# DETECTION OF PERIODONTAL PATHOGENS IN SALIVA USING THE PCR INVADER METHOD IN PATIENTS WITH CHRONIC PERIODONTITIS AND IMPLANT THERAPY

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## ABSTRACT

***Objective:*** *To determine the periopathogens both prior and after the dental implantation.*

***Materials & Methods:*** *This study was conducted from March 26, 2007, to March 31, 2012, in 78 patients (21 males and 57 females) with their informed consent. As a general rule, patients who underwent tooth extraction, and received antibiotic agents due to the incision of an abscess at their initial visit were excluded. The patients were comprised of 28 chronic periodontitis patients (average age of 59.5) with a periodontal pocket exceeding seven mm;39 chronic periodontitis patients (average age of 56.0) with a periodontal pocket of 6 mm or less; and 11 patients (average age of 58.5) with healthy periodontal tissues.*

*The index of periodontitis, including the number of existing teeth, probing depth, plaque index, and bleeding on probing (BOP) of the patients, were measured.*

*The amount of each of the six different periodontal pathogens, Aggregatibacter actinomycetem-comitans(A.a.), Porphyromonas gingivalis (P.g.), Tannerella forsythia (T.f.), Treponema denti-cola(T.d.), Prevotella intermedia(P.i.) and Fusobacterium nucleatum(F.n.), in the saliva of every patient was tested using the PCR-Invader method by outsourcing BML, INC., and the data were statistically processed with the Mann-Whitney U test.*

***Results:*** *In the patient group with a periodontal pocket exceeding 7 mm, there was a significant difference ($P<0.05$) in ratios of P.g. (0.27%) also, T.f., when compared to those of the patient group with a periodontal pocket of 6 mm or less, and those of healthy subjects (0.03%). Among patients with periodontal diseases, patients with a periodontal pocket exceeding 7 mm showed significantly higher BOP, values of the plaque index, when compared to those of patients with a periodontal pocket of 6 mm or less ($P<0.05$)*

***Conclusion:*** *The detection of periodontal pathogens would be useful in determining the risks of periodontitis. Also, it would be necessary to conduct examinations, before treatment of implants, for the risks of peri-implantitis.*

***Keywords:*** *Periodontal pathogens, Implant therapy, PCR invader, chronic periodontitis*

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## INTRODUCTION

Periodontitis is a multi-bacterial infection that affects the periodontal tissues and eventually leads to loss of teeth.[1] Both Periodontitis and peri-implantitis

are chronic inflammatory diseases caused by host factors, as well as by environmental factors and periodontal pathogens.[2] Periodontitis is a chronic inflammatory disease that prevails in a range of clinical entities that are characterized by immunological destruction of the tooth-supporting structures in response to a chronic challenge by specific bacteria in sub-gingival biofilm.[3] It is a complex disease that involves microbial components, environmental factors, and host genetic variations in its development.[4] Introduction of new techniques in the research fields has improved understanding of the microbiology of periodontitis in the past few decades. It is widely accepted that the conversion from periodontal health to disease accompanies a shift in the indigenous flora of the plaque biofilm from gram-positive facultative to gram-negative anaerobic motile microorganisms.[5,6]

Some molecular techniques have been employed for detection and quantification of periodontal pathogens in plaque samples including DNA-DNA hybridization, conventional and real-time PCR, and 16S rRNA clone sequencing.[7] Real-time PCR is the most sensitive, allowing detection of as low as 1.6 cells per reaction.[8,9] Surprisingly, real-time PCR has not been as widely used in the study of the microbiology of periodontitis in many countries of the world.

Periodontists applied multiple techniques to screen out microbiota, which directly destroys the periodontal tissue. Currently, microbial tests are used to identify pathogenic bacteria, identify patients with a high risk of periodontal disease, motivate and educate patients, and provide an indication in the choice of antibiotics. Keeping in view the motivation and education of the patients, saliva test, however, reflects the microbial status of the entire oral cavity and may serve as a useful screening technique in the reduction of risk of oral diseases as well as periodontitis before implant therapy.

In the Japanese population, molecular techniques have been in practice to detect periodontal pathogens in the diagnosis of periodontitis and in determining the effectiveness of the treatment of periodontitis. Therefore, this study aimed to determine whether periodontal pathogens in the saliva of the periodontitis patients showed a correlation with dental implant therapy. A periodontal pathogen test that could easily be applied in a clinical setting would serve as an effective tool in identifying the cause of and risk of periodontitis and peri-implantitis. In this study, to analyze levels of periodontal pathogens, we detected and quantified periodontal pathogens in saliva in patients with periodontal diseases before dental implant therapy using PCR (Invader Method).

