# The Use of Polymerase Chain Reaction (PCR) for Indentifying Periodontopathogenic Bacteria - therapeutic Implications in Periodontal Disease

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*Polymerase chain reaction (PCR) shows a high specificity and allows us to identify pathogenic periodontal bacteria. We chose 45 patients wich were divided into three groups with various types of treatment: (I) - SRP; (II) - SRP, followed by topical application of antiseptics (SRP + local); (III) - SRP followed by systemic administration of antimicrobial agents (SRP + systemic). We collected samples from the initial time (TO) and one month after the treatment (T1) for each patient. For the microbiological assessment of periodontal therapy, we analyzed 90 subgingival plaque samples using PCR technique which provides qualitative data on five periodontopathogenic bacteria species: A. actinomycetemcomitans, P.gingivalis, P.intermedia, T.forsythia, and T.denticola. The treatment was followed by a qualitative change of the bacteria detected previously in a different ratio depending on the treatment. We found the inefficiency of mechanical treatment regarding the reduction of periodontal bacteria in patients belonging to group I, an improvement in the results of group II, while the treatment in group III proved to be the most effective . In patients detected A.a+ and/ or P.g+ a systemic antibiotic treatment is required because these periodontal bacteria penetrate the tissue and mucosal surfaces of the oral cavity.*

*Keywords: PCR, periodontopathogenic bacteria, periodontal treatment*

Periodontal disease is a multifactorial disease for which reason both its diagnosis and treatment is complex. Different inflammatory changes occur in the development of the disease, changes which are destructive of the tissues supporting the teeth and lead toattachment loss and bone resorption, the formation of periodontal pockets and/ or gingival retractions. These changes can be apreciated by registering a few indices: gingival index(GI) wich shows the degree of gingival inflamation, papilary bleeding index(PBI) wich quantifies the presence of bleeding on probing. Measuring the depth of the periodontal pockets(PPD), in mm, is an important element in apreciating the degree of distruction of the periodontal tissue.

The most important role of all the factors involved in developing this disease is played by the microbial factor, all the other ones being favoring or predisposing factors. Some bacterial species in the subgingival plaque, periodontal pathogens, interact with host tissues and cells and lead to the release of cytokines and other mediators of inflammation, resulting in the destruction of periodontal structures [1-3]. The *red complex*, consisting of *Porphyromonas gingivalis, Treponema denticola*, and *Tannerella forsythia*, is heavily involved in the destructive stages of certain types of periodontal disease [4-7]. Most often periodontal treatment is non-specific, it is limited to a mechanical smoothing treatment of the root surface. Mechanical treatment can be effective for a large number of patients, but there is a significant percentage of patients or sites for which it is insufficient, due to the inability to penetrate into the deeper areas of sinuous deep pockets or root levels. The introduction of antimicrobial agents with local or systemic administration can improve results obtained by mechanical treatment [8-10]. Periodontal antimicrobial therapy is an important step that must be taken regardless of the form or severity of periodontal disease. Therefore, identifying periodontal microorganisms and choosing a targeted therapy is the key to success in our approach to improve periodontal status. Polymerase chain reaction (PCR) shows a high specificity and allows us to identify pathogenic periodontal bacteria, being more sensitive than bacterial culture because it identifies germs according to DNA. Evaluation of subgingival microbiological flora by analyzing pathogen markers with the aid of PCR kits (Micro-IDent®) provides qualitative data on five species: *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, and *Treponema denticola*. Knowledge of the composition and concentration of bacterial load can bring a range of benefits related to early and proper diagnosis, it allows choosing targeted local and/ or general medication, and it prevents relapses as a result of choosing ineffective therapies [11-13]. The aim of this study was to identify microorganisms present in periodontal pockets which are responsible for the appearance of inflammatory changes in periodontal tissue that lead us to an early diagnosis and enable us to formulate a treatment protocol with

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maximum effectiveness in reducing the pathological changes caused by periodontal disease.

