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The impact of the presence of Porphyromonas gingivalis on periodontal health in a group of patients with periodontitis in Erbil

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Abstract

Background and objective: Chronic periodontitis is the destruction of the tooth supporting structures as a result of a complex interaction between bacteria colonizing the gingival crevice and host's immune responses. Porphyromonas gingivalis is one of the main periodontopathogens with multiple virulence factors. The aim of this study was to investigate the detection rate of Porphyromonas gingivalis in chronic periodontitis patients versus healthy subjects using PCR assay, and its association with increased pocket depth and clinical attachment loss.

Methods: Seventy subjects (35 patients with chronic periodontitis and 35 healthy subjects) meeting the inclusion criteria of this study were selected. All the subjects were clinically assessed for probing pocket depth and clinical attachment loss then subgingival microbial samples were collected using sterile paper points and analyzed for the presence of Porphyromonas gingivalis using polymerase chain reaction assay.

Results: A significant difference in Porphyromonas gingivalis detection rate between chronic periodontitis and healthy groups was recorded. Porphyromonas gingivalis was significantly associated with deep pockets. The detection rate increased with the increase in the severity of the disease, although, this correlation was not statistically significant.

Conclusion: A positive association was observed between Porphyromonas gingivalis and increased pocket depth. The recovery rate was higher in severe cases.

Keywords: CAL, chronic periodontitis, PCR, P. gingivalis, PPD.

Introduction

Periodontitis is a local infection and inflammation of the supporting structures of the tooth. The most common form is chronic periodontitis (CP) which is characterized by localized or generalized destruction of the periodontal ligament, alveolar bone and gingiva resulting in various degrees of periodontal attachment loss. In the absence of treatment, the disease can ultimately results in loss of the affected tooth.[1,2] Anaerobic, gram-negative bacteria present in subgingival biofilm are usually involved in the initiation and progression of the disease. One of these bacteria is Porphyromonas gingivalis (P. gingivalis) that is frequently detected from subgingival plaque samples of chronic periodontitis patients, whereas much lower detection rate is usually recorded in periodontally healthy individuals.[3-5] This bacterium was known to be a potent periodontopathogen because of a number of virulence factors possessed by the organism such as cysteine proteases, collagenases, heamagglutinins, lipopoly-saccharide (LPS), and fimbriae that facilitate local colonization and tissue destruction through direct cytotoxic effect of these products on the periodontium and, on the other hand, induction of a potent immune response that results in a further damage.[6,7] Diagnosis and initial treatment of periodontal diseases are usually based on certain clinical parameters such as clinical attachment loss (CAL) and probing pocket depth (PPD).[8] To complement the clinical diagnosis, monitor the response

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to therapy during the course of periodontal treatment, make decision for more advanced treatment procedures, previous studies have suggested the importance of performing the microbiological diagnosis besides the clinical diagnosis.[9,10] Bacteria that inhabit the periodontal region are usually anaerobic, slow-growing with complex nutritional requirements. Dependently, identification of these bacteria using conventional culture method is time-consuming and difficult to manage, in addition to the need for further laboratory procedures such as biochemical tests for the presumptive identification of the isolate. For the above reasons, investigators adopted DNA-based molecular methods for diagnostic purposes, especially for the identification of unculturable bacteria.[11,12] Polymerase chain reaction (PCR) is the most widely used molecular method for the in-vitro identification of microorganisms.[13] A number of comparative studies suggested that the use of PCR offers significant advantages with respect to the rapidity and sensitivity of detection of even low number of P. gingivalis in subgingival plaque samples over the conventional culture method.[14-16] The role of P. gingivalis in oral health and disease has been studied in different parts of the world using various laboratory methods. Undoubtedly, different results have been obtained from these studies due to differences in the environmental factors, habits, and medical education specific for the area of each study.[17,18] There was a gap in the information regarding this issue from our region. The aim of this study, therefore, was to investigate the detection rate of P. gingivalis in CP patients compared with periodontally healthy subjects using PCR assay, and study the correlation of P. gingivalis with PPD and CAL.

