Periodontal Status in Relation to the Pathogens *Porphyromonas gingivalis* and *Tannerella forsythensis* in the Three Main Ethnic Groups in Malaysia.

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Sponsoring information:
This study is supported by University of Malaya Research Fund 02/11.

**Abstract:**
**Objective:** To evaluate the periodontal status in relation to the periodontopathogens *Porphyromonas gingivalis* (Pg) and *Tannerella forsythensis* (Tf) in the three main ethnic groups in Malaysia.

**Materials and Methods:** A total of 44 adult subjects (16 Malays, 16 Indians and 12 Chinese) aged 35-65 years were selected. Periodontitis patients (PP) were associated with pocket depth of ≥ 6mm at ≥ 4 sites. Healthy subjects (HS) had probing depths ≤ 3mm and no evidence of attachment loss clinically or bone loss radiographically. Subgingival plaque samples were taken from the deepest pockets ≥ 6mm in the periodontitis subjects and from areas without bleeding upon probing in the healthy subjects. Clinical parameters included plaque index, gingivitis index, bleeding on probing, probing pocket depth, clinical attachment level, and number of missing teeth were measured. The presence of Pg&Tf was assessed by polymerase chain reaction.

**Results:** The prevalence of Pg and Tf in the Indians was highest (Pg 75%, Tf 62.5%), followed by the Chinese (Pg 53.8%, Tf 50%) and the Malays (Pg 43.8%, Tf 50%). The differences among PP were
significant for prevalence of Tf (P <0.05) and among HS were significant for prevalence of Pg (P <0.05).

The Indian participants were associated with higher levels of periodontal clinical scores as compared to the subjects of the other 2 ethnic groups.

**Conclusion:** The study suggests that there are ethnic differences in bacterial colonization of periodontal pockets both in disease & health participants. It was also noted that the Indians were highly predisposed to periodontal infection by Pg & Tf.

**Keywords:** Periodontal disease, periodotopathogens. Porphyromonas gingivalis, Tannerella forsythensis.

1. **Introduction:**

A large number of epidemiological studies have led to the widely accepted conclusion that the prevalence and severity of periodontal disease differed in different populations (Ali et al., 1997; Baelum et al., 1996; Dzink et al., 1988). Knowledge of the variability of disease occurrence between and within populations is important because such knowledge may assist in identifying the determinants in disease occurrence. Marked differences between populations would indicate the causes of disease are not inevitable.

Multiple risk markers or putative risk factors for periodontal diseases have been examined in many studies (Beck et al., 1990; Graig et al., 2002, Meurman et al., 1997). Increased odds ratios for severe disease has been documented for certain “background” factors (Offenbacher, 1996) such as ethnic group, low socioeconomic or educational status, certain systemic conditions such as diabetes; smoking; and occurrence of certain bacteria such as *Porphyromonas gingivalis*, Bacteroides forsythus (*Tannerella forsythensis*), Actinobacillus actinomycetemcomitans and *Prevotella intermedia* in subgingival plaque (Dahlen et al., 1988). *Porphyromonas gingivalis* and *Tannerella forsythensis* occur in elevated proportions in periodontal pockets experiencing active periodontal breakdown and possess numerous virulence factors (Ebersole et al., 2008; Grenier et al., 1995; Schenkein et al., 1993).

The prevalence of some subgingival periodontal pathogens seem to vary among subjects of different ethnic backgrounds (Oliver et al., 1991; Ebersole et al., 2008; Wara-aswapati et al., 2009), an observation which in part may explain the race/ethnicity-related differences in periodontal disease severity. Differences in global periodontal disease severity, in part, may be due to varying oral hygiene practices. There may also be a possibility that certain populations harbour high levels of particularly virulent periodontal microorganisms.

