International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

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

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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.(1)They can adhere and rapidly invade oral epithelial cells (2).its fimbriae mediates initial attachment and subsequent invasion (3). 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

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Bleeding on probing elicited using a straight probe

Probing pocket depth elicited using a UNC-15 probe.

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 Trypticicase Soy Serum Bacitracin Vancomycin agar medium was incubated in 10% CO2 at 37°C for four days. Thencolonies 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.



Collecting sub-gingival plaque using absorbent paper point.

Collected sample is transferred into a transport medium (thyoglycolate)



TSBV Media is poured on plates

Transferring sample on to medium



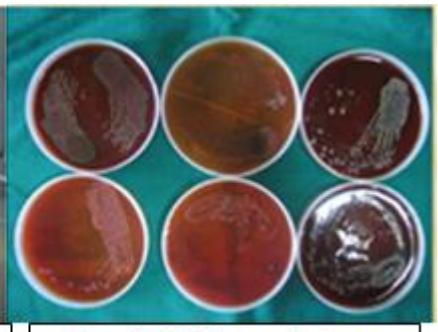
Incubating sample in 10% CO2 at 37°c for 4days

Colonies formed after incubation

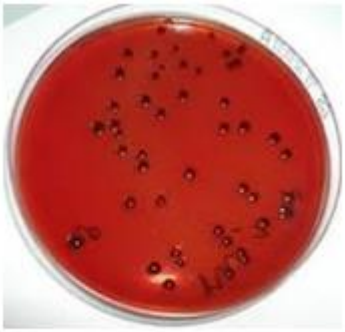
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After incubation inoculate micro organisms on non-selective media

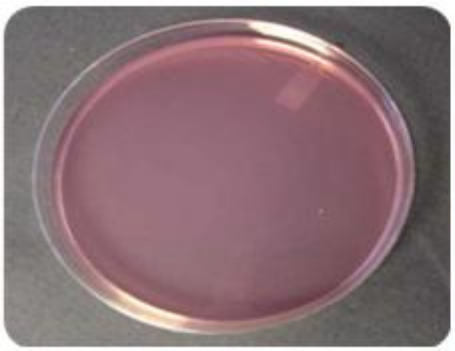


Growth of different colonies on non- selective media.



Growth of black-pigmented colonies on non selective blood agar

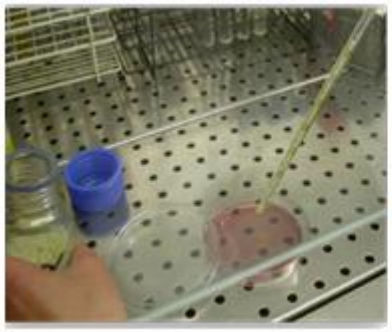
Isolation of Gramnegative enteric rods by culture:
Afterplacement for 20 s, the paper points were pooled into avial containing thyoglycolate transport medium.The sample vials were maintained at room temperature,transferred to the laboratory, and processed within 4 h aftersampling. After the vials were placed in an incubator for30 min at 37°C, bacterial plaque was mechanically dispersedwith a test tube mixer at the maximal setting for 60 s. Serial10fold dilutions were prepared in pepton water, and aliquotswere plated on MacConkey agar. The plates were incubatedaerobically at 37°C for 24 h. Each isolate was characterizedaccording to colonial and cellular morphology and Gramstaincharacteristics. Gramnegative enteric rods were speciatedusing a standardized biochemical test.Total viable counts weredefined as the total number of colony forming units obtainedon non selective media plates. Species found on selective mediawere enumerated and presented as counts $\times 10^5$.