Methods

2.1 Study population

This is a case-control study and subjects were selected from patients attending the clinics of the College of Dentistry, Hawler Medical University, Erbil, between April 2012 and June 2013. Thirty five subjects having the clinical signs of chronic periodontitis (clinical attachment loss (CAL) and probing pocket depth (PPD) ≥3mm)[19] were selected to be the subjects of the chronic periodontitis group (CP group). There was no limitation for age and gender. Another 35 subjects age (categories of 10 years) and sex-matched with those in the CP group without clinical signs of periodontal disease (CAL and PPD <3mm) were served as a periodontally healthy group. Subjects having systemic diseases, those who in the previous three months had been treated with antibiotics and/or inflammatory drugs, those who received periodontal therapy in the preceding six months and smokers and pregnant women were excluded from the study. The study was independently reviewed and approved by the ethical board of Hawler Medical University. The purpose of the study was explained for the participants and their informed consents were obtained to participate in the study.

2.2 Probing and sample collection

Assessment of both PPD and CAL was made for all the subjects at four sites per tooth (mesiobuccal, distobuccal mesiolingual and distolingual) WHO periodontal probe (Dental care, USA). Assessment of the depth of the pocket was done by measuring the distance between the base of the pocket and gingival margin, whereas that of loss of attachment was accomplished by measuring the distance between the base of the pocket and the cementoenamel junction. Sampling procedure was proceeded by a careful removing of supragingival plaqe from the examined tooth surface (to avoid bleeding) by means of sterile cotton rolls to minimize contamination of the collected sample with undesired bacteria. Then, subgingival microbial samples was obtained by inserting four medium-sized sterile paper points (Dentsply, UK) deep into the pocket and kept in place for 60 seconds. Later, the paper points pooled into an

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eppindorf tube containing 250µl of sterile phosphate-buffered saline solution (PBS) and stored at –70°C for further analysis.[19]

2.3 DNA extraction and PCR assay

Extraction of DNA from the collected samples and amplification using polymerase chain reaction (PCR) were done in the Medical Research Center, HMU, Erbil, between November 2013 and February 2014. In order to extract DNA from the stored samples, frozen suspensions were thawed and DNA extraction was done using Clonit Srl DNA extraction kit (Melano, Italy). The procedure required 35-55 minutes. DNA isolation was based on lyses of the cells and subsequent selective DNA precipitation. Finally, the insoluble DNA was washed and desalted by washing buffer. Then, the extracted DNA were amplified through PCR assay using P. *gingivalis* PCR kit (Genekam Biotechnology AG, Germany), which is designed for qualitative detection and amplification of *P. gingivalis* DNA in serum and plasma of human blood and other human samples by the method of polymerase chain reaction. In both techniques, the manufacturers' instructions were followed. Later, the amplified DNA fragments in the specimen were separated by gel electrophoresis and visualized by staining with a dye that bound to DNA and fluoresced when examined by the Ultraviolet Light (UV). The method described by Sambrook and Russel[20] was followed for this purpose. In this method, 2% agarose gel in Tris-Borate EDTA buffer was prepared and let to solidify. Then, the gel was soaked in a gel tank with TBE buffer. The amplified DNA fragments from each sample were added to the gel. The marker DNA (DNA fragment with known molecular weights measured in base pairs), positive control (P*. gingivalis* DNA fragment of 197 base pairs) and negative control (DNA-free solution) were also added. Electrophoresis was run using 80 volt (10 volt/cm) for 15-30 minutes. A positive sample for P*. gingivalis* was decided when a DNA fragment in the same position of the positive control band was observed.