*Pg* and *Tf* are the strongest bacterial markers for destructive periodontal disease and have been isolated frequently in subjects with periodontitis and sometimes in those without disease (20). Retrospective studies have suggested that microbiological assays for critical levels of the target bacteria such as *Pg* and *Tf* might be of diagnostic value (Jenkinson & Dymok, 1999; Papapanou, 1998; Shelburne et al., 2008).

The purpose of this study was to assess the periodontal status in the 3 main ethnic groups in Malaysia in relation to the identified pathogens, *Pg* and *Tf*.

2. **Materials and Methods**

2.1 **Study sample and patient selection**

A cross-sectional study was carried out on subjects who were referred to the Specialist Clinic, Faculty of Dentistry, University of Malaya. All subjects received detailed information sheet concerning the nature of
procedures involved in the study. Informed consent of the patients was obtained according to the ethical guidelines of the Medical Ethical Committee at the Faculty of Dentistry, University of Malaya. Ethical approval was obtained prior to the commencement of the study [Ethics approval No. DFPE0301/0002(P)]. Inclusion criteria required subjects who were of Malay, Indian or Chinese descent, aged between 35 to 65 years, had a minimum of 20 teeth, had not received periodontal or antibiotic therapy within the previous six months, female participants were not pregnant, and did not require antibiotic prophylaxis before dental treatment. The study sample included 16 Malays, 16 Indians and 12 Chinese. The diseased subjects (22 subjects) were those diagnosed with advanced chronic periodontitis who were matched by ethnicity, age and gender with periodontally healthy subjects (22 subjects). Periodontitis patients were selected among those presenting with probing depths of ≥ 6 mm at ≥ 4 teeth (van Winkelhoff et al. 2002), bleeding on probing and radiographic evidence of bone loss at these sites. The periodontally healthy subjects were those with probing depths of ≤ 3mm, gingivitis score of <20% and no evidence of attachment loss either clinically or radiographically (Slots & Listgarten, 1988).

2.2 Clinical measurements
A series of clinical indices were used to record the baseline clinical measurements for the involved teeth excluding the third molars. The plaque index and gingivitis index included 4 sites of the tooth, while, bleeding index, probing pocket depth and loss of attachment assessment were carried out at 6 sites of the tooth using William’s periodontal probe.

2.3 Collection of Subgingival Plaque Samples
Supragingival plaque was first removed from the sample site with a hand curette. The site was then cleaned with cotton pellets and dried. The subgingival plaque was then collected with sterile Gracey curettes applied to the root surface. In the periodontitis group, subgingival plaque was obtained from 8-12 pockets (per patient) with ≥ 6 mm probing depth. In the periodontally healthy group, subgingival plaque was collected from 9-10 sites (for each subject) that did not show any sign of bleeding on probing (Slots & Ting, 1999). Subgingival plaque sample was pooled and resuspended in a tube containing 1 ml of 10% phosphate buffer solution and stored at – 80 °C to be assessed later by polymerase chain reaction (PCR).

2.4 Polymerase Chain Reaction Protocol
Patients’ plaque samples were thawed and then centrifuged at 2100 x g for 15 sec, and a 5 µl aliquot of the supernatant were added to the PCR reaction mixture (Loesche et al., 1992). Hot start technique was utilized to obtain maximum sensitivity and specificity. The primers were selected from the DNA sequence of the fimbrial protein of P. gingivalis and were used at a final concentration of 250ng/25 µL reaction mixture (Meurman et al., 1997). Sense primer was 5' ATAATGGAGAACAGCAGGAA 3', and the antisense primer was 5' TCTTGCCAACCAGTTCCATTTGC3'. For T. forsythensis, the following primers were selected: upper primer, 5' AAA ACA GGG GTTCCG CAT GG 3' (identical to the upper strand, bases 180-199; the respective bases in E. coli 16S rRNA sequence are 148-167); and lower primer, 5' TTC ACC GCG GAC TTA ACA GC 3' (identical to the lower strand, bases 586-605; the respective bases in E. coli 16S rRNA sequence are 568-587). The thermocycler used in this study was PTC-100 Programmable Thermal Controller (MJ Research, Inc., Watertown, MA, USA). The completed reactions were chilled to 4°C. Ten µl of the PCR reaction products from each reaction tube were then mixed with 2 µl of orange blue loading dye and loaded into the tank wells containing 1.5% agarose gel
stained with 10mg/ml ethidium bromide. After 45 min of electrophoresis at 100V, the gel was placed in the gel analyzer and the PCR products were visualized by UV illumination to detect the presence of 426bp and 131bp PCR products for *Tf* and *Pg* respectively.