## Experiemntal part

*Materials and methods*

We chose 45 patients who presented to the Department of Periodontology, the Faculty of Dentistry of the University of Medicine and Pharmacy of Tigu Mures, who met our criteria: (a) to have a form of periodontal disease, (b) to have at least 15 odonto-periodontal units in the oral cavity of which at least 5 could provide probing pocket depths between 5 and 7 mm; (c) to be free of general conditions which may influence periodontal health (diabetes, immune disorders); (d) not to have received any periodontal treatment; (e) not to have received antibiotics in the last six months or prolonged treatment with anti-inflammatory drugs. All patients were informed about the protocol, the benefits and risks of participating in this study and informed consent was obtained from all participants. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Tirgu Mures, Decision 40 of July 2 2014. Clinical assessment of periodontal status was done for each patient by registering the gingival index (GI), papillary bleeding index (PBI), and measuring the probing pocket depth (PPD) at the beginning of the study (TO) and one month after the treatment (T1). For therapeutic assessment, following clinical and initial microbiological evaluation (T0), the patients were divided into three groups of 15 patients each (11 diagnosed with severe chronic periodontitis, 4 with severe aggressive periodontitis): (I) - the group of patients receiving manual and/ or ultrasound scaling and scaling root planning (SRP); (II) - the group of patients receiving manual and/ or ultrasound scaling and scaling root planning, followed by topical application of antiseptics (SRP + local); (III) - the group of patients who underwent manual and/ or ultrasound scaling and scaling root planning, followed by systemic administration of antimicrobial agents (SRP + systemic).

Samples

For the microbiological assessment of periodontal antimicrobial therapy, we analyzed 90 subgingival plaque samples using PCR kits (Micro-IDent®) providing qualitative data on five periodontal bacteria species: *A. actinomycetemcomitans, P.gingivalis, P.intermedia, T. forsythia,* and *T. denticola*. Supragingival plaque was removed, the tooth was isolated and subgingival plaque sampling was performed using sterile paper points (Micro-Ident Sampling Set, Hain Lifescience GmbH, Germany). Sterile paper points were inserted to the base of the periodontal pocket where it was held for 30 s. After removal, it was placed in a transfer tube. We collected samples from the initial time (TO) and one month after the treatment (T1) for each of the 45 patients. Samples were collected from 5 different pockets, clinically detected as having the highest PPD value and were placed in a single transfer tube, which was accompanied by a patient data sheet.

Polymerase Chain Reaction (PCR)

Samples were taken to the Microbiology Laboratory of the University of Medicine and Pharmacy of Tîrgu Mure', where molecular genetic diagnosis of periodontal pathogenic markers was performed with the aid of the PCR method using microIDent test and the existing equipment (GeneAmp® PCR System 9700). The microIDent test sets new standards in the quality of diagnosis of periodontal bacteria by combining DNA amplification with hybridization using specific oligonucleotide probes. The test is based on DNA•STRIP® technology which ensures accurate microbiological diagnosis. An amplification of DNA isolated from the sample occurs in the first phase, and hybridization on strips in the next phase, which virtually eliminates obtaining false positive or false negative results. Laboratory steps were: (1) isolation/ extraction of DNA; (2) preparing the mix for amplification: extracted DNA, primers, nucleotides; (3) the amplification process – PCR – Polymerase Chain Reaction; (4) hybridization in the Twincubator device – denaturation to obtain singlestranded DNA, which will subsequently bind to the specific oligonucleotide probe during hybridization. Non-specific amplicons were removed during washing. During the conjugation reaction, the bound amplicon was stained with alkaline phosphatase and detected colorimetrically by forming specific DNA•STRIP® (fig.1).

*Statistical analysis*

InStat GraphPad software version 3.06 was used for the statistical analysis. $\chi^2$ test (Chi Square Tests), was used for each analysis, the threshold was considered $p < 0.05$.

## Results and discussions

Microbiological results on the prevalence of periodontopathogenic bacteria in subgingival plaque samples in the three groups of patients are summarized in tables 1, 2 and 3.

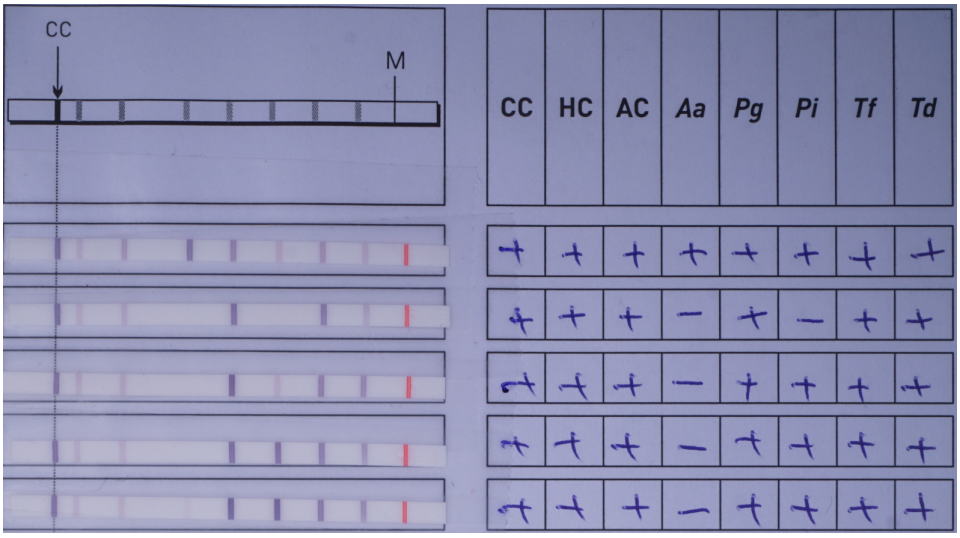


Fig.1 The detection of specific fragments of DNA for periodontopathogenic bacteria

