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Polymerase chain reaction significance in the diagnosis of periodontal diseases

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Absrtract

Background. Polymerase chain reaction (PCR) is a revolutionary technique for rapidly amplifying millions of copies of a specific segment of DNA, which can be used to make more accurate analysis. PCR is well-known for its sensitivity and specificity to determine various microbiota and initially stood out as a novel molecular diagnostic method for different fields of medicine, over time it became increasingly popular in the field of dentistry, particularly periodontology.

Methods. The systematic review adhered to PRISMA guidelines and databases of PubMed, ScienceDirect, and The Cochrane Library were used to perform the search.

Aim. To evaluate the importance of polymerase chain reaction in diagnosing periodontal diseases.

Results. 1356 adult patients with periodontitis and healthy group were evaluated microbiologically. All 7 articles agreed that individual microbial species and total bacterial count in dental plaque samples may be accurately quantified using Q-PCR or real-time PCR. Comparing Culture method and PCR, polymerase chain reaction showed better results for the detection of *F nucleatum* (53 % and 73 % respectively), *P. gingivalis* (84 % and 94 % respectively) and *T. forsythensis* (56 % and 93 % respectively).

Conclusions. PCR as a diagnostic tool upgrades the diagnostic field of periodontology and allows dentists to recognize periopathology in the early stage and select the approprate treatment.

Keywords: polymerase chain reaction; PCR; periodontology; diagnostic tool; periodontal pathogens;

1. Introduction

Periodontal diseases affect approximately 20-50 % world's population [1]. The onset of periodontitis is triggered by the presence of periodontal pathogens, particularly Gram-negative bacteria such as *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*, which are commonly detected within subgingival dental plaque among patients with periodontal disease [2]. According to recent literature, there are three main classifications periodontal diseases are specified into. The first one focuses on the amount of bone loss around teeth (localized or generalized). In the meantime, the second classification is based on the severity of periodontal disease (stage 1 –least severe; stage 4 – most severe). The risk and rate of disease progression (3rd classification) have been divided into three grades from the lowest risk of progression (grade A) to the highest (grade C) [3]. Accurate diagnosis of the disease is one of the most critical pre-treatment steps. While the traditional clinical routine of diagnosing periodontal disease (e.g. clinical attachment loss, radiographic bone loss or bleeding on probing (BOP)) does not include the cause, progression, and prediction of the disease, the scientists initiated a comparative analysis of various microbial detection techniques and came up with a new approach to identify perio-pathogens – a polymerase chain reaction [4].

Polymerase chain reaction (PCR) is a revolutionary technique for rapidly amplifying millions of copies of a specific segment of DNA, which can be used to make more accurate analysis. [5]. PCR involves the use of short synthetic DNA fragments (primers) to select a specific segment of the genome for amplification, followed by multiple steps of DNA synthesis to further amplify that segment [2, 4-8, 10, 20, 24]. PCR can identify even one copy of the DNA targets from clinical samples.

The aim of the article is to show the importance of PCR in the detection of microbial dysbiosis of oral microbiome.

2. Materials and methods

2.1 Methods

The systematic review adhered to PRISMA (Preferred Reporting Items for Systematic Review) guidelines. A focused question was formed according to the PICOS model: how is polymerase chain reaction a significant tool in the diagnostics of periodontal diseases?

2.2 Search strategy

A comprehensive literature search using advanced features of Pubmed, Science Direct, Cochrane Library, and Scopus databases was carried out. The following search terms were used: "polymerase chain reaction" OR "PCR" AND "periodontology" AND "diagnostic tool" OR " periodontal pathogens". The search was supplemented using additional articles in references and lists of similar studies.

2.3 Eligibility criteria

The inclusion criteria for the studies were: studies in English language, studying periodontal patients' microbiota with PCR. The articles describing PCR process and its relation to periodontology were collected and used to prepare a concise review. Case reports, systematic reviews, meta-analyses, and animal studies were excluded from the search. There was no limitation for publication time.

2.4 Study selection and data collection process

Firstly, the possible studies from the initial search were selected for further screening based on the title

and the abstract by the authors. Secondly, the selected studies were analyzed and the ones that did not match the inclusion criteria were discarded. Randomized controlled trials, as

well as comparative studies, double-blinded, controlled clinical trials were included in the article.

