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ORIGINAL ARTICLE

Prevalence of *Porphyromonas gingivalis* isolated from oral cavity in Indian population - A culture-based study

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Abstract

Background: Among the many oral bacteria residing in the oral cavity, *Porphyromonas gingivalis* is known to be the major organism associated with periodontitis. Different methods have been used for identification. However, culture is the gold standard in identification. Culture helps in characterization of the physiologic characters, identification pathogenic traits, virulence factors, etc. Gene alterations can be studied better if the organism is cultivated in the laboratory.

Aims and Objectives: The aims and objectives were to study the isolation of *P. gingivalis* from subgingival plaque samples of periodontally healthy and diseased individuals by culture and phenotypic identification.

Materials and Methods: A total of 400 subjects each with 200 chronic periodontitis and healthy individuals were selected. Patients meeting all criteria's samples were inoculated on Blood agar fortified with Hemin and Vitamin K media. Phenotypic characterization was done by morphological and biochemical analysis to identify *P. gingivalis.*

Results: Of 400 samples, 173 were male and 227 were female. In chronic periodontitis, 119 were female and 81 were male. The total sample positive for *P gingivalis* was 288. In chronic periodontitis, the positivity for *P gingivalis* was 179. The positivity for *P gingivalis* was more in females 168.

Conclusion: Identification of anaerobic microbes can be easily done these anaerobic culture techniques. Hence, we recommend using these techniques in the identification of oral anaerobes as a routine laboratory procedure which is lacking in our country.

Introduction

Periodontal diseases affect a large proportion of the world population and are considered an important health issue in both developed and developing countries.[1] There are several oral bacterial species which mainly reside in the subgingival plaque covering the tooth surface that has been implicated in the etiology of chronic periodontitis.[2] Among them *Porphyromonas gingivalis*, a Gram-negative anaerobic bacterium has been shown to be a keystone pathogen in the etiology of chronic periodontitis.[3-6] Many studies have been conducted over the years to demonstrate the pathogenicity of *P gingivalis* in periodontal diseases.[6] It produces a myriad of virulence factors that cause the destruction of periodontal tissues either directly or indirectly by modulating host immune responses. It is also known to be associated with several systemic diseases such as atherosclerosis, coronary artery disease, preterm labor, rheumatoid arthritis, and oral cancer.[7-9] This organism has been shown to be present even in periodontally healthy sites though in low numbers. However, the prevalence rate varies in different studies.[2] Different methods of identification that includes culture, rapid tests, and molecular techniques such as polymerase chain reaction (PCR), hybridization, and fingerprinting methods have been used to detect the presence of microbes in subgingival plaque samples from human volunteers.[10,11]

Among various methods of identification, traditionally culture has been used for the isolation of oral bacterial species; it has a very high specificity and is considered as a gold standard.[11] Moreover, to study various characteristics of an organism such as physiologic characters, pathogenic traits, virulence factors, gene alterations, and antimicrobial susceptibility, one has

to cultivate the microbe in the laboratory.[12] Even though *P. gingivalis* has been studied extensively over the years, there are not many studies from India and the prevalence rate of this organism in our population is not exactly known. Keeping this in mind, the present study was aimed at isolation of *P. gingivalis* from subgingival plaque samples of periodontally healthy and diseased individuals by culture and subsequent identification by phenotypic methods.

Materials and Methods

The present study was performed in the department of microbiology of our institution.

The study included a total of 400 subjects, of which 200 were apparently healthy individuals (Group I) and 200 were with chronic periodontitis (Group II). The participants for the study were selected from patients visiting the outpatient clinics of the department of oral medicine and radiology of the institute. Ethical clearance was obtained from the Institutional Ethical Committee. A written informed consent was obtained from each participant before enrolling for the study.

Selection criteria

The inclusion criteria for healthy group were as follows: No signs of gingival inflammation, absence of bleeding on probing, and probing depth of ≤ 3 mm, with no clinical attachment loss. The criteria for including chronic periodontitis patients for the study were the presence of gingival inflammation, presence of bleeding on probing, probing depth of ≥ 5 mm, and clinical attachment loss of > 3 mm.