2.4.1 Polymerase Chain Reaction Quality Control
The negative control was a standard PCR mixture with the plaque sample being replaced by 5 µL of sterile deionized water. The positive control used was a standard PCR reaction mixture containing either 5 µL of *Tf* or *Pg* cells instead of the sample. The bacterial strains (*Tf* ATCC 43037 and *Pg* ATCC 33277) used as positive controls were obtained from the Oral Microbiology Services Laboratory at the Institute of Dentistry, University of Helsinki, Finland.

2.5 Statistical Analysis
Statistical differences among groups were determined using Kruskal-Wallis test for non-parametric variables and one way ANOVA was used for parametric data. To examine the relationships between the presence of the specific bacteria and the level of the clinical scores (PI, GI, BS, CAL, PPD, and number of missing teeth), 2-sided 2-sample t-test was used to compare the mean clinical scores of subjects in whom the bacteria were positively or negatively identified.

3. Results
The prevalence of *Tf* in the subgingival plaque of the Indian subjects was highest (62.5%) as compared to the Chinese (50%) and Malay subjects (50%) which however was not significant. The difference among the diseased subjects of the 3 ethnic groups was significant for the prevalence of *Tf* (*p*<0.05), but was not significant for the prevalence of *Pg*. The difference among healthy subjects was significant for the prevalence of *Pg* (*p*<0.05), but was not significant for *Tf* (analysis with the Kruskal-Wallis test) as shown in Table 1.

3.1 Mean clinical scores in *Pg* and *Tf*-positive or negative subjects
In the Malay subjects (diseased and healthy), all clinical scores were higher in *Pg* and *Tf*-positive subjects; which were significant for all clinical scores (except for missing teeth and BOP) in *Pg*-positive subjects, while it was significant only for GS in *Tf*-positive subjects. All clinical scores in the Chinese (diseased and healthy), were higher in *Pg* and *Tf*-positive subjects except for the mean of missing teeth which was higher in *Pg* and *Tf*-negative subjects. However, all the differences between the *Pg* and *Tf*-negative subjects were not significant. In the Indians (diseased and healthy), all clinical scores were higher in *Pg* and *Tf*-positive subjects (Tables 8 and 9). PS, GS, CAL measurements, BOP scores were significantly higher in *Pg*-positive subjects (*p* < 0.05). However, all the clinical scores were not statistically significant in *Tf*-positive Indian subjects (*p* > 0.05) as shown in Table 2.

4. Discussion
A prospective cross-sectional study was conducted on diseased participants who were matched by ethnicity, age and gender with healthy participants. The periodontal pathogens *Pg* and *Tf* were detected by hot start PCR technique which provides reasonably sensitive and specific measures of the putative pathogens that are present in the periodontal pockets. It has been estimated that about half of microorganisms present in the dental plaque are unculturable, which is a major obstacle to the
understanding of the etiology of periodontal infection. Therefore, molecular biology techniques have been introduced as highly specific means for the detection of periodontopathic bacteria. The prevalence rate may be affected by the choice of detection method, with recognized differences in sensitivity and specificity between methods (Loesche et al., 1992). This study revealed that deep pockets were not a prerequisite ecological environments for colonization by putative pathogens (Dahlen et al., 1992), although these species were recovered more frequently from deeper sites & from diseased subjects. Pathogens are often carried in low numbers in mouths free of destructive periodontal diseases (the so-called “carrier state”), making their role in disease more difficult to evaluate.