**Table 1**

THE PREVALENCE OF PERIODONTAL PATHOGENS IN PATIENTS IN GROUP I BEFORE AND AFTER THE TREATMENT

| Periodontal pathogen | Before the treatment N(%) | After the treatment N(%) | p |
|---|---|---|---|
| A.a + | 4 (26.66) | 4 (26.66) | p=0.001 |
| A.a - | 11 (73.33) | 11 (73.33) | |
| P.g + | 15 (100) | 15 (100) | - |
| P.g - | 0 | 0 | |
| P.i + | 13 (86.66) | 12 (80) | p=0.029 |
| P.i - | 2 (13.33) | 3 (20) | |
| T.f + | 14 (93,.33) | 13 (86.66) | p=1.00 |
| T.f | 1 (6.66) | 2 (13.33) | |
| T.d + | 12 (80) | 11 (73.33) | p=0.009 |
| T.d - | 3 (20) | 4 (26.66) | |

**Table 2**

THE PREVALENCE OF PERIODONTAL PATHOGENS IN PATIENTS IN GROUP II BEFORE AND AFTER THE TREATMENT

| Periodontal pathogen | Before the treatment N(%) | After the treatment N(%) | p |
|---|---|---|---|
| A.a + | 4 (26.66) | 3 (20) | p=0.009 |
| A.a - | 11 (73.33) | 12 (80) | |
| P.g + | 15 (100) | 12 (80) | - |
| P.g - | 0 | 3 (20) | |
| P.i + | 10 (66.66) | 4 (26.66) | p=0.231 |
| P.i - | 5 (33.33) | 11 (73.33) | |
| T.f + | 13 (86.66) | 9 (60) | p=0.143 |
| T.f | 2 (13.33) | 6 (40) | |
| T.d + | 13 (86.66) | 5 (33.33) | **p=0.095** |
| T.d - | 2 (13.33) | 10 (66.66) | |

**Table 3**

THE PREVALENCE OF PERIODONTAL PATHOGENS IN PATIENTS IN GROUP III BEFORE AND AFTER THE TREATMENT

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| Periodontal pathogen | Before the treatment N(%) | After the treatment N(%) | P |
|---|---|---|---|
| A.a + | 4 (26.66) | 1 (6.66) | p=0.267 |
| A.a - | 11 (73.33) | 14 (93.33) | |
| P.g + | 15 (100) | 5 (33.33) | - |
| P.g - | 0 | 10 (66.66) | |
| P.i + | 11 (73.33) | 0 | - |
| P.i - | 4 (33.33) | 15 (100) | |
| T.f + | 15 (100) | 4 (33.33) | - |
| T.f | 0 | 11 (73.33) | |
| T.d + | 14 (93.33) | 0 | - |
| T.d - | 1 (6.66) | 15 (100) | |

As can be noticed, *P. gingivalis*, known as a periodontal pathogen with increased virulence, was detected in all 45 samples collected at the time of initial examination (T0). *T. forsythia* was detected prior to treatment in 42 samples (93.33%), followed by *T. denticola* in 39 samples (86.66%), *P. intermedia* in 34 samples (75.55%), and *A. Actinomycetemcomitans* in 12 samples (26.66%). All patients included in the study had an associated bacterial flora on initial examination. The treatment was followed by a qualitative change of the bacteria detected previously in a different ratio depending on the treatment and periodontal bacteria species ( for P.g, P.i, T.d $p<0.001$ and for T.f $p=0.005$). The comparison of the results obtained by PCR, in each of the groups, before and after the treatment, indicates the inefficiency of mechanical treatment regarding the reduction of periodontal bacteria in patients belonging to group I (O.R. = 1.24, C.I. (95%) = 0.59-2.61; $p = 0.71$). We noted an improvement in the results of group II (OR = 3.50; CI (95%) = 1.76-6.95; $p = 0.0005$), while the treatment in group III proved to be the most effective (OR = 23.97; CI (95%) = 10.09-56.95; $p < 0.0001$).

