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PCR-BASED DETECTION OF ORAL MICROORGANISMS IN PERIODONTAL DISEASEDCARDIOVASCULAR PATIENTS AND HEALTHY INDIVIDUALS

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ABSTRACT

Introduction - Periodontal disease is a chronic inflammatory condition associated with microbial dysbiosis within the subgingival environment. The presence of specific pathogenic microorganisms plays a significant role in the initiation and progression of periodontal destruction.

Aim - To compare the prevalence of selected oral microorganisms in periodontitis patients in cardiovascular patients and periodontally healthy individuals.

Materials and methods - A comparative cross-sectional study was conducted among 60 participants, including 30 cardiovascular patients with periodontitis and 30 healthy controls. Subgingival plaque samples were collected and analyzed using polymerase chain reaction (PCR) for the detection of selected oral microorganisms.

Result - Periodontal pathogens were detected more frequently among cardiovascular patients with periodontitis than in healthy controls. A higher prevalence of Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, and Prevotella intermedia was observed in the study group.

Conclusion- The findings indicate a strong association between periodontal pathogens and

cardiovascular patients with periodontal disease. Early microbial assessment may contribute to improved oral and systemic health outcomes.

INTRODUCTION

Periodontal disease is a multifactorial inflammatory disorder affecting the supporting structures of the teeth, including the gingiva, periodontal ligament, cementum, and alveolar bone(1). It develops as a result of complex interactions between pathogenic microorganisms and the host immune response, ultimately leading to periodontal tissue destruction and tooth loss if left untreated(2). The oral cavity harbors a diverse microbial ecosystem composed of numerous bacterial species that exist in a balanced state under healthy conditions. Disruption of this balance can result in microbial dysbiosis, promoting the growth of pathogenic microorganisms that contribute to periodontal inflammation and disease progression(3). Among the microorganisms commonly associated with periodontitis are Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, and Prevotella intermedia (4). These pathogens possess various virulence factors that enable colonization, immune evasion, and tissue destruction within the periodontal environment(5).

Periodontal disease has also been linked to several systemic conditions, particularly cardiovascular disease. Chronic periodontal infections may contribute to systemic inflammation through the dissemination of bacteria and inflammatory mediators into the bloodstream(6). Such mechanisms have been proposed to influence the development and progression of cardiovascular disorders(7). Several studies have identified periodontal pathogens within atherosclerotic plaques and cardiovascular tissues, suggesting a potential biological relationship between oral infections and cardiovascular disease(8). The presence of these microorganisms may contribute to endothelial dysfunction, vascular inflammation, and atherosclerotic

changes. Advances in molecular diagnostic methods such as Polymerase Chain Reaction (PCR) have enabled sensitive and specific detection of periodontal pathogens(9). Therefore, evaluating the oral microbial profile of periodontal diseased cardiovascular patients may provide valuable insights into the relationship between oral health and systemic disease(1).

Periodontal disease is characterized by persistent inflammation resulting from the interaction between pathogenic microorganisms and the host immune response(2). The inflammatory mediators released during periodontal infection, including cytokines and acute-phase proteins, can enter the systemic circulation and contribute to a chronic inflammatory state. This systemic inflammatory burden has been implicated in the pathogenesis of several cardiovascular conditions. The oral cavity serves as a reservoir for numerous microorganisms capable of entering the bloodstream through ulcerated periodontal pockets(3). Transient bacteremia caused by routine activities such as tooth brushing, flossing, and mastication may facilitate the dissemination of periodontal pathogens to distant organs. The presence of these microorganisms within vascular tissues suggests a possible mechanistic link between periodontal infections and cardiovascular disease progression(4).

Recent molecular studies have highlighted the importance of identifying specific periodontal pathogens in patients with systemic diseases(6). Polymerase Chain Reaction (PCR) has emerged as a highly sensitive and specific diagnostic tool for detecting bacterial DNA within subgingival plaque samples(7). The application of PCR in periodontal research enables accurate assessment of microbial profiles and provides valuable information regarding the potential association between oral pathogens and cardiovascular health in chennai population(10).

MATERIALS AND METHODS

Study Design and Study Population- This comparative cross-sectional study was conducted to evaluate the prevalence of oral microorganisms in periodontal diseased cardiovascular patients and healthy individuals. A total of 60 participants were enrolled in the study and divided into two groups. Group I consisted of 30 cardiovascular patients diagnosed with chronic periodontitis, while Group II consisted of 30 periodontally and systemically healthy individuals who served as controls. Participants were recruited from the Department of Periodontics and associated medical clinics after obtaining informed consent. Ethical clearance for the study was obtained from the Institutional Ethics Committee prior to the commencement of the study.

