varied from $r=0.14$ for Pg ($p=0.01$) to $r=0.39$ for Tf ($p=0.001$). Table 3 presents the results of the regression analysis for the clinical findings.

Table 1. Demographic characteristics and clinical features.

Variables	Mean	SD
Age	43.0	±1.45
Probing Pocket Depth (mm)	6.0	± 1.0
Clinical Attachment Loss (mm)	8.0	± 2.0
% Sites with Bleeding on Probing	28.0	± 0.05

SD = Standard Deviation.

cc BY

Table 2. Prevalence of Cg, Fn, Pg and Tf.

Microorganisms	Frequency of Detection		Bacterial Load
	N (%)	Confidence Interval (95%)	10^5 cells
Cg	45 (16.5)	19-23	0.5
Fn	254 (94.0)	95-97	9.25
Pg	214 (80.0)	75-79	8.25
Tf	221 (82.0)	87-91	11.45

Table 3. Regression analysis of Cg, Fn, Pg and Tf in relation to clinical findings.

Microorganisms	Probing Pocket Depth (mm)		Number of Sites with Bleeding on Probing		Age	
	OR	CI (95%)	OR	CI (95%)	OR	CI (95%)
Cg	1.45	1.25 (1.35)	1.25	1.15 (1.68)	1.25	1.28 (1.42)
Fn	1.85	1.35 (1.75)	1.65	1.45 (1.14)	1.12	1.29 (1.68)
Pg	1.68	1.27 (1.45)	1.25	1.16 (1.93)	1.20	1.94 (1.47)
Tf	1.24	1.12 (1.52)	1.95	1.15 (1.60)	1.01	1.05 (1.18)

CI = Confidence Interval.

Discussion

Periodontitis is caused by ecological disturbances in subgingival communities [16-18]. Recent studies have compared dental plaque microbiota in individuals from different geographic locations, which have shown differences in microbial composition [7,8,10]. Proceedings from these studies were related to European countries, South America and recently in Asiatic populations. Findings were related to the different number of sample sites examined in each subject and the microbial technique employed. Therefore these two factors can strongly impact on the proportion of subjects positive for specific bacterial species.

Hence this study was conducted to evaluate the prevalence of novel newly identified periodontal pathogens in plaque samples of Saudi population to relate it with geographic regions of Arabian Peninsula. The findings from the current study contribute to determine the epidemiological distribution of four main periodontal pathogens (Cg, Fn, Pg, Tn) detected by PCR in chronic periodontitis patients. This study revealed a broad range of taxa present disease providing a global-scale view of subgingival microbial communities.

The prevalence of Fn was assessed in 94% of patients, which was in accordance with the reported prevalence rates of 80%-100% [9,19,20]. Recent studies have shown that Fn is one of the furthermost abundant gram-negative anaerobes in mature supragingival and subgingival plaques of both healthy subjects and patients with periodontitis [21,22]. The less prevalent microorganism was Cg, affecting only 39 patients with chronic periodontal disease (16.5%). These results are in agreement with previous studies conducted with Brazilian [23], Chilean [24] and German patients [25].

Findings from the present study also reveal that there was high prevalence of Tf (82%) in the assessed plaque samples. This finding was in accordance to studies done in Japanese population [26]. Tf has been assessed in other studies with high prevalence rate more than 90% [27-30].

With regards to clinical variables correlated to Pg, the present findings are also confirmed by the results of previous authors who reported only a significant positive correlation between the number of deep sites (pocket depth >4 mm) and the prevalence of Pg positive sites [31]. Pg is associated with disease progression and the proportion of this species was found to be increased in deeper pockets [32,33]. Tf was strongly associated with number of bleeding sites thereby supporting previous data and its load statistically correlated with the numbers of sites and suppuration [7].

Conclusion

It was confirmed the presence of main putative periodontal pathogens detectable in Saudi periodontal subjects, also providing a comprehensive view for correlation of these putative periodontal pathogens with the increase in probing pocket depth to the presence and load of all the bacterial species. To obtain more geographic data on prevalence of these periodontal pathogens similar investigations in other countries are needed to disclose any microbiological differences among populations, leading to more specific approaches in prevention and therapy.

Financial Support: This research was funded by Jouf University (Grant no. 39/400), Al Jouf, Kingdom of Saudi Arabia.

Conflict of Interest: The authors declare no conflicts of interest.