2.4 Statistical analysis

The statistical package for the social sciences (version 20.0) was used to analyze data and Excel 2007 for figures. Student t-test was used to find out differences between the means of the study and control groups, whereas Pearson Chi-square test (χ^2) was applied to assess the association between two categorical variables. A *P* value <0.05 considered as statistically significant**.**

Results

Demographic and clinical features of the study population are shown in Table 1. A total of 70 subjects met the inclusion criteria for this study was included; 35 subjects with chronic periodontitis (CP) and 35 healthy subjects. The mean age ± SD of CP group was 37.23 ± 9.77 years, and that of the healthy group was 34.51 ± 7.90 years. The difference between them was not significant (*P* = 0.206). Male and female subjects were equal in number in both groups. Significant differences were observed between the mean of the study groups regarding PPD and CAL (*P* = 0.006 and <0.001, respectively).

Table 1: Demographic and clinical characteristics of the study groups (SD: standard deviation, PPD: probing pocket depth, CAL: clinical attachment loss).

Description	CP group	Healthy group	*P* value
Subjects	35	35	
Age (year)			
Range	20-62	20-63	
Mean ± SD	37.23±9.77	34.51±7.90	
Gender			
Female no.(%)	18(51)	18(51)	
Male no. (%)	17(49)	17(49)	
PPD (mm)			
Range	3.25-6.25	1-3	0.006
Mean± SD	4.40±0.88	2.02±0.57*	
CAL (mm)			
Range	4.50-10.50	0-3	<0.001
Mean ± SD	6.84±1.70	0.79±0.80*	

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The rate of detection of P. gingivalis in the study groups is shown in Table 2. Out of 35 subjects with chronic periodontitis, 26 (74%) were positive for P. gingivalis, whereas only 11 (31%) healthy subjects were positive. The difference in the detection rate between the two groups was statistically significant (*P* <0.001). Figures 1 and 2 reveal the correlation between the rate of detection of P. gingivalis in samples obtained from CP group and each of PPD and CAL, respectively. P. gingivalis was detected more frequently in deeper pockets and more severe chronic disease. However, the statistical analysis using Chi square test revealed a significant correlation between the rate of detection of P. gingivalis and PPD (P = 0.017), but a non-significant correlation with CAL (P = 0.104).

Table 2: Frequency of detection of Porphyromonas gingivalis in the study groups.

P. gingivalis	CP group no. (%)	Healthy group no. (%)	*P* value
Positive	26 (74)	11 (31)	<0.001
Negative	9 (26)	24 (69)	<0.001
Total	35 (100)	35 (100)	

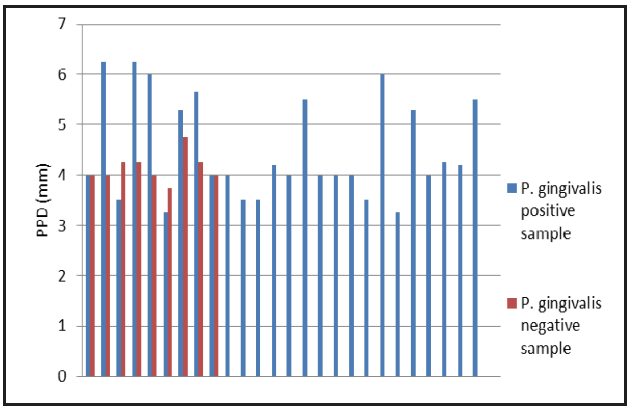

Figure 1: The correlation between *P. gingivalis*-positive versus *P. gingivalis*-negative samples and probing pocket depth (PPD) in CP group.