In the present study, it appeared that *P. gingivalis* and *T. forsythensis* were identified in a number of the healthy subjects with no clinical periodontal symptoms observed. In healthy subjects, the number of pathogens may be very low which however can be detected by PCR technique, but detection of their presence may identify these subjects as carriers and are at risk of developing periodontitis in the future (van Winkelhoff et al., 2002). The study suggests that there are ethnic differences in the bacterial colonization of periodontal pockets both in disease & health. The results indicated that 87.5% of the Indian healthy subjects appeared to have a significant prevalence of *Pg* (p<0.05) as compared to the healthy Malays and Chinese, indicating a possible greater potential in future breakdown of the periodontium of the Indians. In addition, the prevalence of *Tf* in the Indian diseased subjects was significant (p< 0.05) as compared to diseased Malays and Chinese correlating to the greater extent of periodontal destruction in this ethnic group.

The relationship between the presence of specific bacteria and the level of clinical scores was investigated. In the diseased group, the mean PS, GS, BOP, CAL, PPD and number of missing teeth were higher in *Pg* and *Tf*-positive patients as compared to *Pg* and *Tf*-negative patients. In the healthy groups, *Tf*-positive subjects were associated with higher means of plaque and gingival scores as compared to *Tf*-negative healthy subjects. In *Pg*-positive healthy individuals, mean PS and GS were higher than in *Pg*-negative subjects, which however were not significant. This indicates that diseased subjects with these pathogens had greater periodontal destruction reinforcing observations by other investigators on these organisms pathogenicity (Wilson et al., 1995; Meurman et al., 1997; Holt et al., 1999).

A higher number of Indian subjects were found to harbour *Pg* and *Tf* more than the Malays and Chinese, which may explain, to a certain extent, why the Indian subjects had a higher level of clinical periodontal destruction as compared to the participants of the other 2 ethnic groups.

5. Conclusion

*P. gingivalis* & *T. forsythensis* were more prevalent in the diseased than healthy subjects. The study suggests that there are ethnic differences in bacterial colonization of periodontal pockets both in disease & health. It was also noted that the Indians were highly predisposed to periodontal infection by both *Pg* & *Tf* and were associated with higher levels of clinical scores as compared to the Malays & Chinese. *P. gingivalis* & *T. forsythensis* were identified in a number of control subjects. This may indicate that the deep pockets are not a prerequisite for colonization by putative pathogens and the presence of periodontal pathogens may be associated with no clinical signs.

Acknowledgment

This study is supported by University of Malaya Research Fund 02/11.
References


Table 1: Prevalence of *P* gand *T* f in the Malay, Chinese and Indian subjects

<table>
<thead>
<tr>
<th></th>
<th>Malys</th>
<th>Chinese</th>
<th>Indians</th>
<th>Total</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HS</td>
<td>CP</td>
<td>total</td>
<td>HS</td>
<td>CP</td>
<td>total</td>
<td>HS</td>
</tr>
<tr>
<td><em>P</em> g</td>
<td>25% (n=2)</td>
<td>62.5% (n=5)</td>
<td>43.8% (n=7)</td>
<td>16.7% (n=1)</td>
<td>100% (n=6)</td>
<td>58.3% (n=7)</td>
<td>50% (n=4)</td>
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<td><em>T</em> f</td>
<td>37.5% (n=5)</td>
<td>62.5% (n=3)</td>
<td>50% (n=8)</td>
<td>16.7% (n=1)</td>
<td>83.3% (n=5)</td>
<td>50% (n=6)</td>
<td>25% (n=2)</td>
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</table>

HS: Healthy subjects; CP: Chronic periodontitis patients, n: number of subjects
K.W: Kruskal-Wallis test.
* : Significant differences among healthy or disease subjects
Table 2: Mean clinical scores in \textit{Pg} and \textit{Tf} positive or negative subjects