References

[1] Pérez-Chaparro P, Goncalves C, Figueiredo L, Faveri M, Lobão E, Tamashiro N, et al. Newly identified pathogens associated with periodontitis: A systematic review. J Dent Res 2014; 93(9):846-58. https://doi.org/10.1177/0022034514542468

[2] Griffen AL, Beall CJ, Campbell JH, Firestone ND, Kumar PS, Yang ZK, et al. Distinct and complex bacterial profiles in human periodontitis and health revealed by 16S pyrosequencing. ISME J 2012; 6(6):1176-85. https://doi.org/10.1038/ismej.2011.191

[3] Shchipkova AY, Nagaraja HN, Kumar PS. Subgingival microbial profiles of smokers with periodontitis. J Dent Res 2010; 89(11):1247-53. https://doi.org/10.1177/0022034510377203

[4] Matarazzo F, Ribeiro AC, Feres M, Faveri M, Mayer MP. Diversity and quantitative analysis of Archaea in aggressive periodontitis and periodontally healthy subjects. J Clin Periodontol 2011; 38(7):621-7. https://doi.org/10.1111/j.1600-051X.2011.01734.x

[5] Uzel NG, Teles FR, Teles RP, Song XQ, Torresyap G, Socransky SS, et al. Microbial shifts during dental biofilm re-development in the absence of oral hygiene in periodontal health and disease. J Clin Periodontol 2011; 38(7):612-20. https://doi.org/10.1111/j.1600-051X.2011.01730.x

[6] Abusleme L, Dupuy AK, Dutzan N, Silva N, Burleson JA, Strausbaugh LD, et al. The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. ISME J 2013; 7(5):1016-25. https://doi.org/10.1038/ismej.2012.174

[7] Haffajee A, Bogren A, Hasturk H, Feres M, Lopez N, Socransky S. Subgingival microbiota of chronic periodontitis subjects from different geographic locations. J Clin Periodontol 2004; 31(11):996-1002. https://doi.org/10.1111/j.1600-051X.2004.00597.x

[8] Papapanou PN, Teanpaisan R, Obiechina NS, Pithpornchaiyakul W, Pongpaisal S, Pisuithanakan S, et al. Periodontal microbiota and clinical periodontal status in a rural sample in southern Thailand. Eur J Oral Sci 2002; 110(5):345-52. https://doi.org/10.1034/j.1600-0722.2002.21361.x