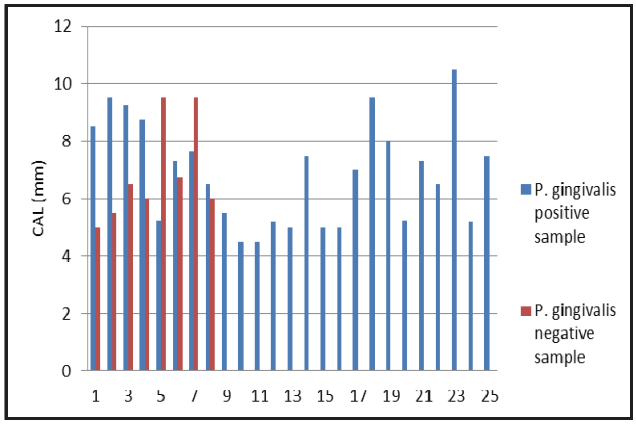

Figure 2: The correlation between *P. gingivalis*-positive versus *P. gingivalis*-negative samples and clinical attachment loss (CAL) in CP group.

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Discussion

This study was done to investigate the detection rate of Porphyromonas gingivalis in chronic periodontitis patients versus healthy subjects, and its association with increased pocket depth and clinical attachment loss. In the present study, PCR method was successfully used to detect P. gingivalis in subgingival microbial samples. Our results demonstrated that P.gingivalis was detected in both periodontally healthy subjects and those with chronic periodontitis with higher detection rate in the latter (74%) than the former (31%). Nearly, these results were consistent with those obtained by the previous studies regardless the nature of the method used for detection of P. gingivalis in each research.[4,5,21] Lower prevalence rate (49.1%) was reported by Boutaga et al[14] using Real-Time PCR. On studying the microbiology of subgingival plaque samples from patients with severe chronic periodontitis, Boyanova and co-workers[22] were reported a much lower detection rate (25.9%) of P. gingivalis yielded from anaerobic culturing. However, all the mentioned references agreed on that the rate of detection is higher in disease than health. Presence of this bacterium in low number of healthy individuals (9/35) and in a significantly higher number of subjects in CP group indicates that it is an opportunistic pathogen. Healthy periodontium is maintained through a good oral hygiene of the individual. Opportunity for higher growth rate of P. gingivalis is usually generated through plaque accumulation in the sub-gingival area in which the growth of early plaque colonizers (gram positive cocci and rods) provide necessary growth factors such as attachment sites, substrate, reduced oxygen tension and an area away from host's oral immunity.[23,24] In this study, the association between the rate of detection of P. gingivalis and PPD in CP group was of particular interest, because, the organism was recovered more frequently in pockets with ≥5mm depth than shallower ones (Figure 1). This association was also statistically significant (P = 0.017). This finding is in agreement to that observed by Vajawat el al[2] and Kawada el al.[25] Boyanova and his colleagues [21] have studied the relationship between the number of P. gingivalis and periodontal status using real-time PCR, and found a significant correlation between the number of P. gingivalis and PPD in which there was a 10-fold increase in P. gingivalis number for every 1mm increase in pocket depth. In our study, it was also found that the rate of detection of P. gingivalis was higher in samples obtained from CP patients with mean CAL of > 6mm (Figure 2). However the statistical analysis of the results failed to proove this association (P = 0.104). Tannner and his colleagues[26] reported low P. gingivalis detection rate (15 -40%) during early periodontitis in two groups of patients in which their mean CAL was <2mm, indicating low detection rate in non-sever cases. Subgingival microbial profile in CP patients from different geographical locations has been studied by Herrera and co-workers[5] in which the recorded CAL was higher than 5mm and the minimum detection rate was 65.9%. This result is consistent with those obtained in this study in that the detection rate of this organism is high in severe cases. The non-significant correlation obtained from this study between the rate of detection of P. gingivalis and CAL could be due to that P. gingivalis is not the only pathogen capable of causing periodontal destruction, as in the chronic periodontitis multiple pathogenic bacteria are associated with periodontal lesion.[4]

Conclusion

The rate of P. gingivalis was strongly higher in specimens obtained from CP group than in healthy subjects. In CP group, P. gingivalis was significantly associated with deep pockets, but not with the disease progression. Further studies are required to investigate the role of other periodontopathogens in chronic periodontitis.

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Conflicts of interest

The authors report no conflicts of interest.

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