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ORIGINAL **R**ESEARCH

Assessment of microbial flora in periodontal pockets of non-vital teeth with advanced periodontitis

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ABSTRACT:

Background: The progression of periodontal disease leads to destruction of the periodontal tissue, allowing bacterial plaque to progress deeper and deeper into the periodontal pocket. More than 500 different bacterial species have been estimated to reside in the subgingival plaque. Hence; the present study was undertaken for assessing the microbial flora in periodontal pockets of non-vital teeth with advanced periodontitis. **Materials & Methods:** A total of 12 patients with advanced periodontitis were enrolled. Among these 12 patients, a total of 25 non-vital teeth involved with apical periodontitis were assessed. Swabs were obtained from the periodontal pockets with the help of sterilized paper points. Sampling of subgingival pocket plaque was done from the deepest portion of the periodontal pocket. Assessment of the bacterial colonies was done by culture methods. **Results:** In 60 percent of the teeth, cocci were identified, while in 52 percent of the teeth, rods were identified. In 44 percent of the teeth, spirochetes were identified. Streptococcus and Peptococcus species were found to be present in 40 percent and 44 percent of the teeth. Eubacterium and Actinomyces were found to be present in 28 percent and 24 percent of the teeth. **Conclusion:** Streptococcus and Peptococcus were the predominant micro-organism in periodontal pockets of non-vital teeth of patients with advanced periodontitis. **Key words:** periodontal pockets, micro-organisms, Flora

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INTRODUCTION

The progression of periodontal disease leads to destruction of the periodontal tissue, allowing bacterial plaque to progress deeper and deeper into the periodontal pocket. The periodontal diseases are a diverse group of clinical entities in which induction of an inflammatory process results in destruction of the attachment apparatus, loss of supporting alveolar bone, and, if untreated, tooth loss. Periodontal disease is one of the most common diseases of the oral cavity and is the major cause of tooth loss in adults.¹⁻³

More than 500 different bacterial species have been estimated to reside in the subgingival plaque. However, studies from the 1930s to 1970s focused on the nonspecific plaque hypothesis which implicated the overall mass of the microbiota as the key factor in the initiation of tissue destruction rather than stressing the significance of specific bacterial species. This theory was subsequently challenged when different bacterial species were seen to play a pivotal role in the initiation and progression of periodontal disease, which supported the specific plaque hypothesis.^{4, 5}

As the biofilm begins to mature, there is a progressive shift from a gram-positive, facultative flora to one predominated by gram-negative, anaerobic species. This shift is associated with the development of the biofilm beneath the gingival surface. The supragingival (above the gingival surface) and subgingival (beneath the gingival surface) habitats differ in terms of pH, redox potential, and nutrient availability.⁵⁻⁷ Hence; the present study was undertaken for assessing the microbial flora in periodontal pockets of non-vital teeth with advanced periodontitis.

MATERIALS & METHODS

The present study was conducted with the aim of assessing the microbial flora in periodontal pockets of non-vital teeth with advanced periodontitis. A total of 12 patients with advanced periodontitis were enrolled. Among these 12 patients, a total of 25 non-vital teeth

involved with apical periodontitis were assessed. Before the starting of the study, written consent was obtained from all the patients after explaining in detail the entire research protocol. Only those patients were included in which carried free teeth were found to have advanced periodontitis and were diagnosed of having non-vital pulp as assessed by electric pulp testing. Swabs were obtained from the periodontal pockets with the help of sterilized paper points and were placed carefully in 2 ml of anaerobic transport culture medium, and subsequently transferred quickly into an anaerobic glove box. Sampling of subgingival pocket plaque was done from the deepest portion of the periodontal pocket. Assessment of the bacterial colonies was done by culture methods. All the results were recorded in Microsoft excel sheet and were analysed by SPSS software.

RESULTS

In the present study, a total of 12 patients with advanced periodontitis were analysed. Mean age of the patients was found to be 43.8 years. 8 patients were males while the remaining 4 patients were females. Majority of the patients belonged to the age group of more than 40 years. In 60 percent of the teeth, cocci were identified, while in 52 percent of the teeth, rods were identified. In 44 percent of the teeth, spirochetes were identified.

In the present study, Streptococcus and Peptococcus species were found to be present in 40 percent and 44 percent of the teeth. Eubacterium and Actinomyces were found to be present in 28 percent and 24 percent of the teeth.

