International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

Detection of *Peptostreptococcus Micros* in Periodontal Health and Disease Using Culture Technique

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Abstract: ***Context**: Periodontal diseases are infectious diseases resulting in inflammation within the supporting tissues of the teeth. There are various periodontopathogens that cause periodontal diseases. One such micro-organism is Peptostreptococcus micros. However, they are often overlooked and they are very difficult to isolate. Hence the aim of the present study is to determine an association of Peptostreptococcus micros in periodontal health and disease. **Aims**: To detect Peptostreptococcus micros in patients with healthy periodontium and chronic periodontitis using culture technique and to compare the prevalence of Peptostreptococcus micros between these two groups. **Methods and Material**: Seventy five subjects were recruited in the study. Based on clinical examination, the subjects were divided into Periodontally healthy individuals and chronic periodontitis individuals. The subgingival plaque samples collected from each subject were inoculated on selective media, the colonies were identified and colony forming units (CFU) counted. **Statistical analysis used**: Comparison of CFU counts in healthy and chronic periodontitis group was done by one way ANOVA and Correlation between CFU counts with clinical parameters by Spearmans rank correlation test. **Results**: The mean log of CFU counts in chronic periodontitis group was significantly more than that in healthy group. Also, statistically significant values were found between the log Colony forming unit counts and the clinical parameters. **Conclusions**: The present study ascertains the correlation of the prevalence of Peptostreptococcus micros to the severity of periodontal disease.*

Keywords: *Peptostreptococcus micros*, periodontitis, culture method

1. Introduction

Periodontal diseases are infectious diseases resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss and bone. The etiology of periodontal diseases is multifactorial and depends on many factors like social factors, environmental factors, genetic and other factors with microbial dental plaque being the initiator of the disease. It is known that the initiation and progression of the periodontal disease is by the gram negative micro-organisms that are present in the subgingival plaque.

There are various known periodontopathogens that cause periodontal diseases. One such micro-organism that is associated with periodontitis is *Peptostreptococcus micros* (formerly known as *Micromonas micros* and currently known as *Parvimonas micra*) and has been more frequently and in higher numbers isolated from patients having active sites in periodontitis. *Peptostreptococcus micros* is a gram positive cocci and is considered to be the normal commensal of the oral cavity[1]. *Peptostreptococcus micros (P.micros)* is also associated with other mixed anaerobic infections of the oral cavity, like the periodontal abscesses, endodontic abscesses and peritonsillar infections[2].

P.micros are the second most frequently recovered anaerobes and account for approximately one quarter of anaerobic isolates found. They are often overlooked and they are very difficult to isolate. Hence the aim of the present study is to determine an association of *Peptostreptococcus micros* in periodontal health and disease.

2. Objectives of the Study

The objective of the study was to detect *Peptostreptococcus micros* in patients with healthy periodontium and chronic periodontitis using culture technique and to compare the prevalence of *Peptostreptococcus micros* between healthy and chronic periodontitis subjects.

3. Materials and Method

The subjects for the study was selected amongst the consecutive patients who were referred to the Periodontology department of our institute, for an assessment of oral health status; and the samples were analyzed at the Department of Molecular Biology and Immunology at Maratha Mandal's Nathajirao G Halgekar Institute of Dental Sciences & Research Centre.

Methods of collection of data

A total of 185 patients were screened. Seventy five subjects, aged 20 to 65 years, satisfying the inclusion and exclusion criteria were explained about the study in their vernacular language and after signing a written informed consent were recruited in the study. The subjects for chronic periodontitis were selected based on the criteria as described in detail by AAP International Workshop for classification of periodontal diseases, 1999[3].

Based on clinical examination, the subjects were divided into Periodontally healthy individuals (Group I) and chronic periodontitis individuals (Group II). All subjects selected had a minimum of 20 natural teeth.

The inclusion criteria of Group I was that there should be no clinical signs of gingival inflammation, absence of bleeding on probing, probing depth ≤ 3mm and no clinical Attachment Loss. The inclusion criteria of Group II was that there should be presence of clinical signs of gingival inflammation, presence of bleeding on probing, probing depth ≥ 5mm and clinical

Attachment Loss ≥ 3mm. The subjects who have received

periodontal therapy or antimicrobial therapy within 3months before sampling, history of any systemic diseases/ conditions, habit of smoke/smokeless tobacco, pregnant woman and lactating mother were excluded from the study.