Inclusion and Exclusion Criteria- Patients aged between 30 and 65 years with a confirmed history of

cardiovascular disease and clinical evidence of chronic periodontitis were included in Group I. Periodontitis was diagnosed based on clinical parameters including probing pocket depth, clinical attachment loss, bleeding on probing, and radiographic evidence of bone loss. Healthy controls were selected based on the absence of periodontal disease and systemic illness. Individuals who had received periodontal therapy or antibiotic treatment within the previous three months, smokers, pregnant or lactating women,

and patients with other systemic diseases such as diabetes mellitus, autoimmune disorders, or immunocompromised conditions were excluded from the study.

Clinical Examination- A comprehensive periodontal examination was performed for all participants using a mouth mirror and William's periodontal probe under adequate illumination. Clinical parameters recorded included Plaque Index (PI), Gingival Index (GI), Probing Pocket Depth (PPD), and Clinical Attachment Loss (CAL). For cardiovascular patients, relevant medical records were reviewed to confirm the diagnosis and duration of cardiovascular disease.

Sample Collection- Subgingival plaque samples were collected under aseptic conditions. In the periodontitis group, plaque samples were obtained from the deepest periodontal pocket after isolating the area with cotton rolls and gently removing supragingival plaque. In healthy controls, samples were collected from the gingival sulcus of posterior teeth. A sterile Gracey curette was used to collect the plaque samples, which were immediately transferred into sterile microcentrifuge tubes containing transport medium and stored at -20°C until further analysis.

DNA Extraction and PCR Analysis- Genomic DNA was extracted from the collected plaque samples using a commercially available DNA extraction kit according to the manufacturer's instructions. The extracted DNA was quantified and assessed for purity before analysis. Polymerase Chain Reaction (PCR) was performed using species-specific primers targeting Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Prevotella intermedia, and Streptococcus sanguinis. PCR amplification was carried out in a thermal cycler under standardized conditions, including initial denaturation, repeated cycles of denaturation, annealing, and extension, followed by a final extension step.

Detection of PCR Products- The amplified PCR products were subjected to electrophoresis on a 1.5% agarose gel containing ethidium bromide. The gels were visualized under ultraviolet illumination using a gel documentation system. The presence of specific amplification bands corresponding to the target

microorganisms was recorded as positive detection.

Statistical Analysis- The collected data were entered into Microsoft Excel and analyzed using the Statistical Package for Social Sciences (SPSS) software version 25.0. Descriptive statistics were used to determine frequencies and percentages of detected microorganisms. Comparisons between the study and control groups were performed using the Chi-square test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Table 1. Distribution of Microorganisms in the Study Groups

Microorganism	Periodontal group (n=30)	Healthy controls (n=30)
Porphyromonas gingivalis	22 (73.3%)	4(13.3%)
Aggregatibacter actinomycetemcomitans	18(60.0%)	3(10.0%)
Fusobacterium nucleatum	24(80.0%)	8(26.7%)
Prevotella intermedia	19(63.3%)	5(16.7%)
Streptococcus sanguinis	8(26.7%)	23(76.7%)

PCR analysis revealed a significantly higher prevalence of periodontal pathogens among cardiovascular patients with periodontitis compared to healthy controls. Fusobacterium nucleatum showed the highest detection rate, followed by Porphyromonas gingivalis and Prevotella intermedia. Conversely, Streptococcus sanguinis was more frequently detected among healthy individuals, indicating the predominance of health-associated microbial flora. The findings suggest a microbial shift toward pathogenic species in periodontal diseased cardiovascular patients.

Further analysis revealed that Porphyromonas gingivalis was detected in 73.3% of cardiovascular patients with periodontitis, compared to only 13.3% of healthy controls. Similarly, Aggregatibacter actinomycetemcomitans was identified in 60.0% of the study group and 10.0% of the control group. These findings indicate a greater colonization of established periodontal pathogens among cardiovascular patients affected by periodontal disease. The detection rate of health-associated microorganisms differed considerably between the two groups. Streptococcus sanguinis was identified in 76.7% of healthy individuals, whereas only 26.7% of cardiovascular patients with periodontitis showed its presence. This reduction in beneficial commensal bacteria, along with the increased prevalence of pathogenic microorganisms, suggests a significant alteration in the subgingival microbial composition associated with periodontal disease in cardiovascular patients.

DISCUSSION

The present study compared the prevalence of selected oral microorganisms in periodontal diseased cardiovascular patients and healthy individuals using PC analysis of subgingival plaque samples. The findings

demonstrated a higher prevalence of periodontal pathogens among cardiovascular patients with periodontitis, supporting the role of microbial dysbiosis in both periodontal and systemic diseases. Among the microorganisms evaluated, Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans were detected more frequently in cardiovascular patients with periodontal disease. These pathogens possess virulence factors capable of inducing chronic inflammation, tissue destruction, and immune dysregulation. Their increased prevalence supports previous reports linking periodontal infections with cardiovascular complications. Fusobacterium nucleatum and Prevotella intermedia were also identified at higher frequencies in the study group. These microorganisms contribute to biofilm maturation and facilitate the colonization of other pathogenic species within periodontal pockets(11). Their presence may further enhance inflammatory responses and contribute to disease progression. The association between periodontal pathogens and cardiovascular disease may be explained by the ability of oral microorganisms to enter the systemic circulation during routine activities such as chewing and tooth brushing. Once in the bloodstream, these pathogens and their by-products may stimulate inflammatory pathways and contribute to endothelial dysfunction and atherosclerotic plaque formation(12).

