Isolation and Evaluation of Microbial Flora in Patients with Chronic Periodontitis: A Microbiological Study

Mitesh Parekh¹, Venkataramana Pammi², S Bharath Varthdana¹, Dharam M Hinduja³, Mohil M Asnani⁴, Anis Ahmed⁵

Abstract

Background: The periodontal diseases comprise of a group of diseases involving gingiva, periodontal ligament, cementum, and alveolar bone. Although multifactorial, the main etiological factor in causing periodontitis is the presence of microorganisms. Animal model studies have shown a positive significant correlation between the presence of dental plaque and occurrence of periodontal pathologies. Hence, we aim to isolate and evaluate the microbial flora in patients with chronic periodontitis: A microbiological study.

Materials and Methods: A total of 100 patients reporting with the problem of chronic periodontitis were included in the study. Samples were collected twice. Initially, before the commencement of any treatment and second, following scaling and root planning treatment along with antibiotic therapy after 1 week. Specimens were sent to the microbiology laboratory for evaluation and assessment. McConkey agar, 5% sheep blood agar and agar plates containing hematin and Vitamin K were used for assessment of growth of colonies of microorganisms.

Results: Average age of the patients was between 45 and 65 years. 76 of the isolates from the patients were polymicrobial in nature. 34 polymicrobial specimens were combination of 2 isolates while 42 samples were a combination of 3 or more isolates. 89% of the total isolates were strictly anaerobes while remaining were aerobic in nature. Fusobacterium species were the most common among the anaerobes.

Conclusion: Periodontitis patients comprises of a wide morphological diverse microbial flora which should be considered while planning treatment.

Key Words: Anaerobes, microorganisms, periodontitis

Introduction

Periodontal diseases refer to a group of diseases involving tooth supporting structures. Gingivitis precedes the development of periodontitis. Gingivitis refers to the inflammation of the gingivae while inflammation of tooth supporting structures (periodontal ligament, bone, etc.) is referred to as periodontitis. Host, pathogenic species, and the absence "beneficial bacteria" are generally regarded as the principle factors responsible for causing periodontitis. One of the primary and etiologic factors in the causation of periodontal pathologies is dental plaque. Experimental studies in both humans and animals have revealed a positive significant correlation between the presence of dental plaque and occurrence of periodontal pathologies. Some of these studies also show that the inflammatory process associated with periodontal pathologies gets reversed when appropriate measures are taken for plaque control. Most commonly microflora associated with periodontal diseases are Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, and Bacteroides forsythus. Salivary microbial flora count has been regarded by some authors as a risk factor for the occurrence of periodontitis. Those people are regarded as high-risk individuals for the development of periodontitis, who have salivary count of Streptococcus mutans and P. gingivalis of more than 10⁷ and 4 × 10⁸ colony forming units/mL. Therefore, we undertook this study to isolate and evaluate the microbial flora in patients with chronic generalize periodontitis.

Materials and Methods

The study comprised 100 patients reporting in the dental wing of hospitals with problem of chronic periodontitis. The patients aged between 45 and 65 years and were predominantly males. The periodontal examination was done, and samples were obtained from the subgingival part of the periodontal pockets for evaluation. Patients with any history of systemic illness, diabetes, hypertension, or any known drug allergy were excluded from the study. Patients on any kind of antibiotic therapy in the last 4 months were also excluded from the study. Sample collection was done 2 times. Initially, the samples were collected before the commencement of any treatment and second, after 1 week following scaling and root planning.
treatment along with antibiotic therapy. For collection of the sample, first of all, surfaces of the tooth were dried using gauze piece to prevent saliva contamination. Samples were collected from the subgingival region using a periodontal curette and were sent to microbiological laboratory, in a test tube containing transporting medium, and immediate processing of the specimen sample was done. Modification of Fontana method and Gram-staining method was used for anaerobes identification.³⁹ McConkey agar, 5% sheep blood agar and agar plates containing hematin and Vitamin K were used for assessment of growth of colonies of microorganisms. Anaerobic microorganisms were evaluated semi-quantitatively by counting the number of colonies. Organisms with a significant number of colonies were again subcultures in Brucella agar media containing Hemin and Vitamin K (BRU). After assessment of the morphology of the colonies along with pigmentation, fluorescence and hemolysis in BRU plates, Gram-staining, tryptophan peptone broth test, and catalase test were done. Antibiotics (kanamycin and vancomycin) were added for further identification of anaerobes. Method described by Vaidhyalingam and Laxminarayana was used for obtaining the anaerobes.⁴⁰ Antimicrobial testing was done using Kirby’s method. All the results were analyzed using SPSS software. Paired t-test was used to measure the level of significance.

