Original Research Article

Detection of *Treponema denticola* by PCR in patients with different periodontal status

Varsha Chaudhary\(^1\), Bhat Kishore\(^1\), Sunil Rao\(^2\), Manohar Kugaji\(^1\) and Preeti Ingalagi\(^1\)

\(^1\)Department of Microbiology, Maratha Mandal’s NGH Institute of Dental Sciences and Research Centre, Belgaum, India

\(^2\)Department of Microbiology, Yenepoya Medical College, Yenepoya University, Mangalore, India

*Corresponding author

**ABSTRACT**

The aim of the study was to determine the prevalence of *T. denticola* by 16S rRNA-based PCR technique in subgingival plaque of periodontally diseased and healthy subjects and its relationship with the periodontal status. *T. denticola* is a highly motile, aerotolerant and anaerobic spirochete. It has been predominantly associated with the incidence and severity of human periodontal disease. As it is difficult to isolate and identify *T. denticola* from clinical plaque samples, currently, 16S rRNA-based PCR method continues to be one of the most sensitive, rapid, and cost-effective methods for determining the prevalence of such microorganisms. A total of three hundred subjects divided into three categories namely; Chronic periodontitis (CP=100), Aggressive periodontitis (AP=100) and controls (C=100) were selected for the study in an age range of 20 to 50 years. Subgingival plaque samples were collected and forwarded for PCR analysis. The PCR was performed by using specific primers for the 16S rRNA gene of *T. denticola* and quantification was done using standard strain of *T. denticola*. Detection frequencies of *T. denticola* in plaque samples from AP patients and CP patients were reported much higher than those from healthy subjects. There was a significant difference between the PCR detections of CP and Control groups. Results of PCR depicted that Female were prone to chronic periodontitis than male in all age groups. This study demonstrated the utility of a 16S rRNA-based PCR detection method for identifying *T. denticola* and strong association between *T. denticola* and periodontitis.

**Keywords**

Aggressive periodontitis; Chronic periodontitis; PCR; *Treponema denticola.*

**Introduction**

Periodontitis is a disease of multifactorial nature with pathogenesis related to several risk factors, including bacterial pathogens, host responses, and possibly certain genetic traits (Michalowicz *et al.*, 2000). It is characterized by the irreversible destruction of collagen fibers and other matrix constituents of the gingiva, periodontal ligament, and alveolar bone around the teeth in conjunction with the formation of periodontal pockets due to the apical migration of the junctional
epithelium (Page and Kornman, 1997). The prevalence of periodontitis in humans is approximately 30%, of which 10-15% is severe forms of the disease (Oliver et al., 1998).

Spirochetes of genus Treponema are a major component of the bacterial flora of the oral cavity (Correla et al., 2004; Paster et al., 2001). Of these, Treponema denticola has been predominantly associated with the incidence and severity of human periodontal disease such as chronic periodontitis, early-onset periodontitis and necrotizing ulcerative gingivitis (Loesche and Grossman, 2001). T. denticola is a highly motile, aerotolerant and anaerobic spirochete dwells in a complex and diverse microbial community and especially prevalent in the anaerobic gingival crevice (Paster et al., 2001). The accumulation of these bacteria and their products in the pocket may render the surface lining periodontal cells highly susceptible to lysis and damage (Sela, 2001).

In the periodontal pocket, T. denticola has been found close to the junctional epithelium. It has shown to adhere to epithelial cells, fibroblasts and to other bacteria as well as to matrix proteins (Thomas, 1996) and to collagen used as a barrier for guided bone generation (Sela et al., 1999). T. denticola also shows nutritional interactions with other periodontal bacteria, especially Porphyromonas gingivalis. Moreover, due to oral treponemes having adherent properties to epithelial cells and coaggregation abilities to important biofilm-bridging organisms (Yao et al., 1996), the mechanical dispersal of dental plaque containing treponemes may lead to the colonization of new intraoral habits after periodontal therapy (Ehmke et al., 2004).

