Association between Gram-Negative Enteric Rods, Porphyromonas gingivalis and Changes in Clinical Parameters in Chronic Periodontitis: An Observational Study


Department of Periodontology, Faculty of dental Sciences, Ramaiah University of Applied Sciences, Bangalore-560054

Abstract: The correlation between Gram negative enteric rods and Porphyromonas gingivalis in periodontal diseases has received little attention in the literature. The objective of this study was to investigate the association between gram negative enteric rods and Porphyromonas gingivalis and changes in clinical parameters in chronic periodontitis subjects. Occurrence of Gram negative enteric rods, Porphyromonas gingivalis and clinical parameters were examined in 30 subjects with chronic periodontitis. Sub gingival plaque samples were collected from the deepest periodontal pocket and transported in thyoglycolate broth and organisms were cultured. Chi-square, Mann-Whitney and Spearman rank correlation coefficient were used to assess the clinical data. Gram negative enteric rods, Porphyromonas gingivalis were detected in 20 subjects. There were significantly positive correlations between Gram negative enteric rods, Porphyromonas gingivalis and bleeding on probing, probing depth, clinical attachment loss. This study suggests that presence of enteric rods and Porphyromonas gingivalis were related to adverse periodontal conditions. These results could have an impact on periodontal treatment and should be taken into account in the mechanical and antimicrobial treatment of periodontal disease in some populations.

Keywords: Gram negative rods, Porphyromonas gingivalis, chronic periodontitis

1. Introduction

Periodontitis, a biofilm-related infection with mixed microbial etiology. Sub gingival biofilm hosts a variety of bacterial species and only a few have been associated positively with disease progression. Porphyromonas gingivalis is present in 85% of the diseased sites in chronic periodontitis. They can adhere and rapidly invade oral epithelial cells and subsequent invasion. Gram negative enteric rods have shown the capacity to invade human tissue and produce enterotoxins. Hence the aim of the study was to investigate the association between Gram negative enteric rods, Porphyromonas gingivalis and clinical parameters in subjects with chronic periodontitis.

2. Methodology

A total of 30 subjects reported to the Department of Periodontology, Faculty of Dental Sciences, Ramaiah University of Applied Sciences in Bangalore, who were diagnosed with chronic periodontitis were included. Informed and written consents were obtained from each participant. The study design was approved by the Ethical Committee and study is carried out for one month i.e. from December 2015 – January 2016.

Inclusion criteria’s
- Age group 25 - 65 years
- Pocket depth ≥ 4mm
- Bleeding on probing
- Clinical attachment level ≥ 5mm

Exclusion criteria’s
- Subjects with history of any systemic diseases.
- Subjects who smoke.
- With history of periodontal treatment in previous 6 months.
- Who are pregnant or lactating
- Who used antibiotic or other drugs that affect periodontal status in the past 6 months.
- Regularly using CHX mouthwash.

Clinical Evaluation
Microbial sampling and isolation of *Porphyromonas gingivalis* by culture

Microbial sampling on periodontitis patients was performed on pockets ≥4 mm. The deepest pockets were selected for sampling. After removing supragingival plaque with curettes and isolating the area with cotton pellets, the absorbent paper points were inserted into each periodontal pocket for 20 seconds. The paper points were transferred to a tube with thyoglycolate medium. All samples were labeled properly and processed within four hours after sampling. The samples were analyzed using microbial culture techniques for the presence of periodontopathic bacteria, most samples were processed at room temperature (25°C) and incubated in CO2 and anaerobic culture systems. The Trypticase Soy Serum Bacitracin Vancomycin agar medium was incubated in 10% CO2 at 37°C for four days. Then colonies are seen on culture plates which were seen as black pigmented colonies after gram staining. Total viable counts (TVC) were defined as the total number of colony forming units obtained on non-selective media plates. Species found on selective media were enumerated and their percentage of TVC was calculated.
Isolation of Gramnegative enteric rods by culture:
After placement for 20 s, the paper points were pooled into a vial containing thymoglycolate transport medium. The sample vials were maintained at room temperature, transferred to the laboratory, and processed within 4 h after sampling. After the vials were placed in an incubator for 30 min at 37°C, bacterial plaque was mechanically dispersed with a test tube mixer at the maximal setting for 60 s. Serial 10-fold dilutions were prepared in pepton water, and aliquots were plated on MacConkey agar. The plates were incubated aerobically at 37°C for 24 h. Each isolate was characterized according to colonial and cellular morphology and Gram stain characteristics. Gramnegative enteric rods were speciated using a standardized biochemical test. Total viable counts were defined as the total number of colony forming units obtained on non-selective media plates. Species found on selective media were enumerated and presented as counts × 10^5.
3. Statistical Analysis

