



Microbiota of Chronic Periodontitis and their Association with Severity of the Disease

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ABSTRACT

Aim: To know the role of these microorganisms as etiological agents in chronic periodontitis and their role in the severity of the disease.

Material and Method: A total of 300 patients with chronic periodontitis and 300 age and sex matched controls were enrolled with prior informed consent. Sub gingival plaque specimens was collected and processed for bacterial and yeast etiology. The data was analyzed by using SPSS software and Chi square test with p-value of <0.0001 was applied.

Results: The most common age group affected were 31 to 40 years and male were outnumbered the female patients but the difference was not statistically significant. Overall detection rate of aerobes, anaerobes and yeasts were 47.3%, 78.3% and 4.6% respectively. The most common etiological agents significantly associated belong to anaerobes and yeasts. Anaerobes were found to be associated significantly with severity of disease.

Conclusion: *Peptostreptococcus anaerobius*, *Veillonella parvula* and *Porphyromonas gingivalis* were predominant etiological agents implicated as etiological agents in chronic periodontitis. Aerobes were significantly associated with mild chronic periodontitis while anaerobes found to be significantly associated with severe chronic periodontitis.

Key Words: Chronic periodontitis, Microbiota, Anaerobes, Aerobes, Yeasts, Severity

INTRODUCTION

Chronic periodontitis is the most common oral disease affecting worldwide especially in India which leads to tooth loss if kept untreated for long (1). It is initiated by plaque which consists of bacteria that are responsible for initiation and further progression of the disease (2). Aerobes, anaerobes and possibly yeasts could play a crucial role in the initiation of chronic periodontitis (3). The present study has evaluated the role of microorganisms in chronic periodontitis and in the severity of the disease.

odontitis (100 each from mild, moderate and severe) as per classification of American Association of Periodontologist (4) and 300 age and sex matched controls were enrolled with prior informed consent. An inclusion and exclusion criterion was applied before obtaining specimens from patients as well as healthy controls. Subgingival plaque specimen was collected and transferred to brain heart infusion (BHI) broth and Robertson cocker meat (RCM) medium and processed for cultivation of aerobes and anaerobes by standard methods (5). The data was analyzed with the help of SPSS software v17.0 and Chi square test with p-value of <0.0001 was used to find out any significant associations.

MATERIAL AND METHODS

The present study was a prospective case control study. The ethical approval was taken from Ethical Committee (D-1210169-71) and the study period was from June 2011 to December 2014. A total of 300 patients with chronic peri-

RESULTS

The most common age group affected were 31 to 40 years (43.49±8.48) and male patients outnumbered female patients (p>0.05). The overall detection rate of aerobes,

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anaerobes and yeasts was 47.3, 78.3 and 4.6 % respectively (Table 1). The aerobes were significantly associated with mild while anaerobes were significantly associated with severe chronic periodontitis (Table 2). The common etiological agents include *Peptostreptococcus anaerobius* (37.3 %), *Veillonella parvula* (34.3 %), *Enterococcus faecalis* (33.0 %) and *Porphyromonas gingivalis* (25 %) (Table 3). Anaerobes (*P. gingivalis*, *B. fragilis*, *F. nucleatum*, *V. parvula*) were found to be associated significantly with increasing severity of the disease (Table 4).

DISCUSSION

In adult patients there is a complex interplay of the mixed polymicrobial infection and host response. The present study has evaluated the role of these microorganisms as etiological agents in chronic periodontitis and their association with severity of the disease. Anaerobic bacteria (78.3 %) were the commonest bacterial pathogen detected from patients with chronic periodontitis (Table 1). Various studies have reported anaerobes in periodontitis with isolation rates ranging from 57.0 % to 93.0 % (6-11). The anaerobic flora plays an important role in the progression of chronic periodontitis. This could be due to the fact that the more the pocket depth and attachment loss an anaerobic environment is created, which is ultimately favorable to the growth of anaerobic pathogens. They establish in the depth of the oral pockets and cause tissue destruction.

The anaerobes were significantly associated with the severity ($p < 0.0001$) of chronic periodontitis (Table 2). Various studies have reported anaerobes from severe chronic periodontitis than mild periodontitis (10, 12, 13, 14). *Peptostreptococcus anaerobius* (37.3 %), *Veillonella parvula* (34.3 %) and *Porphyromonas gingivalis* (25.0 %) were predominantly detected from patients with chronic periodontitis and found to be associated significantly with the disease ($p < 0.0001$) (Table 3).

Peptostreptococcus anaerobius

P. anaerobius is found to be associated with adult periodontitis. They possess capsule which is an important virulence factor and can produce abscesses. *P. anaerobius* was the commonest anaerobe (37.3 %) detected in the present study (Table 3). Younis et al (15) and Sixou et al (16,17) have reported *P. anaerobius* in 2.7%, 5.8% and 14.2 % from patients with periodontitis. However, Koll-Klais et al (18) have reported *Peptostreptococcus spp.* in 90.0 % from patients with periodontitis. They did not speciate the *Peptostreptococcus spp.* isolated in their study. From the findings of the current study, *P. anaerobius* is reflected to be a major pathogen in the etiology of chronic periodontitis. *P. anaerobius* was found to be associated significantly with mild chronic periodontitis.