The exclusion criteria for both groups included patients with diabetes or any other systemic illness, patients having the habit of tobacco chewing, patients on any types of medication, pregnant women's and lactating mothers, and patients who had undergone periodontal treatment antimicrobial therapy for a period of 3 months before study and subjects with <20 teeth.

Microbiological sampling

After stripping of the supragingival plaque, the subgingival samples were collected for microbiological study using sterile endodontic paper points: At least 4 teeth were sampled, with four deepest pockets in healthy subjects or most diseased sites in chronic periodontitis patients. All paper points from each subject were put in one vial containing reduced transport fluid (RTF) and transferred to the laboratory at the earliest.[13]

Immediately on receipt in the laboratory, each sample was Vortex (Biorad BR-200 vortexer) for 30 s and was serially diluted in RTF and plated on to enriched blood agar (with Hemin and Vitamin K) and kanamycin blood agar (with Hemin and Vitamin K). The plates were incubated anaerobically in an anaerobic jar with modified gas pack system for a period of 72 h. At the end of the incubation period, plates were inspected for the presence of small, shiny, circular, black-pigmented, and mucoid colonies with or without hemolysis [Figure 1]. The number of colonies was recorded, and the morphology was confirmed by Gram staining. Isolated colonies were subcultured on a fresh enriched blood agar medium and reincubated anaerobically to obtain pure colonies. Colonies were subjected to biochemical characterization. Those colonies which were catalase negative, indole positive reduces nitrate, did not ferment any carbohydrates [Figure 2] and did not show fluorescence under UV light were considered as *P. gingivalis*.[14] The number of colonies on the original plate was multiplied by dilution factor and expressed as colony-forming units (CFUs).

Results

The present study was aimed at isolation and identification of *P. gingivalis* from subgingival plaque. The study involved a

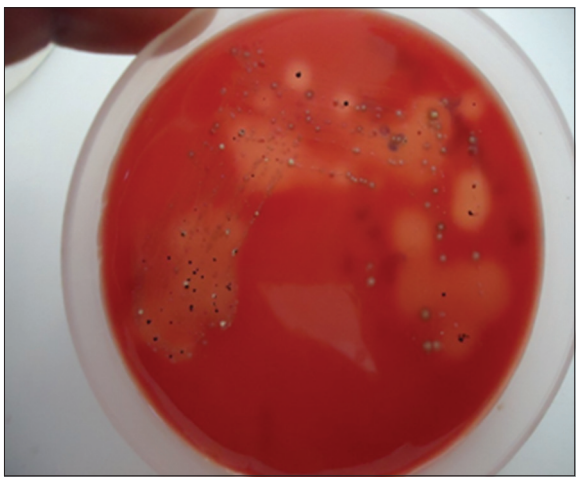

Figure 1: Photograph showing black-pigmented colonies on blood agar with Hemin and Vitamin K medium

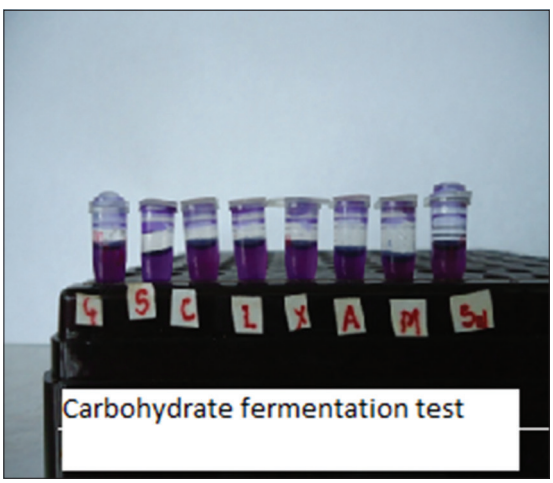

Figure 2: Photograph showing carbohydrate fermentation analysis for *Porphyromonas gingivalis.* Were labels indicates: G-glucose, S-sucrose, C-cellobiose, L-lactose, X-xylose, A-arabinose, M-maltose, S-salicin

total of 400 participants, of which 200 were apparently healthy individuals [Group I] and another 200 were patients with chronic periodontitis [Group II]. In Group I, 109 participants were male and 91 were female while in Group II males were 81 and females 119. The overall prevalence rate *P. gingivalis* was high in Group II with 179 (89.5%) patients showing *P. gingivalis* in comparison to 109 (54.0%) subjects in Group I. These values were statistically significant (*P* value–0.0045; Chi-square value 10.82) [Table 1].