Mac conkey agar plate



Streaking the plate

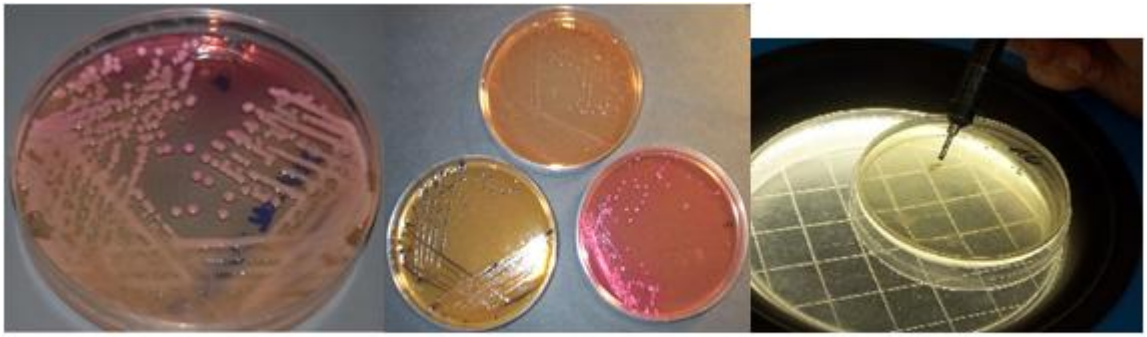


Processing the sample with in 4hrs



Incubated at 37°C for 24hours

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Colonies formed on mac conkey agar plates

Colony forming units are counted

3. Statistical Analysis

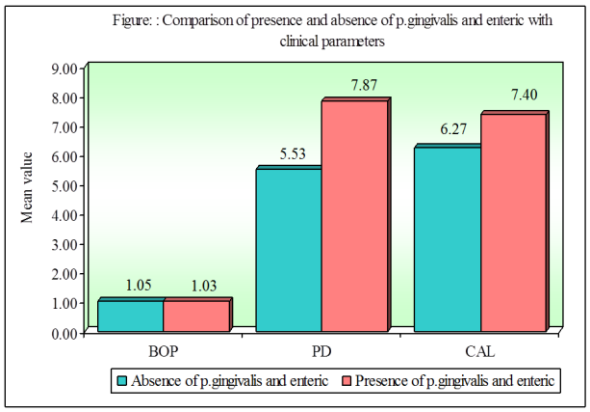
Data were entered into an Excel, The database was subsequently locked, importedinto Statistical Package for Social Sciences (SPSS) for Windows,formatted, and analyzed. Indicators of desciptive statistics wereused, such as frequencies, percentage, average, variance, andstandard deviation. The presence of *Porphyromonas gingivalis*and Gramnegative enteric rodspositive individuals weredescribed as the percentage of individuals with at least infected pocket. PD and CAL differences and the presence or absence of*Porphyromonas gingivalis* and Gramnegative enteric rods weredetermined by the MannWhitney test. Association among *Porphyromonas gingivalis* and Gramnegative enteric rods wasexpressed through a nonparametric correlation coefficient(Spearman rank). Only sites presenting concomitantly CALand PD of 4 mm or more at baseline were considered in theanalyses of CAL, PD, and BOP. The significance level was setat 0.05 for all test.

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 was 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

Variables	Absence of p.gingivalis and gram-ve enteric rods			Presence of p.gingivalis and gram –ve enteric rods			U-value	Z-value	P-value
	Mean	SD	Sum of ranks	Mean	SD	Sum of ranks			
BOP	1.05	0.09	241.50	1.03	0.13	223.50	103.50	-0.3733	0.7089
PD	5.53	0.99	145.50	7.87	1.73	319.50	25.50	-3.6086	0.0003*
CAL	6.27	1.22	145.50	7.40	1.35	284.00	61.00	-2.1361	0.0327*



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

Variables	Absence of p.gingivalis			Presence of p.gingivalis			U-value	Z-value	P-value
	Mean	SD	Sum of ranks	Mean	SD	Sum of ranks			
BOP	1.07	0.10	180.00	1.03	0.11	285.00	75.00	-1.0999	0.2714
PD	5.10	0.74	68.50	7.50	1.67	396.50	13.50	-3.8055	0.0001*
CAL	6.20	1.40	68.50	7.15	1.31	351.00	59.00	-1.8038	0.0713