[9] Sanz M, van Winkelhoff AJ, Herrera D, Dellemijn-Kippuw N, Simón R, Winkel E. Differences in the composition of the subgingival microbiota of two periodontitis populations of different geographical origin. A comparison between Spain and The Netherlands. Eur J Oral Sci 2000; 108(5):383-92. https://doi.org/10.1034/j.1600-0722.2000.108005383.x
[10] Yano-Higuchi K, Takamatsu N, He T, Umeda M, Ishikawa I. Prevalence of Bacteroides forsythus, Porphyromonas gingivalis and Actinobacillus actinomycetemcomitans in subgingival microflora of Japanese patients with adult and rapidly progressive periodontitis. J Clin Periodontol 2000; 27(8):597-602. https://doi.org/10.1034/j.1600-051x.2000.027008597.x
[11] Claus D, Berkeley R. Genus Bacillus Cohn. In: Sneath PHA, Mair NS, Sharpe ME, Holt JG. (Eds.). Bergey's Manual of Systematic Bacteriology. vol. 2: Baltimore: The Williams & Wilkins Co., 1986. pp. 1105-1139.
[12] Gerhardt P, Wood WA. Methods for General and Molecular Bacteriology: American Society for Microbiology, 1994. 791 pp.
[13] Barrow GI, Feltham RKA. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd. ed. Cambridge: Cambridge University Press, 1993.
[14] Barrow GI, Feltham RKA. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd. ed. Cambridge University Press, 2003. 331 pp.
[15] Cappuccino JG, Sherman N. Microbiology: A Laboratory Manual. 5th. ed. California: The Benjamin/Cummings Publishing Co., 1999. 477 pp.
[16] Marsh PD, Moter A, Devine DA. Dental plaque biofilms: Communities, conflict and control. Periodontol 2000 2011; 55(1):16-35. https://doi.org/10.1111/j.1600-0757.2009.00339.x
[17] Diaz PI. Microbial diversity and interactions in subgingival biofilm communities. Front Oral Biol 2012; 15:17-40. https://doi.org/10.1159/000329669
[18] Diaz PI, Dupuy AK, Abusleme L, Reese B, Obergfell C, Choquette L, et al. Using high throughput sequencing to explore the biodiversity in oral bacterial communities. Mol Oral Microbiol 2012; 27(3):182-201. https://doi.org/10.1111/j.2041-1014.2012.00642.x
[19] Ali RW, Velcescu C, Jivanescu MC, Lofthus B, Skaug N. Prevalence of 6 putative periodontal pathogens in subgingival plaque samples from Romanian adult periodontitis patients. J Clin Periodontol 1996; 23(2):133-9. https://doi.org/10.1111/j.1600-051X.1996.tb00546.x
[20] Han YW. Fusobacterium nucleatum: A commensal-turned pathogen. Curr Opin Microbiol 2015; 23:141-7. https://doi.org/10.1016/j.mib.2014.11.013
[21] Krishnan K, Chen T, Paster BJ. A practical guide to the oral microbiome and its relation to health and disease. Oral Dis 2017; 23(3):276-86. https://doi.org/10.1111/odi.12509
[22] Colombo A, Paster B, Grimaldi G, Lourenço T, Teva A, Campos-Neto A, et al. Clinical and microbiological parameters of naturally occurring periodontitis in the non-human primate Macaca mulatta. J Oral Microbiol 2017; 9(1):1403843. https://doi.org/10.1080/20002297.2017.1403843
[23] Cortelli JR, Cortelli SC, Jordan S, Haraszthy VI, Zambon JJ. Prevalence of periodontal pathogens in Brazilians with aggressive or chronic periodontitis. J Clin Periodontol 2005; 32(8):860-6. https://doi.org/10.1111/j.1600-051X.2005.00777.x
[24] Montanares H, Opazo F, Herrera L, Villanueva M-P, Medina G, Fernández H. Campylobacter species in chilean patients with chronic periodontitis. Biosci J 2018; 34(5):1455-62.
[25] Henne K, Fuchs F, Kruth S, Horz HP, Conrads G. Shifts in Campylobacter species abundance may reflect general microbial community shifts in periodontitis progression. J Oral Microbiol 2014; 6:25874. https://doi.org/10.3402/jom.v6.25874
[26] Tomita S, Komiya-Ito A, Imamura K, Kita D, Ota K, Takayama S, et al. Prevalence of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis and Tannerella forsythia in Japanese patients with generalized chronic and aggressive periodontitis. Microb Pathog 2013; 61:11-5. https://doi.org/10.1016/j.micpath.2013.04.006
[27] Ertugrul AS, Arslan U, Dursun R, Hakki SS. Periodontopathogen profile of healthy and oral lichen planus patients with gingivitis or periodontitis. Int J Oral Sci 2013; 5(2):92-7. https://doi.org/10.1038/ijos.2013.30
[28] 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(2):106-13. https://doi.org/10.1111/j.1600-051X.2007.01170.x

[29] Thiha K, Takeuchi Y, Umeda M, Huang Y, Ohnishi M, Ishikawa I. Identification of periodontopathic bacteria in gingival tissue of Japanese periodontitis patients. Oral Microbiol Immunol 2007; 22(3):201-7. https://doi.org/10.1111/j.1399-302X.2007.00354.x

[30] Albandar JM, Olsen I, Gjermo P. Associations between six DNA probe-detected periodontal bacteria and alveolar bone loss and other clinical signs of periodontitis. Acta Odontol Scand 1990; 48(6):415-23.

[31] Mombelli A, Casagni F, Madianos PN. Can presence or absence of periodontal pathogens distinguish between subjects with chronic and aggressive periodontitis? A systematic review. J Clin Periodontol 2002; 29(Suppl 3):10-21.

[32] Chen C, Wang T, Chen W. Occurrence of Aggregatibacter actinomycetemcomitans serotypes in subgingival plaque from United States subjects. Mol Oral Microbiol 2010; 25(3):207-14. https://doi.org/10.1111/j.2041-1014.2010.00567.x

[33] Faveri M, Figueiredo LC, Duarte PM, Mestnik MJ, Mayer MPA, Feres M. Microbiological profile of untreated subjects with localized aggressive periodontitis. J Clin Periodontol 2009; 36(9):739-49. https://doi.org/10.1111/j.1600-051X.2009.01449.x