We further compared the prevalence of *P. gingivalis* between subjects of same gender between the two groups. The results showed that males in Group II had significantly higher prevalence (84%) of *P. gingivalis* compared to those of Group I (56.5%). The difference was statistically significant when compared by Fisher's exact test ($P \leq 0.001$) [Table 2].

Similarly, among women also, subjects in Group II had significantly higher prevalence (93.3%) of *P. gingivalis* compared to those in Group I (52.8%). This result also was statistically significant with $P < 0.001$ [Table 2]. We also compared the results among total number of males and females who participated in the study. The females showed slightly higher prevalence (74%) of *P. gingivalis* compared to males (69.4%). The difference was not statistically significant when compared by Fisher's exact.

When we compared the culture results between males and females in each group, it could be seen that the females had slightly higher prevalence (93.3%) of *P. gingivalis* compared to males (84%) in Group II. The difference was not statistically significant when compared by Fisher's exact test ($P < 0.058$). On the other hand, in Group I, the males had slightly higher prevalence (56.5%) of *P. gingivalis* compared to females (52.8%). The difference was also not statistically significant when compared by Fisher's exact test ($P < 0.669$) [Table 3].

All subjects from each group were further categorized into four divisions (18–27 years, 28–37 years, 38–47 years, and 48–60 years) based on age. Analysis showed that the prevalence of *P. gingivalis* was higher in Group II when compared to Group I. However, the value was statistically significant only within the age categories of 18–27, 28–37, and 38–47 years when compared by Fisher's exact test. For the age category of 48–60 years, the difference was not statistically significant [Table 4].

We also performed the quantitative analysis by comparing the CFUs in positive samples between the two groups and also between males and females in each group. The results showed that participants in Group II had higher median CFUs when compared to Group I. Further, in both the groups, males had higher median CFUs compared to females. All these results were statistically significant [Table 5].

However, within each group, there was no significant difference in the colony count between males and females [Table 6]. We also compared the median CFU count within different age groups. Here, the value was statistically significant only within the age categories of 18–27, 28–37, and 38–47 years when compared by Mann–Whitney U-test. For the age category of 48–60 years, the difference was not statistically significant.

We tried to study the correlation between the age and CFUs by Spearman's correlation coefficient. The analysis shows that overall there is a significant positive correlation between age and CFU. The correlation is significantly positive when compared only within healthy group. The correlation is positive but not significant when compared within chronic periodontitis patients.

Table 1: Prevalence of *Porphyromonas gingivalis*

Group	Culture (%)		Total (%)	Fisher's exact test
	Negative	Positive		
Chronic periodontitis	21	179	200	<0.001, significant ($P < 0.001$)
	10.5	89.5	100.0	
Healthy	91	109	200	
	45.5	54.5	100.0	
Total	112	288	400	
	28.0	72.0	100.0	

Table 2: Prevalence of *Porphyromonas gingivalis* within males and within females

Group	Culture (%)		Total (%)	Fisher's exact test
	Negative	Positive		
Males				
Chronic periodontitis	13	68	81	<0.001, significant
	16.0	84.0	100.0	
Healthy	40	52	92	
	43.5	56.5	100.0	
Females				
Chronic periodontitis	8	111	119	<0.001, significant
	6.7	93.3	100.0	
Healthy	51	57	108	
	47.2	52.8	100.0	

Table 3: Comparison of the *prevalence of Porphyromonas* gingivalis between males and females within healthy and within chronic periodontitis