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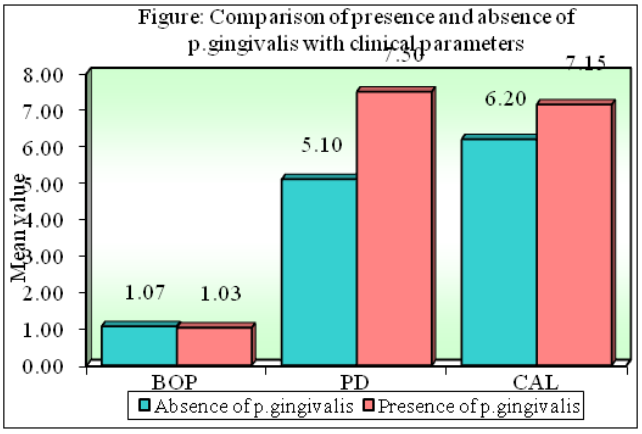


Table 3: Comparison of presence and absence of gram negative enteric rods with clinical parameters by Mann-Whitney U test

Variables	Absence of gram negative enteric rods			Presence of gram negative enteric rods			U-value	Z-value	P-value
	Mean	SD	Sum of ranks	Mean	SD	Sum of ranks			
BOP	1.06	0.07	169.50	1.03	0.12	295.50	85.50	-0.6379	0.5235
PD	5.80	1.03	115.50	7.15	1.98	349.50	60.50	-1.7378	0.0823
CAL	6.70	1.25	115.50	6.90	1.48	315.00	95.00	-0.2200	0.8259

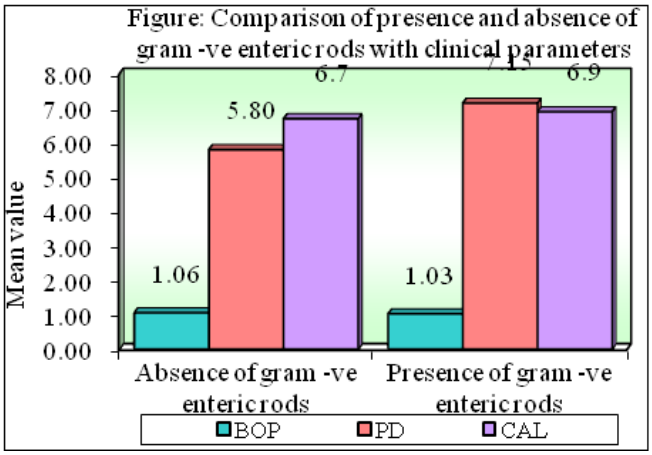


Table 4: Correlation between CFU counts of p. gingivalis and gram -ve enteric rods with clinical parameters by Spearman's rank correlation

Clinical parameters	N	Spearman R	t-value	p-level
BOP	30	-0.3325	-1.8657	0.0726
PD	30	0.7820	6.6385	0.0001*
CAL	30	0.2529	1.3834	0.1775