Methodologies involving culture, flourescence microscopy tests, DNA probes, and the polymerase chain reaction (PCR) are currently available for investigating periodontopathic bacterial infections in lesions. It is time-consuming work to evaluate the prevalence of these bacteria by the use of culture or flourescense microscopy tests from the same clinical sample (Ashimoto et al., 1996; Takamatsu et al., 1999; Watanabe and Frommel, 1996). As it is difficult to isolate and identify T. denticola from clinical plaque samples, currently, 16S rRNA-based PCR method continues to be one of the most sensitive, rapid, and cost-effective methods for determining the prevalence of such microorganisms (Sakamoto et al., 1999; Sakamoto et al., 2001). The aim of the study was to determine the prevalence of T. denticola by 16S rRNA-based PCR technique in subgingival plaque of periodontally diseased and healthy subjects and its relationship with the periodontal status.

Materials and Methods

Sample Collection

The study was performed in the department of Microbiology, Maratha Mandal’s NGH Institute of Dental Sciences, Belgaum. A total of three hundred subjects divided into three categories namely; Chronic periodontitis (CP=100), Aggressive periodontitis (AP=100) and controls (C=100) were selected for the study. Informed consent was obtained from each participant before enrolling in the study. The study subjects were adults in an age range of 20 to 50 years and belonged to both the sex. The inclusion criteria for patients were: Chronic periodontitis, patients having periodontal pockets of ≥4mm, bleeding on probing, CAL of ≥3 mm² and radiographic
evidence of bone loss; Aggressive periodontitis, subjects with a pattern of severe destruction, with attachment loss of at least 4-7 or 8 mm, medical history nil and familiar aggregation of cases. Subject with a probing depth of 2-3mm and without any evidence of inflammation were selected as controls. Pregnant women and lactating mothers, those who had received systemic antibiotics and/or had undergone periodontal treatment during the last six months were excluded from the study.

Subgingival plaque samples were collected using 2-3 sterile paper points inserted for 30 sec. into the diseased sites of subjects with chronic and aggressive periodontitis. Same procedure has been followed for collection of samples from healthy ones. Paper points has to be pooled and placed in the eppendorf tubes containing transport media-Reduced Transport Fluid (RTF) and then samples were forwarded for PCR analysis.

**DNA Analysis**

DNA extraction was carried out for the samples. For this the samples were centrifuged at 5,000rpm for 5 min. Supernatant was discarded, 200 µl of fresh T.E. buffer was added and centrifuged for 3-4 minutes. Above procedure was repeated for 3-4 times with fresh T.E. buffer. Again the supernatant was discarded and 50 µl lysis buffer (Tris HCL: 50mM (pH 8.0), KCL : 50mM ,MgCl2: 2.5mM, Tween 20: 0.45 %, Nodient P-40: 0.45%)and proteinase –K (10mg/ml) was added. It was kept in water bath for 2 hrs at 60⁰C then kept in boiling water bath for 10 minutes. The extracted DNA is stored at -20⁰C and used for PCR analysis.

The PCR was performed in a verity thermal cycler (Applied biosystem) by using specific primers for the 16S rRNA gene of *T.denticola*. PCR primers (Forward primer: 5’-TAA TAC CGA ATG TGC TTT ACA T-3’ and Reverse primer: 5’- TCA AAG AAG CAT TCC CTC TTC TTA-3’) of 316 bp length were used in the current study. The PCR protocol was as follows: 95⁰C for 5 min followed by 40 cycles of 95⁰C for 30 S, 60⁰C for 1 Min, 72⁰C for 1Min, and a final step of 72⁰C for 5 min. Amplified products were analyzed by 2% agarose gel electrophoresis, stained with 0.5 µgm/ml ethidium bromide, visualized and digitized using a BioRad Gel Doc 2000 system (Fig.1). As molecular size marker, 100bp DNA ladder (Chromous Biotech) was used. Quantification was done using standard strain of *T.denticola*. (Fig.1)