Furthermore, PCR offers a highly sensitive and specific method for detecting periodontal pathogens, even when present in low concentrations. Compared with conventional culture methods, PCR provides rapid and accurate microbial identification, making it a valuable diagnostic tool in periodontal research(13). Despite the significant findings, the study had certain limitations, including a relatively small sample size and the evaluation of only selected microorganisms. Future studies involving larger populations and advanced molecular techniques are recommended to further explore the relationship between oral microbial dysbiosis, periodontal disease, and cardiovascular health. The high prevalence of Porphyromonas gingivalis observed in the present study may be of particular clinical significance(14). This microorganism has been extensively studied for its ability to invade epithelial and endothelial cells and induce a sustained inflammatory response. The detection of this pathogen in a large proportion of cardiovascular patients with periodontitis supports the hypothesis that periodontal infections may contribute to systemic inflammatory processes associated with cardiovascular disease. Aggregatibacter actinomycetemcomitans was also frequently detected among cardiovascular patients with periodontal disease. This bacterium produces several virulence factors, including leukotoxins and endotoxins, which can impair host immune defenses and promote periodontal tissue destruction. The increased prevalence of this microorganism among diseased individuals further emphasizes its role in periodontal pathogenesis and potential systemic implications(15). The predominance of Fusobacterium nucleatum in the study group is consistent with its recognized role as a bridging organism within dental biofilms. It facilitates the attachment and colonization of various pathogenic species, thereby enhancing microbial complexity and virulence. Its high detection rate among cardiovascular patients may indicate a more mature and pathogenic subgingival biofilm compared to healthy individuals.

The lower prevalence of Streptococcus sanguinis among cardiovascular patients with periodontitis reflects the disruption of a health-associated microbial ecosystem. Streptococcus species are considered beneficial early colonizers that contribute to microbial homeostasis and inhibit the growth of pathogenic bacteria(16). A reduction in these protective microorganisms may create favorable conditions for the establishment and persistence of periodontal pathogens. The findings of the present study highlight the importance of maintaining optimal periodontal health, particularly among individuals with cardiovascular disease. Regular periodontal

evaluation, effective plaque control measures, and timely therapeutic interventions may help reduce the burden of pathogenic microorganisms and associated inflammatory responses(17). Further longitudinal studies are recommended to investigate the causal relationship between periodontal pathogens and cardiovascular disease and to evaluate the impact of periodontal therapy on cardiovascular outcomes.

In addition to their direct role in periodontal tissue destruction, oral microorganisms have gained considerable attention due to their potential influence on systemic health(18). The relationship between periodontal disease and cardiovascular disease is believed to involve multiple biological mechanisms, including chronic systemic inflammation, endothelial dysfunction, immune activation, and bacterial translocation. Periodontal pathogens and their virulence factors may enter the bloodstream through inflamed periodontal tissues and contribute to vascular inflammation, thereby increasing the risk of cardiovascular complications. The higher prevalence of periodontal pathogens observed among cardiovascular patients in the present study further supports the concept of an oral-systemic connection(19). Although the exact nature of this association remains under investigation, the findings emphasize the importance of integrating oral health assessment into the overall management of cardiovascular patients. Collaborative efforts between dental and medical professionals may facilitate early detection of risk factors, improve patient outcomes, and contribute to a more comprehensive approach to healthcare(20).

CONCLUSION

The present study demonstrated a higher prevalence of periodontal pathogens, including *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Prevotella intermedia*, among cardiovascular patients with periodontitis compared to healthy individuals. In contrast, health-associated microorganisms such as *Streptococcus sanguinis* were more frequently detected in the control group, indicating a significant alteration in the subgingival microbial profile of diseased patients.

The findings support the concept that periodontal disease is associated with microbial dysbiosis and suggest a potential relationship between periodontal pathogens and cardiovascular disease. The increased detection of pathogenic microorganisms in cardiovascular patients may contribute to systemic inflammatory responses and further emphasize the importance of maintaining periodontal health in individuals with cardiovascular conditions.

Within the limitations of the present study, PCR proved to be a sensitive and reliable method for detecting periodontal pathogens in subgingival plaque samples. Further large-scale longitudinal studies are recommended to better understand the causal relationship between periodontal infections and cardiovascular disease and to evaluate the impact of periodontal therapy on systemic health outcomes.

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