Results
We evaluated a total of 100 patients of chronic periodontitis which were 45-65 years of age with an average age of 54 years. 76 of the isolates from the patients were polymicrobial in nature (P < 0.05) (Table 1). Further, 34 out of 76 polymicrobial specimens were combination of 2 isolates, whereas 42 samples were a combination of 3 or more isolates (Table 2). 89% of the total isolates were strictly anaerobes, whereas rest of 11 were aerobic in nature (Table 3). Out of 89, 69 isolates consisted of Gram-negative anaerobes while rest of them were Gram-positive anaerobes (Table 4). Among the anaerobes, Fusobacterium species were the most common one (Graph 1).

Discussion
A total of 100 patients having chronic periodontitis were selected for the study. The patients were between 45 and 65 years of age with average age of 54 years. Benachinmardi et al., in their study, observed maximum cases of periodontitis between 38 and 47 years of age group.¹⁰ Antony et al. also found maximum cases between 30 and 60 years of age.¹¹ The increased prevalence of periodontitis with age is supported by old fact that its prevalence with age increases due to slow progressive loss of tooth supporting periodontal structures.¹²

Our results showed that more maximum isolates (76%) from the patients were polymicrobial in nature (Table 1). Saini et al., in their study on microbial flora assessment, found similar results showing polymicrobial nature of the disease.¹³ Similar results were seen in the studies of Salari and Kadkhoda and Nonnenmacher et al., who confirmed the presence of polymicrobial isolates in their samples.¹⁴,¹⁵ Studies depicting contrary results are also mentioned in the past literature. Results of studies of Salari and Kadkhoda showed less than 20% polymicrobial isolates in the whole of their study samples.¹⁴ The difference in results of varying studies depicting difference in rates of both quantity and quality of bacteria can be attributed to the difference in patient selection criteria in all the studies, the difference in sites from which samples were selected, type of culturing methods and tests employed and specificity and sensitivity of those tests for detecting microorganisms.¹³ On further assessing the polymicrobial isolates, we could not find any significant difference between types of microorganisms.

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Table 1: The nature of microbial flora in periodontitis patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total cases (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymicrobial</td>
<td>76 (76)</td>
<td>0.0014 (s)</td>
</tr>
<tr>
<td>Monomicrobial</td>
<td>14 (14)</td>
<td></td>
</tr>
<tr>
<td>Oral commensal</td>
<td>10 (10)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: The nature of polymicrobial isolates in periodontitis patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Out of total 76 cases (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination of 2 isolates</td>
<td>34 (45)</td>
<td>0.251 (n.s)</td>
</tr>
<tr>
<td>Combination of 3 or more isolates</td>
<td>42 (55)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: The division of microbial flora in periodontitis patients on the basis of oxygen requirement.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total cases (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobes</td>
<td>89 (89)</td>
<td>0.0021 (s)</td>
</tr>
<tr>
<td>Aerobes</td>
<td>11 (11)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: The division of anaerobes in periodontitis patients on the basis of Gram-staining.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Out of total 89 cases (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-negative anaerobes</td>
<td>69 (77.5)</td>
<td>0.0012 (s)</td>
</tr>
<tr>
<td>Gram-positive anaerobes</td>
<td>20 (22.5)</td>
<td></td>
</tr>
</tbody>
</table>