**Results and Discussion**

A Microbiological study was conducted to detect *T.denticola* by PCR in Chronic periodontitis (CP), Aggressive periodontitis (AP) and control groups (C). Comparison was done between the results obtained in all the above three groups. Detection frequencies of *T.denticola* in plaque samples from AP patients (67%) and CP patients (81%) were much higher than those from healthy subjects (55%, Graph 1). In Aggressive and Chronic periodontitis patients *T.denticola* was detected frequently at sites that showed deep periodontal pockets and severe attachment loss. There was a significant difference between the PCR detections of CP and Control groups (P value <0.001 is significant). While, there was no significant difference between the results of healthy (control group) and AP patients. Results were also compared against age & sex. Age wise distribution was done as 20-30, 31-40 and 41-50. Results of PCR
depicted that Female were prone to chronic periodontitis than male in all age groups. When compared to control group female in age group 41-50 were most affected for chronic periodontitis i.e. the age of CP group was significantly greater than AP and healthy subjects. Age wise results of AP group were showing that female in age group 20-30 were more positive for T. denticola (Graph 2-4).

Bacterial infections may cause disease manifestations through multiple mechanisms, including, direct effects of bacterial products, effect of the host immune response to the organism, and the persistent actions of the host immune response after clearance of the organism (Lin et al., 2000). Yet it is controversial that the signs and/or symptoms of periodontal disease result from the presence of the microorganisms, the cause of periodontal breakdown in patients with AP and CP (Tamura et al., 2006). The present study was conducted to evaluate the prevalence of T. denticola in subgingival plaque samples of patients with CP/AP and periodontally healthy subjects and its relationship with the periodontal status.

Several studies using subgingival plaque samples have shown that T. denticola is more frequently detected in periodontally diseased subjects than in healthy subjects (Riviere et al., 1995; Simonson et al., 1988; Takeuchi et al., 2003). The results of Statistics in the present study showed a positive association between CP subjects and T. denticola. This finding concurs with previous reports where T. denticola has been specifically implicated in CP (Sela, 2001). More recently, it has been also suggested that T. denticola may be one of the principal bacteria observed in AP (Takeuchi et al., 2003). In this study, a significantly higher detection frequency of T. denticola was obtained in subgingival plaque samples from CP patients (81%) in comparison with those from AP and healthy subjects (67% and 55% respectively). The differences in T. denticola detection between AP and healthy subjects were not statistically significant. These results suggested that the mechanism by which the severe and rapid periodontal breakdown occurs in AP might be independent of the presence of T. denticola and it is likely to be by the involvement of other bacteria with higher pathogenicity, such as Tannerella forsythensis and P. gingivalis, as noted by others (Takeuchi et al., 2003). Periodontal destruction in AP is also affected by several factors that affect the immune response of the host among them neutrophil function abnormalities (Page et al., 1985) or excessive production of inflammatory cytokine/prostaglandin (Offenbacher, 1996).

On the other hand, it is given that a pathogen may need to be in numbers sufficient to exceed a particular threshold to initiate disease, T. denticola may be found in healthy subjects who may be referred to as being in a carrier state (Watanabe and Frommel, 1996). Consequently, although the concentration of periodontal pathogens in subgingival sites is the critical factor that will determine whether disease will occur or progress. The results of this study revealed that the patients in the CP group were significantly more positive for T. denticola than AP and healthy subjects.

In summary, the findings, when used in conjunction with an optimized clinical examination protocol, the assay may offer
**Fig.1** Lane numbers 2, 3, 6, 7, 8, 10 and 11 showing bands of amplified DNA products at 316 bp position in Chronic Periodontitis samples.

**Graph.1** Showing PCR results for T. denticola.

**Graph.2** Showing Age wise results of control samples.
Graph.3 Showing Age wise results of Chronic periodontitis samples

Graph.4 Showing Age wise results of Aggressive periodontitis samples.

a rapid, useful, and cost-effective tool for monitoring the presence of T.denticola in sub gingival plaque samples from both healthy and diseased patients and correlating it with the amount and extent of periodontal breakdown.

The study concluded that 16S rRNA-based PCR technique is a rapid, useful, and cost-effective tool for monitoring the presence of T.denticola in sub gingival plaque samples from both healthy and diseased patients.

**References**


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