Data were entered into an Excel. The database was subsequently locked, imported into Statistical Package for Social Sciences (SPSS) for Windows, formatted, and analyzed. Indicators of descriptive statistics were used, such as frequencies, percentage, average, variance, and standard deviation. The presence of Porphyromonas gingivalis and Gram-negative enteric rods was determined in individuals with at least one infected pocket. PD and CAL differences and the presence of Porphyromonas gingivalis and Gram-negative enteric rods were determined by the Mann-Whitney test. Association among Porphyromonas gingivalis and Gram-negative enteric rods was assessed through a nonparametric correlation coefficient (Spearman rank). Only sites presenting concomitantly CAL and PD of 4 mm or more at baseline were considered in the analyses of CAL, PD, and BOP. The significance level was set at 0.05 for all tests.

4. Results

Among 30 patients examined, Gram-negative enteric rods and Porphyromonas gingivalis were detected in 20 individuals, respectively. A total of 20 (66.66%) patients harbored both microorganisms studied. Gram-negative enteric rods in periodontal pockets were highly significant and positively correlated with presence of P. gingivalis (r=0.652, P<0.0001) and also both organisms were highly significant and positively correlated with PD, CAL (Table 1 and Fig 1).

Table 1: Comparison of presence and absence of Porphyromonas gingivalis and Gram-negative enteric rods with clinical parameters by Mann-Whitney U test

<table>
<thead>
<tr>
<th>Variables</th>
<th>Absence of p. gingivalis and gram-negative enteric rods</th>
<th>Presence of p. gingivalis and gram-negative enteric rods</th>
<th>U-value</th>
<th>Z-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Sum of ranks</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>BOP</td>
<td>1.05</td>
<td>0.09</td>
<td>241.50</td>
<td>1.03</td>
<td>0.13</td>
</tr>
<tr>
<td>PD</td>
<td>5.53</td>
<td>0.99</td>
<td>145.50</td>
<td>7.87</td>
<td>1.73</td>
</tr>
<tr>
<td>CAL</td>
<td>6.27</td>
<td>1.22</td>
<td>145.50</td>
<td>7.40</td>
<td>1.35</td>
</tr>
</tbody>
</table>

Porphyromonas gingivalis is highly and significantly correlated with PD when compared to CAL and BOP (Table 2 and figure 2).

Table 2: Comparison of presence and absence of Porphyromonas gingivalis with clinical parameters by Mann-Whitney U test

<table>
<thead>
<tr>
<th>Variables</th>
<th>Absence of p. gingivalis</th>
<th>Presence of p. gingivalis</th>
<th>U-value</th>
<th>Z-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Sum of ranks</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>BOP</td>
<td>1.07</td>
<td>0.10</td>
<td>180.00</td>
<td>1.03</td>
<td>0.11</td>
</tr>
<tr>
<td>PD</td>
<td>5.10</td>
<td>0.74</td>
<td>68.50</td>
<td>7.50</td>
<td>1.67</td>
</tr>
<tr>
<td>CAL</td>
<td>6.20</td>
<td>1.40</td>
<td>68.50</td>
<td>7.15</td>
<td>1.31</td>
</tr>
</tbody>
</table>
Table 3: Comparison of presence and absence of gram negative enteric rods with clinical parameters by Mann-Whitney U test

<table>
<thead>
<tr>
<th>Variables</th>
<th>Absence of gram negative enteric rods</th>
<th>Presence of gram negative enteric rods</th>
<th>U-value</th>
<th>Z-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Sum of ranks</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>BOP</td>
<td>1.06</td>
<td>0.07</td>
<td>169.50</td>
<td>1.03</td>
<td>0.12</td>
</tr>
<tr>
<td>PD</td>
<td>5.80</td>
<td>1.03</td>
<td>115.50</td>
<td>7.15</td>
<td>1.98</td>
</tr>
<tr>
<td>CAL</td>
<td>6.70</td>
<td>1.25</td>
<td>115.50</td>
<td>6.90</td>
<td>1.48</td>
</tr>
</tbody>
</table>