Group	Culture (%)		Total (%)	Fisher's exact test
	Negative	Positive		
Chronic periodontitis				
Male	13	68	81	<0.058, not significant
	16.0	84.0	100.0	
Female	8	111	119	
	6.7	93.3	100.0	
Healthy				
Male	40	52	92	<0.669, not significant
	43.5	56.5	100.0	
Female	51	57	108	
	47.2	52.8	100.0	

Table 4: Comparison of the prevalence of *Porphyromonas gingivalis* in males according to age distribution

Age categorized (year)	Group	Culture (%)		Total (%)	Fisher's exact test
		Negative	Positive		
18–27	Chronic periodontitis	9	39	48	<0.001, significant
		18.8	81.3	100.0	
	Healthy	38	34	72	
		52.8	47.2	100.0	
	Total	47	73	120	
		39.2	60.8	100.0	
28–37	Chronic periodontitis	6	64	70	<0.001, significant
		8.6	91.4	100.0	
	Healthy	21	37	58	
		36.2	63.8	100.0	
	Total	27	101	128	
		21.1	78.9	100.0	
38–47	Chronic periodontitis	2	61	63	<0.001, significant
		3.2	96.8	100.0	
	Healthy	28	27	55	
		50.9%	49.1	100.0	
	Total	30	88	118	
		25.4	74.6	100.0	
48–60	Chronic periodontitis	4	15	19	>0.05, not significant
		21.1	78.9	100.0	
	Healthy	4	11	15	
		26.7	73.3	100.0	
	Total	8	26	34	
		23.5	76.5	100.0	

Table 5: Comparison of median CFU between healthy and chronic periodontitis within males and within females

Category	Group	*n*	Mean	Standard deviation	Minimum	Maximum	Percentiles			Mann–Whitney U-test
							25th	50th median	75th	*Z* value, *P* value, and significance
Overall (All cases)	Chronic periodontitis	200	52,930	49,845	0.00	270,000	16250	37,500	78,750	*Z* value-9.733
	Healthy	200	16,000	23,801	0.00	100,000	0.00	5000	25,000	*P*-value<0.001, significant
Within males	Chronic periodontitis	81	53,061	55,402	0.00	270,000	15000	40,000	80,000	*Z* value-0.521
	Healthy	92	18,163	24,862	0.00	100,000	0.00	5000	33,750	*P*-value<0.001, significant
Within females	Chronic periodontitis	119	52,840	45,924	0.00	230,000	20000	35,000	75,000	*Z* value-0.839
	Healthy	108	14,157	22,812	0.00	100,000	0.00	3500	20,000	*P*-value<0.001, significant

CFUs: Colony-forming units

Discussion

P. gingivalis, a Gram-negative anaerobe, is one of the most extensively researched bacteria of the oral cavity. Studies spanning over the past several years have provided evidence highlighting the contribution of this organism in the initiation and progression of periodontal disease. Of late, we have a better understanding of the various virulence factors produced by *P. gingivalis,* mechanisms by which it can cause periodontitis

Table 6: Comparison of median CFU between males and females within healthy and within periodontitis

Category	Group	*n*	Mean	Standard deviation	Minimum	Maximum	Percentiles			Mann–Whitney U-test
							25th	50th median	75th	(*Z*-value, *P* value, and significance)
Overall (All cases)	Males	173	34,502	45,383	0.00	270,000	0.00	20,000	50,000	*Z* value-0.508
	Females	227	34,436	41,502	0.00	230,000	0.00	20,000	50,000	*P*-value<0.611, not significant
Chronic periodontitis	Males	81	53,061	55,402	0.00	270,000	15,000	40,000	80,000	*Z* value-0.682
	Females	119	52,840	45,924	0.00	230,000	20,000	35,000	75,000	*P*-value<0.495, not significant
Healthy	Males	92	18,163	24862	0.00	100,000	0.00	5000	33,750	*Z* value-1.09
	Females	108	14,157	22,812	0.00	100,000	0.00	3500	20,000	*P*-value<0.285, not significant

CFUs: Colony-forming units

and its probable role in various systemic disorders including oral cancer.[12]