In mild chronic periodontitis, the anaerobic environment is limited, because of which *P. anaerobius* have been detected predominantly in the present study. This also suggests that they come first in the initiation of the disease.

Peptostreptococcus micros

Peptostreptococcus micros were detected from 17.3 % from patients with chronic periodontitis in the present study (Table 3). Mane et al (19) have reported *Peptostreptococcus micros* in 23.0 % from patients with chronic periodontitis. *P. micros* were also found to be associated significantly with mild chronic periodontitis.

Veillonella parvula

V. parvula has been reported to play an essential role as early colonizers in the biofilm formation and thereby facilitate succession in development of oral biofilms which helps other microbiota to establish them. *V. parvula* was detected in 34.3 % of cases of chronic periodontitis in the present study (Table 3). The detection rates of the organism were seen from 9.0 % to 81.0 % in various studies (18, 20). In the present study, *V. parvula* was found to be associated with the increased severity of chronic periodontitis. They were detected in 49.0 % from severe chronic periodontitis as compared to mild and moderate (Table 4) suggesting their role too in the increased severity of the disease.

Porphyromonas gingivalis

P. gingivalis is an important etiological agent which is postulated to play a major role in chronic periodontitis by different mechanisms. They produce many pathogenic virulence factors such as lipopolysaccharides and H_2S , which can induce the host to release interleukin, tumour necrosis factors which precipitated the host's immune response leading to bone resorption and prevent the repairing of osteal tissue. They produce proteases, collagenases which are important in the process of tissue breakdown. They possess fimbriae which are helpful in attachment to host epithelial cells and damage soft tissue directly. *P. gingivalis* was detected in 25.0 % of cases of chronic periodontitis in the present study (Table 3). Different studies have reported different rates ranging from 21.9 to 78.0 % from patients with chronic periodontitis (11, 18, 21, 19, 22).

The most common species associated with severe chronic periodontitis was *Porphyromonas gingivalis* in the present study. They were detected from 59.0 % of severe chronic periodontitis cases suggesting their strong association with the increased severity of the disease (Table 4). Different studies have reported association between *P. gingivalis* detection and severity of chronic periodontitis (23, 24, 25).

The other anaerobes detected were *Bacteroides fragilis* (5.6%) and *Fusobacterium nucleatum* (1.3%) from patients

with chronic periodontitis suggesting their association with the disease in the present study (Table 3). Their association was found to be statistically significant. Various authors have detected various other anaerobes in their respective studies (11, 19, 23). All the above studies and the current study have highlighted the role of *Peptostreptococcus anaerobius*, *Veillonella parvula* and *Porphyromonas gingivalis* as an important etiological agent of chronic periodontitis.

Aerobes establish themselves in supragingival plaque and participate in the disease initiation by deleterious effect during their interactions with the host. Aerobes were detected in 47.3 % of the patients with chronic periodontitis in the present study (Table 1). Other studies have reported aerobes as a causative agent in patients with chronic periodontitis ranging from 7.4 % to 62.3 % (8, 10, 19, 26). In the present study aerobes were detected from cases of chronic periodontitis suggesting their role too as etiological agent in chronic periodontitis.

Enterococcus faecalis

E. faecalis possesses numerous virulence factors which may add to periodontal inflammation and tissue destruction. *Enterococcus faecalis* was the most common (33.0 %) aerobe detected from patient with chronic periodontitis in the present study (Table 3). Different studies have reported different detection rates ranging from 12.0 to 51.8 % in their studies (18, 27, 28). All the above studies and the present study have displayed that *Enterococcus faecalis* plays an important role in the causation of chronic periodontitis. *E. faecalis* were detected from all grades of chronic periodontitis equally. There was no statistical difference found in severity of the disease and detection of *E. faecalis*. The reason might be their facultative nature which allows them to grow in any adverse conditions.

Staphylococcus aureus

The presence of *Staphylococcus aureus* in patients with chronic periodontitis is usually seen in mild and moderate infections. However, they can act as an opportunistic pathogen and their presence must be considered when planning antibiotic therapy. *Staphylococcus aureus* was the second highest (15.0 %) aerobe which was detected from cases of chronic periodontitis (Table 3). Different studies have reported different detection rates ranging from 4.5 % to 42.8 % in their respective studies (29, 30, 18, 8, 6). In the present study, *S. aureus* shown to be associated significantly with mild chronic periodontitis as compared to the severe chronic periodontitis suggesting their initial role in the disease (Table 4).

Other aerobes

The other aerobes detected in the present study were *Streptococcus spp.* (2.6 %), *E. coli* (2.3 %) and *Pseudomonas aeruginosa* (1.6 %) from patients with chronic periodontitis

(Table 3). Other studies have also reported these aerobes in their respective studies (9, 6) from periodontitis patients. Aerobes in subgingival samples indicate that they play a role in initiation of chronic periodontitis and create a suitable environment for invasion by other microbes i.e. anaerobes or Herpes viruses resulting in increase in severity.