Table 4: Correlation between CFU counts of *P. gingivalis* and gram -ve enteric rods with clinical parameters by Spearman’s rank correlation

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>N</th>
<th>Spearman R</th>
<th>t-value</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOP</td>
<td>30</td>
<td>-0.3325</td>
<td>-1.8657</td>
<td>0.0726</td>
</tr>
<tr>
<td>PD</td>
<td>30</td>
<td>0.7820</td>
<td>6.6385</td>
<td>0.0001*</td>
</tr>
<tr>
<td>CAL</td>
<td>30</td>
<td>0.2529</td>
<td>1.3834</td>
<td>0.1775</td>
</tr>
</tbody>
</table>

Table 5: Correlation between CFU counts of *Porphyromonas gingivalis* with gram negative enteric rods by Spearman’s rank correlation

<table>
<thead>
<tr>
<th>Variables</th>
<th>Correlation between CFU counts of <em>P. gingivalis</em> with Gram negative enteric rods</th>
<th>N</th>
<th>Spearman R</th>
<th>t-value</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>0.5297</td>
<td>3.3046</td>
<td>0.0026*</td>
</tr>
</tbody>
</table>
5. Discussion

In this study, we investigated the relationships between P. gingivalis and Gram negative enteric rods and clinical parameters from patients with untreated chronic periodontitis. Information from the present study may have therapeutic implications for the treatment of non oral infections caused by oral pathogens. Dissemination of periodontal pathogens to other body sites frequently occurs and may cause serious diseases.

The ecology of oral sub gingival communities in health and periodontitis and elucidate the relationship between inflammation and the sub gingival micro biome. Supra-and sub gingival bacterial biofilm development assumes a crucial part in the improvement and movement of the infection, with gram-negative anaerobic rods and spirochetes commanding sub gingival polymicrobial biofilms in chronic periodontitis. The results of present study also showed a positive correlation of presence of gram negative enteric rods in sub gingival plaque samples (4).

Carlos M et.al in 2012 found that there were significantly positive correlations between enteric rods and presence of P. gingivalis and both microorganisms were significantly and positively correlated with clinical parameters. The results of the present study shows a significant positive correlation between Porphyromonas gingivalis ,gram negative enteric rods and clinical parameters.(5)

Carlos M et.al in 2011 showed that the mean probing depth (mm) of the sampled sites was significantly deeper in patients with presence of P.gingivalis when compared to Gram negative enteric rods.(6)

Lafaurie et.al in 2007 showed that P. gingivalis occurred in 71.5% and enteric rods occurred in 34.5% of individuals with chronic periodontitis. The present study shows that both P.gingivalis and gram negative enteric rods occurred in 66.66% of individuals with chronic periodontitis subjects.(8)

In a study by Thomasae in 1997 showed negative association between gram-negative enteric rods and A. Actinomycetemcomitansrelated to the ecological interrelationships that occur among subgingival microorganisms inhabiting deep periodontal pockets in humans. The results of present study showed P.gingivalis are inhabited more in deepest pockets than gram negative enteric rods in chronic periodontitis patients.(7,9)

The investigation of the subgingival microbiota in a specific nation gets to be related to distinguish its conceivable effect on results after treatment. A larger examination would be more appropriate to study connections between Gram negative enteric rods and P.gingivalis further. Contrasts in host response, oral cleanliness, oral therapeutic administrations access, and microbial species may clear up these refinements in the clinical articulation of periodontitis in the population concentrated. More thorough examinations tending to the relationship amongst periodontitis and natural and inherited variables are required.(10)

6. Conclusion

This study concludes that presence of gram negative enteric rods and P.gingivalis were related to cause periodontal conditions. These results could have an impact on
periodontal treatment and should be taken into account in the mechanical and antimicrobial treatment of periodontal disease in some populations.

7. Future Scope

Further long term studies have to be carried out for establishment of specific treatment strategies in larger populations in different parts of other countries.

References


Author Profile

**Corresponding author:** Dr. Bhavya. B is Reader, Department of Periodontology, Faculty of Dental Sciences, Ramiah University of Applied Sciences, Bangalore-560054, India

**Dr. Ashwini. S** is Professor and Head of the Department, Department of Periodontology, Faculty of Dental Sciences, Ramiah University of Applied Sciences, Bangalore-560054

**Dr. Shruthi. K. R** is Post graduate Student, Department of Periodontology, Faculty of Dental Sciences, Ramiah University of Applied Sciences, Bangalore-560054