<table>
<thead>
<tr>
<th></th>
<th>Mean Clinical Score (Malays)</th>
<th>Mean Clinical Score (Chinese)</th>
<th>Mean Clinical Score (Indians)</th>
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<tbody>
<tr>
<td>PS</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>\textit{Pg} positive</td>
<td>1.82*</td>
<td>1.56</td>
<td>2.12*</td>
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<tr>
<td>\textit{Pg} negative</td>
<td>1.38</td>
<td>1.51</td>
<td>1.58</td>
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<tr>
<td>\textit{Tf} positive</td>
<td>1.73</td>
<td>1.60</td>
<td>2.0</td>
</tr>
<tr>
<td>\textit{Tf} negative</td>
<td>1.44</td>
<td>1.51</td>
<td>1.67</td>
</tr>
<tr>
<td>GS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{Pg} positive</td>
<td>1.86*</td>
<td>1.47</td>
<td>2.04*</td>
</tr>
<tr>
<td>\textit{Pg} negative</td>
<td>1.31</td>
<td>1.46</td>
<td>1.45</td>
</tr>
<tr>
<td>\textit{Tf} positive</td>
<td>1.77*</td>
<td>1.50</td>
<td>1.91</td>
</tr>
<tr>
<td>\textit{Tf} negative</td>
<td>1.34</td>
<td>1.45</td>
<td>1.52</td>
</tr>
<tr>
<td>PPD</td>
<td></td>
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<tr>
<td>\textit{Pg} positive</td>
<td>3.31*</td>
<td>3.30</td>
<td>3.76</td>
</tr>
<tr>
<td>\textit{Pg} negative</td>
<td>2.47</td>
<td>2.57</td>
<td>2.70</td>
</tr>
<tr>
<td>\textit{Tf} positive</td>
<td>3.10</td>
<td>3.13</td>
<td>3.51</td>
</tr>
<tr>
<td>\textit{Tf} negative</td>
<td>2.62</td>
<td>2.78</td>
<td>2.90</td>
</tr>
<tr>
<td>CAL</td>
<td></td>
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</tr>
<tr>
<td>\textit{Pg} positive</td>
<td>2.85*</td>
<td>3.24</td>
<td>3.65*</td>
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<tr>
<td>\textit{Pg} negative</td>
<td>0.83</td>
<td>2.05</td>
<td>0.91</td>
</tr>
<tr>
<td>\textit{Tf} positive</td>
<td>2.52</td>
<td>2.70</td>
<td>2.92</td>
</tr>
<tr>
<td>\textit{Tf} negative</td>
<td>0.97</td>
<td>1.80</td>
<td>0.75</td>
</tr>
<tr>
<td>Missing teeth</td>
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<td></td>
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<tr>
<td>\textit{Pg} positive</td>
<td>3.75</td>
<td>2.04</td>
<td>3.10</td>
</tr>
<tr>
<td>\textit{Pg} negative</td>
<td>3.0</td>
<td>3.71</td>
<td>1.17</td>
</tr>
<tr>
<td>\textit{Tf} positive</td>
<td>4.11</td>
<td>0.67</td>
<td>2.40</td>
</tr>
<tr>
<td>\textit{Tf} negative</td>
<td>2.43</td>
<td>3.67</td>
<td>2.00</td>
</tr>
<tr>
<td>% BOP</td>
<td></td>
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</tr>
<tr>
<td>\textit{Pg} positive</td>
<td>36</td>
<td>50</td>
<td>53</td>
</tr>
<tr>
<td>\textit{Pg} negative</td>
<td>25</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>\textit{Tf} positive</td>
<td>34</td>
<td>43</td>
<td>48*</td>
</tr>
<tr>
<td>\textit{Tf} negative</td>
<td>23</td>
<td>33</td>
<td>27</td>
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* = \( p < 0.05 \)

PS plaques score, GS gingival score, PPD probing pocket depth, BOP bleeding on probing.