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MOLECULAR-GENETIC ASSESSMENT OF PERIODONTAL PATHOGENS IN SUBGINGIVAL PLAQUE USING REAL-TIME PCR IN HEALTHY INDIVIDUALS AND PATIENTS WITH GINGIVITIS AND PERIODONTITIS

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Abstract:

Periodontal diseases, including gingivitis and periodontitis, are closely associated with the presence and quantity of specific periodontal pathogens. Rapid, sensitive, and specific detection of these microorganisms, including those difficult to culture, can be achieved using real-time PCR. In this study, subgingival plaque samples from healthy individuals and patients with gingivitis and periodontitis were collected and analyzed using the "ParodontoScreen" reagent kit to detect Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythensis, Treponema denticola, and Candida albicans. A. actinomycetemcomitans was most frequently detected in periodontitis patients (60.2%), significantly higher than in gingivitis patients (38%) and healthy individuals (5%). Other pathogens, including P. gingivalis, P. intermedia, T. forsythensis, and T. denticola, were also found more frequently and in higher quantities in diseased groups, with total bacterial load reaching up to 10^7 genome equivalents in periodontitis patients. Associations of pathogens, such as A. actinomycetemcomitans, P. gingivalis, and T. denticola, were common in both gingivitis and periodontitis patients, indicating the importance of microbial interactions in disease progression. These findings emphasize the significance of quantifying periodontal pathogens and their associations for predicting disease progression and guiding appropriate therapeutic interventions, with real-time PCR serving as a reliable tool for detecting species contributing to periodontal tissue destruction.

Keywords: Periodontal pathogens, real-time PCR, Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, subgingival plaque, microbial associations, periodontitis, gingivitis.

МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКАЯ ОЦЕНКА ПАРОДОНТОПАТОГЕНОВ В ПОДДЕСНЕВОМ ЗУБНОМ НАЛЁТЕ С

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ИСПОЛЬЗОВАНИЕМ ПЦР В РЕАЛЬНОМ ВРЕМЕНИ У ЗДОРОВЫХ ЛЮДЕЙ И ПАЦИЕНТОВ С ГИНГИВИТОМ И ПАРОДОНТИТОМ

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Аннотация

Пародонтальные заболевания, включая гингивит и пародонтит, тесно связаны с присутствием и количеством специфических пародонтопатогенных микроорганизмов. Быстрое, чувствительное и специфическое выявление этих микроорганизмов, включая трудно культивируемые виды, возможно с помощью ПЦР в реальном времени. В данном исследовании образцы поддесневого зубного налёта у здоровых лиц и пациентов с гингивитом и пародонтитом были собраны и проанализированы с использованием набора реагентов "ParodontoScreen" для выявления Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythensis, Treponema denticola и Candida albicans. A. actinomycetemcomitans чаще всего обнаруживался у пациентов с пародонтитом (60,2%), значительно чаще, чем у пациентов с гингивитом (38%) и у здоровых лиц (5%). Другие патогены, включая P. gingivalis, P. intermedia, T. forsythensis и T. denticola, также выявлялись чаще и в больших количествах в группах с заболеваниями, с общей бактериальной нагрузкой до 10^7 геномных эквивалентов у пациентов с пародонтитом. Ассоциации патогенов, такие как A. actinomycetemcomitans, P. gingivalis и T. denticola, были распространены как среди пациентов с гингивитом, так и с пародонтитом, что подчеркивает важность микробных взаимодействий в прогрессировании болезни. Результаты исследования подтверждают значимость количественной оценки пародонтопатогенов и их ассоциаций для прогнозирования развития заболевания и выбора адекватной терапии, при этом ПЦР в реальном времени является надежным инструментом для выявления видов, способствующих разрушению пародонтальных тканей.

Ключевые слова: Пародонтопатогены, ПЦР в реальном времени, Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, поддесневой налёт, микробные ассоциации, пародонтит, гингивит.

I. INTRODUCTION

Periodontal diseases, including gingivitis and periodontitis, are among the most common oral health disorders worldwide, leading to the progressive destruction of the supporting structures of the teeth and, ultimately, tooth loss. The etiology of these conditions is multifactorial, with microbial dysbiosis in the subgingival biofilm recognized as a primary pathogenic factor. Specific bacteria, such as *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis*, and *Treponema denticola*, have been consistently associated with the initiation and progression of periodontal tissue destruction. Fungal species, including *Candida albicans*, as well as opportunistic pathogens, may also contribute to disease severity by interacting with bacterial communities.

Traditional microbiological and culture-based methods for detecting these microorganisms are limited by the fastidious growth requirements of many periodontal pathogens. Molecular-genetic techniques, particularly real-time polymerase chain reaction (PCR), provide a rapid, highly sensitive, and specific alternative for identifying these microorganisms, even at low

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concentrations. Real-time PCR allows for both qualitative and quantitative assessment of microbial load, as well as the detection of pathogenic associations, which are critical for understanding the dynamics of periodontal microbiota and its role in disease progression.

Despite extensive research on periodontal pathogens in various populations, regional variations in microbial composition and prevalence highlight the need for localized studies. Assessing the frequency, quantity, and co-occurrence of key periodontal pathogens in patients with gingivitis, periodontitis, and healthy individuals provides essential insights for predicting disease progression and guiding targeted therapeutic interventions. Therefore, the present study aimed to evaluate the presence, quantitative load, and associations of major periodontal pathogens in subgingival plaque samples using real-time PCR, with the goal of elucidating microbial patterns related to periodontal health and disease.