## MATERIALS AND METHODS

It was a prospective longitudinal study. A total of 78 adult Japanese patients requesting dental implant treatment visiting the Katsutadai Dental Clinic Chiba-ken between March 26, 2007, to March 31, in 2012 were evaluated. The health status and background of each patient were recorded, including age, sex, and probing pocket depth (57 women and 21 men). The patients were classified into the following three groups based on age & probing pocket depth: 1st group comprised of 28 chronic periodontitis patients (average age of 59.5) with a periodontal pocket exceeding seven mm (>7mm). The second group consisted of 39 chronic periodontitis patients (average age of 56.0) with a periodontal pocket of 6 mm or less (≤6 mm) the Third group included 11 patients (average age of 58.5) with healthy periodontal tissues. (Table 1).

Pocket probing was carried out with a Williams probe and recorded at six sites (mesiofacial, midfacial, distofacial, mesiolingual, midlingual, and distolingual) in each tooth. All patients were systemically healthy, had a normal salivary flow, and had received no periodontal treatment or antibiotics for at least six months before participating in this study. Whereas the patients who underwent tooth extraction, and received antibiotic agents due to the incision of an abscess at their initial visit were excluded. Informed consent was obtained from each patient.

The index of periodontitis, including the number of existing teeth, probing depth, plaque index, and bleeding on probing (BOP) of the patients, were measured.

The amount of each of the six different periodontal pathogens, Aggregatibacter actinomycetemcomitans (A.a.), Porphyromonas gingivalis (P.g.), Tannerella forsythia (T.f.), Treponema denticola(T.d.), Prevotella intermedia(P.i.) and Fusobacterium nucleatum(F.n.), in the saliva of every patient was tested using the PCR-Invader method by outsourcing BML, INC. (Japan). (Table.1).

Each patient was required to chew gum for at

**Table 1: Classification of experimental groups**

| Classification | Number | Age | Evaluation basis of periodontal status |
|---|---|---|---|
| Chronic Periodontitis Patients | 28 | 59.5 | Periodontal pocket exceeding seven mm (>7mm). |
| Chronic Periodontitis Patients | 39 | 56 | Periodontal pocket of 6 mm or less (≤6 mm) |
| Health Patients without Periodontitis | 11 | 58.5 | With healthy periodontal tissues. |

Subjects were divided into three groups based on the severity of the periodontal disease.

**Table 2: Table of Periodontal pathogens**

| S/No | Perriodontal Pathogens |
|---|---|
| 1 | Aggregatibacter Actinomycetemcomitans(A.a) |
| 2 | Porphyromonas Gingivalis(P.g) |
| 3 | Tannerella Forsythia (T.f) |
| 4 | Treponema Denticola(T.d.) |
| 5 | Prevotella Intermedia(P.i.) |
| 6 | Fusobacterium Nucleatum(F.n) |

least 5 min before collection of saliva samples. A total of 3–5 ml stimulated whole saliva was then collected in an empty, sterile, 50-ml test tube. The patients were instructed not to brush their teeth or eat for up to 1 hour before sampling, which was performed between implant therapy. The whole saliva was stored at -20 °C until processing.

The amount of each of the six different periodontal pathogens, Aggregatibacter actinomycetemcomitans (A.a), Porphyromonas gingivalis (P.g.), Tannerella forsythia (T.f.), Treponema denticola(T.d.), Prevotella intermedia (P.i.)and Fusobacterium nucleatum (F.n.), in the saliva of every patient was tested using the PCR-Invader method by outsourcing BML, INC. (Japan).

## RESULTS

In the patient group with a periodontal pocket exceeding 7 mm, there was a significant difference ($P<0.05$) in ratios of P.g. (0.27%), and T.f. When compared to those of the patient group with a periodontal pocket of 6 mm or less, and those with healthy subjects (0.03%). Among patients with periodontal diseases, patients with a periodontal pocket exceeding 7 mm showed significantly higher BOP, values of the plaque index and amount of tobacco smoked when compared to those of patients with a periodontal pocket of 6 mm or less ($P<0.05$) (Table 2)

## DISCUSSION

The subgingival microbial profile associated with periodontitis has been reported to significantly differ by geographical location independent of other factors known to modify subgingival microbial composition.[8,11] It becomes prudent, therefore, that obtaining more information about the global distribution of periodontal pathogens and patterns of their association with the disease can improve our understanding of the differences in the role they play in periodontitis in different populations. Therefore the objective of the current study was to assess the association of six anaerobic periodontal pathogens with chronic periodontitis in a Japanese population attending Katsutadai Dental Clinic in Chiba using PCR (Invader method).