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Graph 1: Prevalence (%) of various anaerobic Gram-negative microorganisms.
present (Table 2). Our findings were similar to the results obtained by Benachinmardi et al., who also observed non-significant difference in the composition of the polymicrobial isolates.10

The results of our study show the predominance of anaerobic microorganisms (89%) in chronic periodontal lesions (Table 3). This highly significant prevalence of anaerobes in periodontitis cases have also been reported in the literature by Van Winkelhoff et al.,19 Mane et al.20 Literature quotes some studies with decreases amount of strict anaerobes in periodontitis patients. Nonnenmacher et al. detected comparatively lower fraction of anaerobes in their results.15 Difference in site of collection of samples, method employed for the detection of particular microorganism and the sensitivity of these methods may be reason for the difference in results of prevalence of anaerobes in above-mentioned studies.15,16 While evaluating the anaerobic microorganisms, we observed that amount Gram-negative anaerobes were significantly more than Gram-positive anaerobes (P < 0.05). Daniluk et al. and Socransky et al. reported similar findings in their respective studies.18,19 We also further assessed the anaerobic species and observed that out of 89 isolates of anaerobes, 69 samples (77.5%) were of Gram-negative bacilli (GNB) (Table 4). Fusobacterium species predominated out of all (GNB). Other species found among the anaerobes were Gram-positive bacilli such as Micromonas micros, Peptostreptococcus anaerobius, and S. mutans. Out of 11 aerobic samples, species identified were Staphylococcus aureus (6 samples) and Streptococcus pneumonia (5 samples).

The most common anaerobes observed in our study were Bacteroides fragilis, species of Porphyromonas, Fusobacterium, Prevotella intermedia, Fusobacterium nucleatum, and other Prevotella spp. (Graph 1). Studies quoted in the literature also depict presence of anaerobes in different proportions. Mane et al. analyzed the presence of anaerobic microorganisms in periodontitis patients and compared it with normal healthy individuals and found anaerobes in 83% of periodontitis cases consisting chiefly of P. gingivalis (48%), F. nucleatum (24%), Peptostreptococcus micros (23%), and Prevotella spp. (26%). Their results showed diversity of Anaerobes spp. in periodontitis patients.17 Daniluk et al. also evaluated the microbial flora in subgingival and supragingival plaques of adult patients with periodontal disease and observed more prevalence of aerobic (62.3%) than anaerobic bacteria (37.7%).18 According to Socransky et al., some species of Peptostreptococcus belongs to a second major group (complex) of organisms.19 The other major group of microorganisms includes F. nucleatum, Campylobacter rectum, Eikenella corrodens, Eubacterium nodatum, Selenomonos noxia, P. micros, Streptococcus intermedius, and Treponema denticola.19-21 In the initiation and progression of periodontal lesions, the exact precise role of these microorganisms is less defined as compared to that of “Black” complexes.18 We found 11% of the total microorganisms in the aerobic category in our study. The results were in correlation with the results of Daniluk et al., who observed 14% aerobes out of all microorganisms.18 On applying modified Fontana staining, Spirochetes (T. denticola) were observed in 29% of cases. Benachinmardi et al. also observed approximately 30% of spirochetal microorganisms in their study.10

Various culture techniques can be used for the detection of bacteria present in the periodontal pockets. Recognition of bacteria in the periodontal pockets can be treated more efficiently with the help of antibiotics.22

Conclusion
From the above findings, we conclude that periodontitis patients comprise of a wide morphological diverse microbial flora. Therefore, microbial flora should be put into consideration while planning for treatment therapy of periodontal diseases. A variety of microbiological tests and methods are available these days for isolation and identification of different microorganisms. Hence, dental field should also put this microbial flora into considerations in today’s treatment protocols.

References
11. Antony B, Kurian R, Faizal M, Verma BR, Shivananda PG. Actinobacillus actinomycetemcomitans and anaerobes in...