In the Group II subgingival plaque samples were collected from 3 deepest sites with probing depth of ≥ 5mm and in Group I plaque samples were collected from normal gingival sulcus. A thorough periodontal examination was carried out and parameters selected for the study were carefully recorded. Single examiner did all the recordings. The clinical parameters like the plaque index, gingival index, probing depth and clinical attachment loss were recorded.

Clinical samples were collected using strict asepsis. The selected sites were isolated with sterile cotton rolls and air-dried. Supragingival plaque and calculus was removed. The sub gingival plaque samples were collected from each subject from the most apical portion of the accessible probing depth and alongside the root of the tooth at all possible sites using a sterile universal curette, which was then transferred into a micro centrifuge tube containing transport medium (reduced transport fluid) and sent to the laboratory for culture analysis. The subgingival plaque sample was then inoculated on selective media phenyl ethyl alcohol agar (PEA) and incubated aerobically for 72hrs at 37^0C. After 72hrs the colonies were identified (Figure 1) and colony morphology was confirmed by microscopy. Colony forming units (CFU) were counted.

4. Statistical Analysis

Distribution of age, sex predilection and prevalence of P. micros in healthy and chronic periodontitis group was done using Chi – square test. Comparison of colony forming unit (CFU) counts in healthy and chronic periodontitis group was done by one way ANOVA. Kruskal Wallis way ANOVA and Mann - Whitney U test was used for the comparison of clinical parameters. Correlation between CFU counts with clinical parameters was done using Spearmans rank correlation test. Normality of all variables scores in two study groups was done by Kolmogorov Smirnov test.

5. Results

In the healthy subject group out of 25 subjects, 11 were males and 14 females. In chronic periodontitis group 9 were males and 16 females (Graph 1). In healthy subject group, 48% of patients were in the age group of 31-40 yrs and 52% of patients were in the age group of 41-50 yrs in the chronic periodontitis group (Table 1).

The detection of *Peptostreptococcus micros* in periodontal health and disease was done using the culture method. The comparison between the mean log CFU counts of healthy and chronic periodontitis group was done using independent t test. The mean log CFU counts in healthy group was 2.54 ± 1.96 and that of the chronic periodontitis group was 3.79 ± 1.44. Statistically significant value (p=0.0133) was seen when comparison between the healthy and chronic periodontitis group was made (Graph 2).

The correlation between log Colony forming unit counts with clinical parameters like plaque index, gingival index, probing depth and clinical attachment loss was done by the Karl Pearson's correlation coefficient method. Statistically significant values were found between the log Colony forming unit counts and plaque index, gingival index, probing depth and clinical attachment loss with p value 0.0005, 0.0003, 0.0001 and 0.0001 respectively (Table 2). Normality of all variables scores in healthy and chronic periodontitis was done by Kolmogorov Smirnov test (Table 3). All parameters / variables scores in two study groups were found to follow a normal distribution. Therefore, the parametric tests were applied.

6. Discussion

The prevalence of *P. micros* showed that the mean value of log CFU counts increased from healthy individuals to chronic periodontitis subjects. The mean value of log CFU counts in healthy group and chronic periodontitis group was 2.54 ±, 1.96 and 3.79 ± 1.44 respectively. The comparison of log CFU counts between the healthy and chronic periodontitis group was made. The comparison between the healthy and chronic periodontitis group with respect to mean log CFU count was found to be statistically significant with p value 0.0133. This could be because the number of anaerobic organisms increase with increase in the periodontal pocket depth.[4]

This present study was in accordance with the Claudia Nonnenmacher et al[4], found a statistically significant difference when periodontitis patients were compared to healthy subjects for the presence of *P. micros* with p value 0.0001. *P. micros* presented in statistically higher numbers (2.45×10^5) in aggressive periodontitis patients in comparison to chronic periodontics subjects (7.23×10^4) with p value 0.003.

Another study conducted by M .P. Riggio et al.[5] they used the PCR technique for rapid and specific identification of this organism in clinical samples. 68 subgingival plaque samples were analysed of which 19 were positive for *P. micros* DNA. The proportion of patients carrying *P. micros* DNA in at least one sampled site was 11 (61%) of 18. They confirmed the results that *P. micros* may be involved in the etiology of adult periodontitis.

Studies conducted by Moore et al[6] also found that the presence of *P. micros* occurred significantly more frequently and at higher proportions in subgingival samples in active than in inactive deep periodontitis lesions using culture methods.