In the previous studies, 250 bacterial species were recovered from plaque samples while employing only cultivation-based techniques.[10] Recently molecular techniques, mainly Polymerase chain reaction has been extensively used to detect microbiota in the gingival sulcus around the teeth due to which sub-gingival microbiota has increased to more than 700 bacterial species.[11,12,13]

Usually, subgingival microbiota is considered commensal, out of these several species have been implicated as periodontal pathogens. Such as Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola called as red complex have also been implicated in the etiology of chronic periodontitis14. Many other putative pathogens, e.g., Fusobacterium spp., Prevotella spp., Campylobacter rectus, Eubacterium nodatom, and Parvimonas Micra also play their part in the pathogenesis of periodontitis.[14] Therefore, it is believed that periodontal destruction is a response of periodontium to a group of microbial rather than a single pathogen.[15]

Researchers involved in periodontal disease diagnostics are currently investigating the possible use of oral fluids, such as saliva, for disease assessment.[16]

There are different opinions about whether bacterial tests should be conducted in the treatment of

periodontal disease; studies are being conducted by different periodontologists. Although periodontitis is a bacterial infection, there are no established microbiological tests that can be easily applied clinically, and periodontitis is often treated without identifying the responsible bacteria. In recent years, various chair-side microbial tests have been developed aiming at the detection of periodontal disease, including enzyme assay, DNA probe method, and polymerase chain reaction.

The reason why we have used PCR (Invader Method) because previously clinicians employed different techniques for the detection of potential pathogens, such as microbial culture, nucleic acid hybridization assays, and specific polymerase chain reactions (PCR). However, these methods target only predefined or cultivable bacterial phylotypes and are not able to determine the overall microbial diversity within the tested biofilms.[17,18] Therefore, these detection techniques do not adequately identify potential differences in microbial composition in teeth or implants[19] as PCR (Invader Method) can quantify as minimum as possible number of Microbials in the saliva of the patients. None of these tests, however, is widely employed due to issues related to cost and convenience.[20]

Similar to our study, at present, it is possible to quantify periodontal pathogens have been quantified by real-time polymerase chain reaction (RT-PCR), and clinical bacterial tests are being performed much more frequently in the recent past.[21]

In order to obtain Microbial samples we have employed the same technique as in the previous studies, sampling of microbes is usually performed by inserting paper points in periodontal pockets or obtaining samples of saliva, testing saliva as a whole is more convenient and suitable for screening of bacteria in the entire oral cavity, Therefore, in our study, we collected saliva for the quantification of Periodontal pathogens in periodontitis having who attended our clinic office for implant therapy.

Numerous studies have elucidated the pathogenic microbial processes leading from healthy to infected peri-implant tissues. Biofilm formation around implants is characterized by a shift from mainly gram-positive aerobic and facultative anaerobic cocci and rods to a higher proportion of periodontal pathogens.[22,23,24, 25,26]

According to Socransky et al.[27], periodontal pathogens included Aggregatibacter actinomycetemcomitans, as well as species of the red complex, such as Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia, and of the orange complex, such as Fusobacterium nucleatum and Prevotella intermedia.[28,29] Whereas in our study, we have found P.g.and T.f. in high ratios as compared with the healthy group and chronic periodontitis patients having a periodontal pocket of 6 mm or less.

Several studies have focused on periodontal pathogens and have demonstrated the similarity between the microbiota around teeth and implants. It was, therefore, concluded that there is cross-contamination from teeth to implants.[30]

It was suggested that the ratios of P.g. , and T.f. in saliva were related to the condition of periodontitis ($P<0.05$), and detection of the types of periodontal pathogens would be useful in determining the risks of periodontitis. Also, it would be necessary to conduct examinations, before treatment of implants, for the risks of peri-implantitis. Percentages of periodontal pathogens in saliva after treatment of periodontal diseases and aggressive periodontitis have been investigated.

## CONCLUSION

P. gingivalis and T. denticola were frequently detected in periodontitis patients by PCR. The prevalence of these two microorganisms was correlated with various clinical parameters. Our data suggest that their presence is associated with the severity of periodontal tissue destruction.

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