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Detection and Quantification of Periodontopathogenic Bacteria in Subgingival Plaque Samples on Patients Undergoing Orthodontic Treatment

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ABSTRACT

Introduction: According to last years' research, periodontopathogens may have a negative impact on treatment options in patients with periodontal lesions. However, not all infected sites suffer periodontal destructions, which can be explained on the assumption that only a limited number of pathogens present in a sufficient amount, are capable of affecting the periodontal tissue. Thermal cycling polymerase chain reaction (PCR) is a new technique used for the identification and quantification of periodontopathogenic bacteria. The aim of our study was to confirm the presence of periodontal pathogens, and to evaluate the amount of microbacterial pathogens in the periodontal pockets of patients undergoing orthodontic treatment for a more predictable result. **Material and methods:** A total amount of 32 subgingival samples were collected from periodontal pockets ≥6 mm in 8 patients. Clinical examinations, periapical radiographs and periodontal screenings were performed. Only patients undergoing orthodontic treatment with fixed appliances were included in the study. PCR and DNA hybridization-based identification were performed by paper-point sampling using a micro-IDent plus, Hain Lifescience Germany kit. **Results and Discussions:** Results showed that bacterial load may be connected to disease progression. The prevalence of the periodontopathogenic bacteria *Actinobacillus a.* was established in 42.8% of cases, *P. Gingivalis* in 71.42%, *P. Intermedia* 57.14%, *Bacteroides F.* was found in 85.71% of cases, *Treponema D.* in 100% of cases. Extremely high bacterial loads were recorded for *Actinobacillus a., Bacteroides F.* and *Prevotella I.*

Keywords: PCR, periodonthopatogens, micro-IDent plus,orthodontics

INTRODUCTION

Dental plaque is a natural microbial deposit, a true biofilm containing bacteria in a matrix composed mainly of bacterial extracellular polymers and salivary or gingival exudate. The term biofilm describes bacterial communities adhering to the tooth surface and other areas of the mouth. This ability to adhere to surfaces is a general feature of most bacteria.[1] There are various mechanisms that explain the

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adhesion mechanism — electrostatic adhesion, hydrophobic mechanisms, bacterial layers specialized by specific surface receptors and enzymes (adhesins).[2] Maturation of the plaque is achieved by accumulation of bacterial growth and reproduction of bacteria, leading to the release of a number of organic and inorganic metabolites, an accumulation determining the achievement of an interbacterial matrix. This complex matrix is composed of polysaccharides — proteins produced by the microorganisms of the dental plaque.[3] Bacterial plaque growth can be influenced by the surface irregularities, resulting a bacterial colony with similar morphology. In addition, some bacteria are capable of growth through the colonies that are already formed.[4] In the final structures, microorganisms can attach themselves to the tooth surface or other bacteria already attached to contribute to the complexity of the composition of the plaque after a few days. It is generally accepted that the primary etiologic factor in periodontal disease is represented by the dental plaque microorganisms. However, not all infected sites suffer periodontal destructions. This phenomenon can be explained on the assumption that only a limited number of pathogens are present in a sufficient amount, capable of affecting the periodontal tissue, while the same pathogens present in a less amount are compatible with a healthy periodontium. Both gram-positive and gram-negative anaerobic bacteria produce a range of metabolites (butyric acid, propionic acid, amine, indole, ammonia, volatile sulfide) injurious to the host tissue. Most bacteria in the subgingival space produce hyaluronidase, which has the effect of increasing the permeability of intercellular spaces by widening the gingival epithelium.[5] The products dissolved in the saliva are an important source of nutrients for the bacteria whithin the subgingival plaque. In deep periodontal pockets, nutritive conditions are changing because the penetration of dissolved substances by the saliva at this level is very limited. Most nutrient sources for bacteria in deep periodontal pockets are represented in the periodontal tissue and blood.[6]

The aim of this study was to confirm the presence of periodontal pathogens and to evaluate the amount of microbacterial pathogens in the periodontal pockets for a better treatment and a more predictable result.

MATERIAL AND METHODS

Clinical examination

Eight orthodontically treated subjects with clinical and radiological evidence of bone loss and a probing depth ≥6 mm were selected. None of the patients had used antibiotics during at least three weeks before sampling.

One experienced examiner performed all the examinations. Periodontal screening was recorded on six sites per each tooth.

Sampling method

Patients were instructed to refrain from eating and drinking one hour before sampling. Gingival crevicular fluid (GCF) was collected using a single-use sterile paper point number #25 micro-IDent plus, Hain Lifescience Germany.

Each tooth was carefully isolated with sterile cotton pads and a sterile paper point was inserted to the maximum depth previously recorded on the periodontogram. The paper points were held into position for 20 seconds and immediately after were inserted into the transport kit (micro-IDent plus, Hain Lifescience Germany).

Microbiological examination

Polymerase chain reaction (PCR) and DNA hybridization-based identification were performed in a DNA thermal cycler (Perkin Elmer, GeneAmp 2400, Norwalk,USA) programmed for five minutes at 94 degrees Celsius, followed by thirty cycles with adequate annealing temperature to allow the DNA extension, in one private laboratory in Heidelberg, Germany.

Statistical analysis

All statistical analysis was performed using InStat software (Graphpad, USA). Standard methods to calculate statistics were used. The association between microbial detection by PCR and periodontal disease was tested using the chi square test, with two-sided p value and $\alpha = 0.05$. The odds ratio was calculated at 95% confidence interval.

RESULTS

Results showed that bacterial load might be connected to disease progression. The prevalence of the periodontopathogenic bacteria *Actinobacillus actinomicetemcomitans* was found in 42.8% of cases, *Porphiromonas Gingivalis* in 71.42%, *Prevotella Intermedia* 57.14%, *Bacteroides Forsithus* was found in 85.71% of cases, *Treponema Denticola* in 100% of cases. Extremely high bacteria l loads were recorded for *Actinobacillus a*, *Bacteroides F.* and *Prevotella I.*

DISCUSSIONS

PCR thermal cycling analysis was successfully used in

our study to identify periodontopathogens associated with human periodontitis and orthodontic fixed appliances. PCR technique may be useful in investigating bacterial populations in individual patients only. A single method could not be ideal, and using both traditional and molecular methods is recommended in the bacterial detection.

CONFLICT OF INTEREST

Nothing to declare.

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