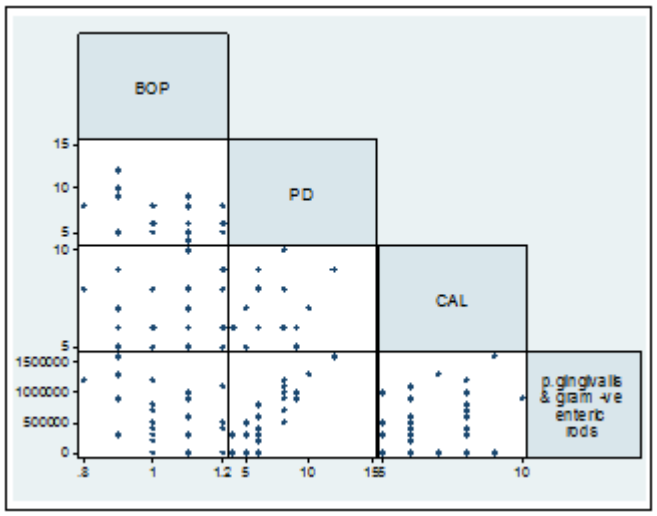
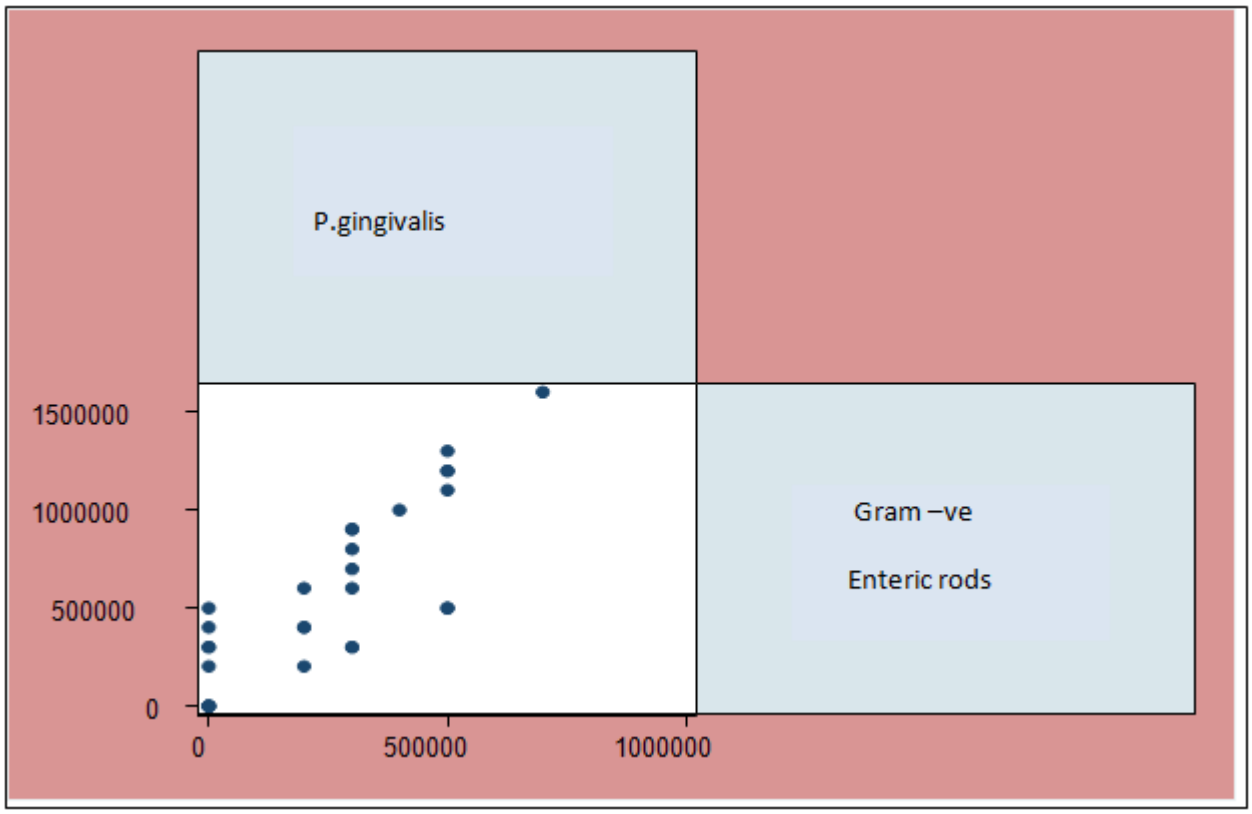


Table 5: Correlation between CFU counts of *Porphyromonas gingivalis* with gram negative enteric rods by Spearman's rank correlation

Variables	Correlation between CFU counts of p. gingivalis with Gram negative enteric rods			
	N	Spearman R	t-value	p-level
	30	0.5297	3.3046	0.0026*

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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 and Gram-negative enteric rods. The present study also shows that the mean probing depth of the sampled sites were 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

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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.

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