The major habitat of *P. gingivalis* is the subgingival plaque of the human oral cavity. The number of *P. gingivalis* has been shown to increase substantially in sites with periodontitis and lower or non-detectable in healthy sites.[15] Studies conducted over the past several years have shown this bacterium to be present in the oral cavity of healthy individuals also, though in much lower numbers than that seen in patients with periodontitis.[16] The prevalence rate varies among different studies. This appears largely due to the site selected, method adopted for sampling and the technique used for the identification of the organism. Unfortunately, even though a large number of studies have been conducted on the prevalence of *P. gingivalis* in healthy and diseased subjects, many of them are qualitative and suffer from poor and inconsistent methodology.[17] In general, culture and other methods yield lower numbers than molecular techniques such as PCR. Even with culture, there appears to be no standard method adopted and variability can be found in the type of media selected, anaerobic conditions, and the duration of incubation of the plates. This could be the reason for the highly variable prevalence rate.

An analysis of the results from various studies over the years shows that *P. gingivalis* has been detected in 0–40% of healthy individuals and 40–100% of diseased subjects.[18] In our study, 54% of healthy individuals and 89.5% of patients with chronic periodontitis were positive for *P. gingivalis*. While these results are in agreement with other investigators for the prevalence in periodontitis, the positivity rate in healthy individuals appears higher than the results of other studies. However, the bacterial counts were significantly lower in healthy individuals in comparison to diseased subjects.

There are not many studies from India on the prevalence of *P. gingivalis*. Most of the studies conducted have used either molecular methods for bacterial detection or where culture is used, the sample size appears to be very small.[18-20] This is the first study in Indian population where a total of 400 participants were involved to study the prevalence rate of *P. gingivalis*.

There could be several reasons for higher positivity rate among healthy subjects in our study. However, we feel that the key factor was incubation of plates for 1 week as against the standard practice of 72 h of incubation.

We categorized the study population into different age groups to assess the presence of *P. gingivalis*. It could be seen that both in men and women, the prevalence of *P. gingivalis* was higher in younger population and was slightly reduced in the age range of 48–60 years. This observation is marginally different from other workers reporting increased frequency of isolation of *P. gingivalis* with age. An extensive study involving 242 patients with mean ages of 16, 25, 35, and 46 revealed that this organism was absent in the youngest patient group but had a prevalence of around 50% in the oldest group.[16] Since we do not have data from any other studies from India, we do not know whether this trend exists in other parts of the country also. Finding of this study, the mean CFUs of bacteria were found to be consistently lower in all age ranges, especially in 18–27 years both among males and females in healthy group as against chronic periodontitis group. This observation is in agreement with the findings of other workers.

Culture techniques have been the classic diagnostic method to detect bacterial species residing in the subgingival plaque. At present, it is the only method to identify new species. Although molecular approach can identify new species of bacteria, culture is the only method that enables us to study their physiological and pathological characteristics and antimicrobial susceptibility pattern, and provide a quantitative measurement of viable bacteria. This is also possible with molecular methods, that is, to quantitate the bacterial species from a sample. Culture is still considered as a gold standard in oral microbiology and remains an important tool of characterizing subgingival microflora.[21,22] Deficiency of the culture procedure is evident while targeting uncultivated bacteria. Fortunately, *P. gingivalis* free from this difficulty as it is cultivated relatively easily and identified in the laboratory.[23,24] In addition, in contrast to other detection methods, a positive finding based on culture can be confirmed by subsequent testing. Hence, we strongly feel that for easily cultivable anaerobic bacteria, anaerobic culture should be advocated and encouraged. This will enable the microbiologists to establish anaerobic culture techniques in laboratories and help generate basic data about various oral anaerobes, which is lacking from our country.

Conclusion

With the present observation of our study we strongly feel that for easily cultivable anaerobic bacteria, anaerobic culture should be

advocated and encouraged. This will enable the microbiologists to establish anaerobic culture techniques in laboratories and help generate basic data about various oral anaerobes, which is lacking from our country.

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