The *Candida spp.* has various virulence factors that can make it possible for them to colonize and propagate in the oral mucosa especially the periodontal pockets. *Candida spp.* is a major component of oral microflora and could play a role in the initiation of periodontitis. The prevalence of *Candida spp.* in the present study was 4.6 % from patients with chronic periodontitis (Table 1). The other studies have reported detection of *Candida spp.* in chronic periodontitis which ranges from 0 to 53.5 % from patients with chronic periodontitis (9, 18, 26, 31-35).

In the present study, *Candida spp.* was detected mainly from patients with mild chronic periodontitis (9.0 %) suggesting their association in the initial stage of the disease (Table 4). Canabaro et al (34) and Jarvensivuet al (32) have reported association of *Candida spp.* with deep pockets suggesting their role in severity of the disease. However, in the present study, *Candida spp.* has been detected more significantly in the mild chronic periodontitis suggesting their role in the initial phase of the disease.

CONCLUSION

Chronic periodontitis is a multi-etiological oral disease affecting wide range of population. It is mainly initiated by aerobic flora and yeasts which is then followed by anaerobes. *Peptostreptococcus anaerobius*, *Veillonella parvula* and *Porphyromonas gingivalis* were the most predominant etiological agents along with *Staphylococcus aureus*. In the severity of the disease aerobes were associated more with mild chronic periodontitis while anaerobes such as *P. gingivalis* were associated with severe chronic periodontitis. Thus, before initiation of any therapeutic treatment it is essential to know the stage of the disease and the etiology of the disease which help in management of the disease.

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Table 1: Overall detection rates of other organisms in patients with chronic periodontitis and healthy controls

Other organisms	Chronic periodontitis (N=300)		Healthy Controls (N=300)	
	Detected (%)	Not detected (%)	Detected (%)	Not detected (%)
Aerobes	103 (47.3)	158 (52.6)	182 (60.6)	118 (39.3)
Anaerobes	235(78.3)	065 (21.6)	15 (5.0)	285 (95.0)
Yeasts	014 (4.6)	286 (95.3)	0 (0.0)	300 (100.0)

Table 2: Association between other organism and severity of chronic periodontitis

Organisms	Severity of Chronic periodontitis			Chi-square	P-Value
	Mild	Moderate	Severe		
Aerobic	61	45	36	12.86	<0.005*
Anaerobic	87	63	85	20.89	<0.0001*
Yeasts	09	04	01	7.17	<0.01*

*statistically significant

Table 3: Detection of other organisms in chronic periodontitis

Other organisms	Chronic Peri-odontitis (N=300)	Control (N=300)	Z value	P-value
1. Aerobes				
<i>Streptococcus</i> sp.	08 (2.6)	176 (58.6)	18.72	<0.0001*
<i>Staphylococcus aureus</i>	45 (15.0)	03 (1.0)	6.54	<0.0001*
<i>Enterococcus faecalis</i>	99 (33.0)	11 (3.6)	10.03	<0.0001*
<i>Escherichia coli</i>	07 (2.3)	00 (0.0)	2.68	<0.01*
<i>Pseudomonas aeruginosa</i>	05 (1.6)	00 (0.0)	2.25	<0.05*
2. Anaerobes				
<i>Porphyromonas gingivalis</i>	75 (25.0)	00 (0.0)	10	<0.0001*
<i>Bacteroides fragilis</i>	17 (5.6)	00 (0.0)	4.24	<0.0001*
<i>Fusobacterium nucleatum</i>	04 (1.3)	00 (0.0)	2.01	<0.05*

<i>Veillonella parvula</i>	103 (34.3)	10 (3.3)	10.58	<0.0001*
<i>Peptostreptococcus anaerobius</i>	112 (37.3)	03 (1.0)	12.74	<0.0001*
<i>Peptostreptococcus micros</i>	52 (17.3)	04 (1.3)	7.01	<0.0001*
3. Yeasts				
<i>Candida</i> spp.	14 (4.6)	01 (0.3)	3.43	<0.001*

Table 4: Association between other microorganisms and severity of chronic periodontitis

Organisms	Severity of Chronic peri-odontitis			p-value
	Mild (n=100)	Moderate (n=100)	Severe (n=100)	
Aerobes				
<i>Streptococcus sp.</i>	07	01	00	<0.005*
<i>Staphylococcus aureus</i>	33	12	00	<0.0001*
<i>Enterococcus faecalis</i>	31	32	36	>0.05#
<i>E. coli</i>	06	01	00	<0.005*
<i>Pseudomonas aeruginosa</i>	05	00	00	<0.01*
Anaerobes				
<i>Porphyromonas gingivalis</i>	02	14	59	<0.0001*
<i>Fusobacterium nucleatum</i>	00	01	03	>0.05#
<i>Bacteroides fragilis</i>	02	02	13	<0.001*
<i>Veillonella parvula</i>	22	32	49	<0.0001*
<i>Peptostreptococcus anaerobius</i>	51	31	30	<0.005*
<i>Peptostreptococcus micros</i>	36	09	07	<0.0001*
Yeasts				
<i>Candida spp.</i>	09	04	01	<0.01*

not significant * significant