Similar supporting study was conducted by Rams T E et al.[7] It was a cross-sectional study involving subjects with advanced adult periodontitis, early-onset periodontitis and localized juvenile periodontitis. *P. micros* was isolated from subgingival samples on anaerobic enriched blood agar plates and identified on the basis of cellular and colonial morphology and selected biochemical tests. *P. micros* demonstrated a significantly higher prevalence in disease active than in disease inactive patients. Tanner et al[8] and Dzink et al[9] also found significant association between *P. micros* and disease-active periodontitis.

Edit Urban et al[10] compared between commercial PCR-based hybridization methods with conventional anaerobic culture technique was done. Subgingival plaque samples were collected from periodontal pockets of pregnant women with chronic localized periodontitis. The overall agreement between both methods was excellent for *P. micros (Micromonas micros)* and pathogens. There was only one sample, where the culture method was negative and the PCR- based method could detect *P. micros.*

Clinical parameters like the plaque score, gingival bleeding score, probing depth and clinical attachment loss value were taken into account in our present study and we found that the clinical parameters are directly proportional to the total number of CFU counts of *P. micros*. Hence according to our present study it can be concluded that *P. micros* has a positive correlation with the clinical parameters.

According to Claudia Nonnenmacher et al[4] a significant correlation was observed between the number of *P. micros* and the clinical parameters like probing depth (PD), bleeding on probing (BOP). A significant positive correlation was observed for higher levels of *P. micros* with PD ≥ 7 mm. Rams et al also found that *P. micros* occurred significantly more frequently and at higher proportions in active than in inactive deep periodontitis pockets.

7. Conclusion

In the present study the prevalence of *Peptostreptococcus micros* increased from health to periodontitis patients thus ascertaining their correlarion to the severity of periodontal disease. The effect of periodontal treatment on the incidence and prevalence of *P. micros* was not evaluated in the present study. For the confirmation of *P. micros*, sequencing can be done using 16S rRNA amplification. Studies can be done to correlate different genotypes with phenotypic traits and its virulence. Thus longitudinal studies and clinical trials are needed to be carried out to know the exact association of *P. micros* in periodontal health and disease.

Foot Notes:

Table 1 and 2:
*PI – Plaque index
† GI – gingival Index
‡ PD – pocket depth
§ CAL – clinical attachment loss

Table 2:
‖ - CFUs – colony forming units

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Figure Legends

Figure 1: Selective media showing *Peptostreptococcus micros*
Graph 1: Distribution of sex predilection in healthy and chronic periodontitis group
Graph 2: Comparison of two study groups with respect to mean log CFU counts

Table 1: Distribution of patients by age groups in two study groups

Age groups	Chronic periodontitis group	%	Healthy group	%	Total
<=30yrs	2	8	8	32	10
31-40yrs	4	16	12	48	16
41-50yrs	13	52	4	16	17
51-60yrs	6	24	1	4	7
Total	25	100	25	100	50

Chi-square=15.9361 P = 0.0010*

Mean age	45.52	34.92	40.22
SD age	7.75	6.18	8.76

*p<0.05

Table 2: Correlation between CFU counts with clinical parameters in Chronic periodontitis group and healthy group by Karl Pearson's correlation coefficient method

Groups	Clinical parameters	Correlation between CFU counts with clinical parameters		
		r-value	t-value	p-value
Chronic periodontitis group	PI*	-0.1685	-0.8196	0.4208
	GI†	-0.2367	-1.1685	0.2546
	PD‡	0.0248	0.1191	0.9062
	CAL§	-0.0607	-0.2916	0.7732
Healthy group	PI	-0.1185	-0.5725	0.5726
	GI	0.0736	0.3539	0.7267
	PD	-0.2269	-1.1174	0.2753
	CAL	--	--	--
Total samples	PI	0.4740	3.7296	0.0005*
	GI	0.4941	3.9374	0.0003*
	PD	0.5407	4.4526	0.0001*
	CAL	0.5464	4.5201	0.0001*

*p<0.05

Table 3: Normality of all variables scores in two study groups by Kolmogorov Smirnov test

Variables	Chronic periodontitis group		Healthy group	
	Z-value	p-value	Z-value	p-value
CFUs‖	0.7870	0.5660	1.0770	0.1960
PI	0.6950	0.7200	1.1760	0.1260
GI	0.5410	0.9320	0.9300	0.3530
PD	1.1750	0.1199	1.0500	0.2200
CAL	0.6420	0.8050	-	-