Assessment of subgingival bacterial flora using PCR is an important element in both the diagnosis and the treatment of periodontal disease. Numerous studies [14-16] have shown the correlation between detection of periodontal pathogens by PCR and the presence of pathological changes in the periodontium. In our study, the increased prevalence of *P. gingivalis* associated with other periodontal bacteria correlated with initial clinical examination allowed us to diagnose most patients with severe chronic periodontitis. Some authors consider the presence of *A. actinomycetemcomitans* in subgingival plaque samples as a marker for the diagnosis of aggressive periodontitis [17], while others consider that the microbiological profile of patients with severe chronic periodontitis is similar to those with aggressive periodontitis [18,19]. A quantitative microbiological analysis for the ratio of *A.a* compared to other periodontal bacterial pathogens could help in the differential diagnosis between the two pathologic entities [20]. Given that our study only provided a qualitative analysis not a quantitative one regarding the composition of subgingival bacterial, the 12 patients found with *A.a*+ were diagnosed with severe aggressive periodontitis taking into account the clinical features and patient age. Mechanical treatment including manual and/ or ultrasound scaling and scaling root planning (SRP) is an important stage in periodontal therapy which has to be applied to each patient with periodontal disease. Regardless of the instruments and the protocol used, the results of numerous studies prove that SRP leads to

improved clinical periodontal status [21, 22]. SRP is a mechanical treatment which cannot completely remove periodontal bacteria due to their presence in periodontal tissues, on the one hand, and to the impossibility to correctly probe deep pockets, on the other hand [23]. Although in patients in group I we noted a reduction in the inflammatory phenomena in the periodontal tissue, we observed no significant changes in the analysis of subgingival plaque samples compared to T0. The use of some antimicrobial agents applied locally to the periodontal pockets after SRP has many benefits in the treatment of periodontal disease [24-26]. The data presented in table 2 demonstrate that the topical application of an antiseptic as an adjunct to SRP resulted in reducing the number of samples tested positive for each of the 5 periodontal bacteria: 8 for *T. denticola*, and 6 for *P. intermedia*. For *A. actinomycetem comitans, P. gingivalis*, and *T. forsythia* the number of samples that did not test positive, compared to the initial examination, was 1, 3, and 4. Each of the patients had samples positive for one ororor more of the bacteria identified by the PCR technique, even if we found a reduction in GI, BPI, or PPD on clinical examination.

Benefits of systemic administration of antimicrobial agents as adjuvant to periodontal disease treatment have been reported by numerous researchers [27-30]. The choice of antibiotics should be made after determining the susceptibility, and their administration should follow the mechanical disruption of the plaque biofilm [31]. After examining the subgingival samples from group III, a total of 9 patients were negative for the 5 periodontal bacteria. The clinical diagnosis of aggressive periodontitis was confirmed by the microbiological results by identifying some samples positive for *A.a*, *P.g*, and *P.i*, and after a systemic antibiotic treatment. In these patients, SRP should be correlated with general and local antimicrobial therapy, followed by surgery to reduce the depth of periodontal pockets.

## Conclusions

The treatment of severe chronic periodontitis and of severe aggressive periodontitis has to reduce or eliminate periodontal bacteria that cause and maintain the pathological changes of periodontal tissue. By removing local irritation factors and the mechanical disruption of the plaque biofilm, SRP lies at the basis of periodontal therapy. The local application of antimicrobial agents in periodontal pockets is an adjunct to mechanical treatment of periodontal disease because it reduces periodontal bacteria, resulting in improved periodontal status. Systemic antibiotic therapy should not be initiated in all patients with periodontal disease, because there are cases in which mechanical and local antimicrobial therapy is sufficient. In patients detected *A.a*+ and/ or *P.g*+ a systemic antibiotic treatment is required because these periodontal bacteria penetrate the tissue and mucosal surfaces of the oral cavity. PCR technique is useful in choosing a correct and effective therapy of periodontal disease.

## Abbreviations

PCR= Polymerase Chain Reaction
A.a = A. actinomycetemcomitans=Aggregatibacter actinomycetem comitans
P.g = P. gingivalis=Porphyromonas gingivalis
P.i = P. intermedia=Prevotella intermedia
T.f = T. forsythia=Tannerella forsythia
T.d= T. denticola=Treponema denticola
GI= gingival index
PBI= papillary bleeding index
PPD= probing pocket depth
SRP= scaling root planning
DNA= Deoxyribonucleic acid
O.R= Odd ratio
C.I= Confidence interval

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