II. MATERIALS AND METHODS

A) Materials

Subgingival plaque samples were collected from healthy individuals, patients with gingivitis, and patients with periodontitis. Patients included in the study had not received antibacterial therapy or used antiseptic agents, and no oral hygiene procedures were performed prior to sampling. Sterile paper points (0.3–0.8 mm), sterile paper pins (sizes #25–30), and disposable sterile probes were used for sample collection. Each sample was immediately placed into 1.5 ml plastic tubes containing transport medium to preserve microbial viability until analysis. Positive controls were considered valid when microbial concentration exceeded a logarithmic value of 3.5. The study targeted five major periodontal pathogens—*Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis*, *Treponema denticola*—as well as the opportunistic fungus *Candida albicans* and other conditional pathogens.

B) Method

Detection and quantification of microorganisms were performed using real-time polymerase chain reaction (PCR) with the "ParodontoScreen" reagent kit (DNA-Technologies, Russia) on a DTprime4 amplifier. The reagent kit included a PCR mix specific for all bacteria (total bacterial load), Taq polymerase solution, mineral oil, an 8-tube paraffin strip, and positive control samples. During amplification, 10 µl of Taq polymerase, 20 µl of mineral oil, and 100 µl of DNA samples or controls were combined in each tube using disposable filtered tips without disturbing the paraffin layer, with DNA volume of 5 µl per reaction. A master mix was prepared to cover all experimental and control reactions, including additional replicates. Fluorescent signals were detected in FAM, HEX, ROX, Cy5, and Cy5.5 channels, allowing simultaneous detection of multiple targets. Tubes were placed in the reaction module, and the amplification program monitored fluorescence in real time. Cycle threshold (Ct) values were used to calculate DNA concentrations, and calibration curves constructed from K1 and K2 DNA standards allowed quantitative determination of DNA in 1 ml of experimental and control samples.

III. RESULTS AND DISCUSSION

A) Results

Real-time PCR analysis of subgingival plaque samples demonstrated significant differences in the presence and abundance of periodontal pathogens among healthy individuals, gingivitis patients, and periodontitis patients. *Actinobacillus actinomycetemcomitans* was the most frequently detected species in periodontitis patients (60.2%), compared with 38% in gingivitis patients and 5% in healthy individuals. Other key pathogens, including *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis*, and *Treponema denticola*, were also more prevalent in diseased groups, with their quantitative loads increasing with disease severity. *Candida albicans* was observed more frequently in periodontitis (37%) and gingivitis (30%) patients than in healthy controls (12%).

Quantitative assessment revealed that the total bacterial load ("bac-mass") rose markedly with disease progression. Healthy individuals had an average load of 10^3 genome equivalents per reaction, gingivitis patients had 10^5, and periodontitis patients reached 10^7. Individual pathogen loads increased proportionally: *T. denticola* ranged from 10^3 in healthy subjects to 5×10^5 in periodontitis; *P. gingivalis* from 7 to 6×10^5; *P. intermedia* from 1 to 2×10^4; *A. actinomycetemcomitans* from 1 to 10^6; *T. forsythensis* from 10^2 to 6×10^5; and *C. albicans* from 10^2 to 10^5 genome equivalents. Microbial associations were also assessed, revealing that combinations of pathogens were more frequent in disease states. For example, *A. actinomycetemcomitans*, *P. gingivalis*, and *T. denticola* were detected together in 42.8% of gingivitis patients and 75% of periodontitis patients. The combination of *T. denticola* and *C. albicans* was observed in 88% of gingivitis and 60.4% of periodontitis cases.

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B) Discussion

The results highlight the critical role of microbial dysbiosis in the pathogenesis of periodontal disease. Elevated prevalence and load of key pathogens, particularly *A. actinomycetemcomitans*, *P. gingivalis*, and *T. denticola*, correlate strongly with disease severity and tissue destruction. The presence of multiple pathogenic species in periodontitis patients, compared to low-abundance, single-species detection in healthy individuals, suggests that microbial interactions and synergistic pathogenicity drive disease progression.

The detection of pathogen combinations, including anaerobic bacteria and opportunistic fungi like *C. albicans*, underscores the complexity of the subgingival microbiome in periodontal disease. These microbial associations likely enhance virulence and contribute to host tissue damage, local inflammation, and immune evasion. Quantitative assessment of bacterial load further demonstrates that microbial overgrowth is a hallmark of periodontitis, reinforcing the need for targeted therapeutic interventions.

Real-time PCR proved to be a highly effective method for rapid and accurate detection of fastidious microorganisms that are difficult to culture, enabling both qualitative and quantitative evaluation. The study's findings emphasize the importance of pathogen profiling in clinical practice to guide personalized periodontal therapy, predict disease progression, and prevent destructive outcomes. Additionally, regional patterns of pathogen prevalence highlight the necessity of localized studies to inform public health strategies and clinical management.

IV. Conclusion

This study demonstrates that the prevalence, quantity, and associations of key periodontal pathogens are significantly higher in patients with gingivitis and periodontitis compared to healthy individuals. *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis*, and *Treponema denticola*, along with *Candida albicans*, were identified as major contributors to microbial dysbiosis in subgingival plaque. The total bacterial load and co-occurrence of multiple pathogens increased with disease severity, indicating that both the composition and abundance of subgingival microbiota play a crucial role in periodontal tissue destruction.

Real-time PCR proved to be a rapid, sensitive, and specific method for detecting fastidious pathogens, providing accurate quantitative and qualitative assessment of microbial communities. The identification of pathogen combinations and high bacterial loads can guide personalized treatment strategies, help prevent progression to severe periodontitis, and reduce the risk of destructive periodontal changes. These findings underscore the importance of pathogen-targeted diagnostics and therapeutic interventions in clinical dentistry, as well as the need for ongoing research into regional microbial patterns and their influence on disease development.

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