DISCUSSION

Periodontal disease is an inflammatory process that affects the protective and supportive tissues around the tooth. Bacterial plaque accumulation on the tooth surface leads to marginal tissue inflammation, known as gingivitis. Gingivitis is fairly common and is present in up to 90% of the US population. If left untreated, gingivitis may progress to periodontitis, which is characterized by loss of periodontal attachment support (clinical attachment loss, [CAL]) and bone resorption, eventually resulting in tooth mobility and loss. Chronic periodontitis is a common disease characterized by a painless, slow progression. Good plaque control, as well as removal of bacteria, calculus deposits, and granulation tissue by mechanical instrumentation constitutes an effective approach to reduce signs of inflammation in most cases of chronic periodontitis.⁵⁻⁷ In periodontitis, pathobionts and kevstone pathogens such as Porphyromonas gingivalis appear in greater proportion in than health. As a keystone pathogen, P. gingivalis impairs host immune responses and appears necessary but not sufficient to cause periodontitis. Historically, dental caries had been causally linked to Streptococcus mutans. Contemporary microbiome studies now indicate that singular pathogens are not obvious in either caries or periodontitis. Both diseases appear to result from a perturbation among relatively minor constituents in local microbial communities resulting in dysbiosis.⁸⁻¹⁰ Hence; the present study was undertaken for assessing the microbial flora in periodontal pockets of non-vital teeth with advanced periodontitis.

In the present study, a total of 12 patients with advanced periodontitis were analysed. Mean age of the patients was found to be 43.8 years. 8 patients were males while the remaining 4 patients were females. Majority of the patients belonged to the age group of more than 40 years. In 60 percent of the teeth, cocci were identified, while in 52 percent of the teeth, rods were identified. In 44 percent of the teeth, spirochetes were identified. Kobayashi T et al assessed the microflora from root canals and periodontal pockets of periodontally affected teeth. Caries-free teeth affected with advanced periodontitis and diagnosed as clinically dead by electric pulp testing were selected. The root canals and periodontal pockets were sampled, and the bacterial flora examined by both culture and interference microscopy. The results indicated that the aerobe/anaerobe ratio in the periodontal pocket was 0.23, while it was 0.0022 in the root canal, the large predominance of obligate anaerobes reflecting the anaerobic environment found in the root canal. Morphological classification obtained from interference microscopy showed similar proportions of morphotypes in the two sites.





Species	Number of teeth	Percentage of teeth
Streptococcus	10	40
Peptococcus	11	44
Unidentified gram positive cocci	9	36
Eubacterium	7	28
Actinomyces	6	24
Veilonella	4	16
Amylophylus	5	20
Fusobacterium	5	20

Table 1: Distribution of bacteria in periodontal pocket of non-vital teeth with advanced periodontitis

Results of anaerobic culture demonstrated a significantly higher rate of detection of facultative Streptococcus bacteria in the periodontal pocket than in the root canal. The predominant bacterial species common to both regions were Streptococcus, Peptostreptococcus, Eubacterium, Bacteroides, and Fusobacterium for obligate anaerobes. As for facultative anaerobes, Actinomyces and Streptococcus were detected predominantly in the periodontal pocket. The occurrence of micro-organisms common to both sites in this study suggested that the periodontal pocket may be a possible source of root canal infections.¹⁰

In the present study, Streptococcus and Peptococcus species were found to be present in 40 percent and 44 percent of the teeth. Eubacterium and Actinomyces were found to be present in 28 percent and 24 percent of the teeth. Scapoli L et al designed a real-time polymerase chain reaction bases assay to detect and quantify red complex species, then used to investigate 307 periodontal pocket samples from 127 periodontitis patients and 180 controls. Significant higher prevalence of red complex species and increased amount of P. gingivalis and T. denticola were detected in periodontal pocket of periodontitis patients. Results demonstrated that the test is a valuable tool to improve diagnosis of periodontal disease. Three bacterial species that constitute the red complex group, Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola, are considered the main pathogens involved in periodontitis.¹¹ Some studies have identified bacteria that appear to be associated with oral health. For instance, a positive association has been observed between oral health and the presence or *Capnocytophaga* of Veillonella ochracea. Additionally. Streptococcus salivarius tends to inhabit the dorsal surface of the tongue of healthy patients who lack halitosis. An ambitious study which attempted to define the normal oral microbiome found that species such as Streptococcus mitis, Gemella haemolysans, and Granulicatella adiacens were common in healthy subjects.12-14

CONCLUSION

From the above results, the authors concluded that Streptococcus and Peptococcus were the predominant micro-organism in periodontal pockets of non-vital teeth of patients with advanced periodontitis.

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