3. Results

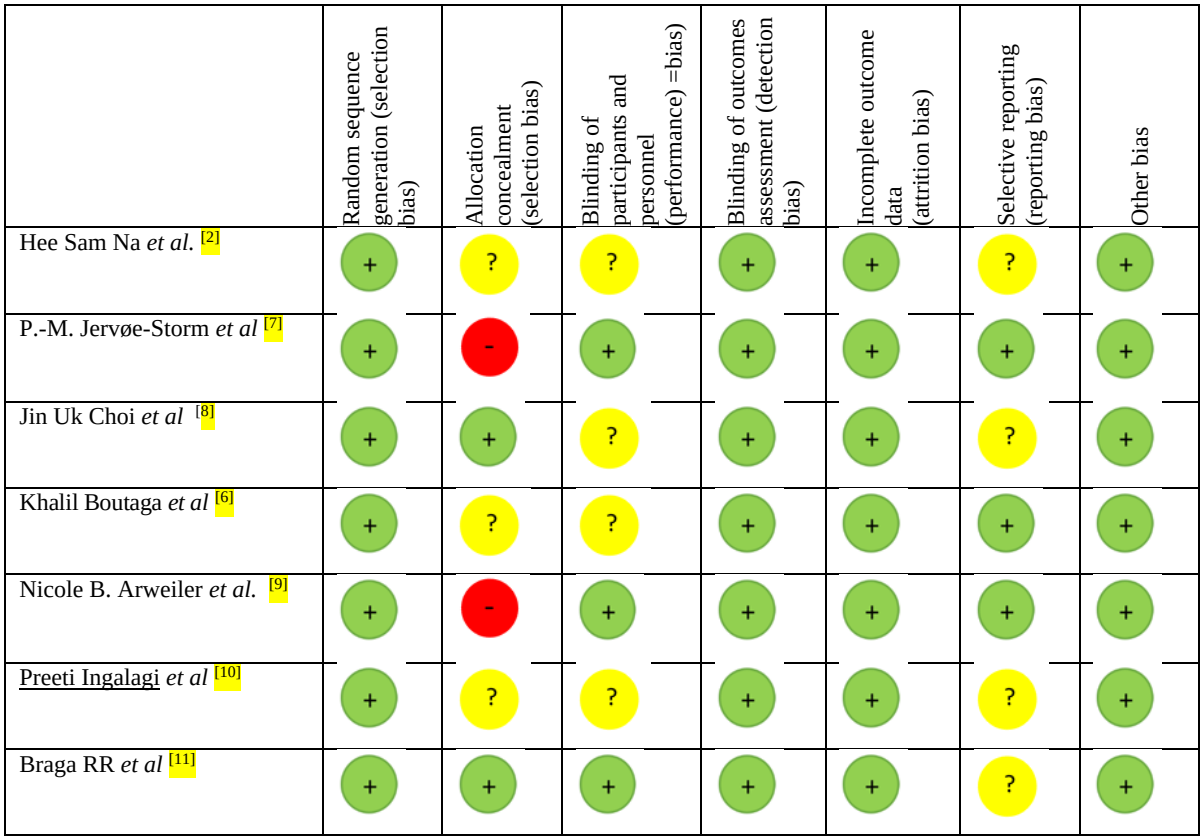
3.1 Study selection

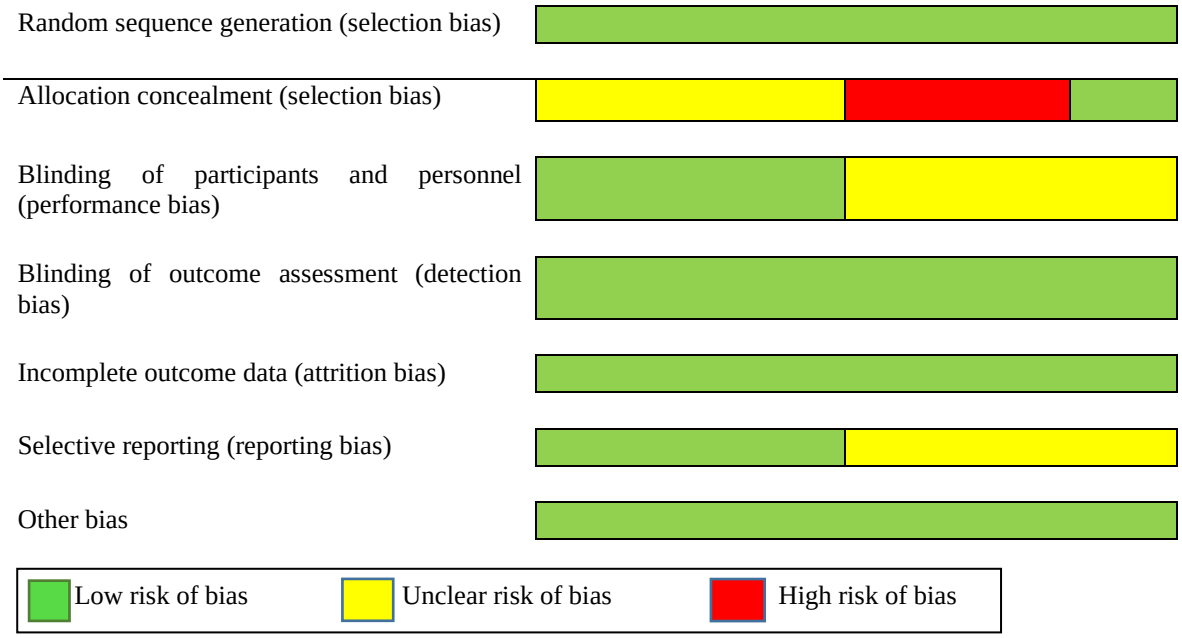
Primary database search yielded 397 results out of which 11 were duplicates and were excluded. Titles and abstracts of 386 articles were screened and after the process, 28 studies were used for further full-text analysis. After checking the content and relevance of the articles, 7 articles were used. 1356 adult patients with periodontitis and healthy individuals (the control group) were evaluated microbiologically.

3.2 Risk of bias assessment

All 7 included studies were evaluated qualitatively by the tools of Cohraine Collaboration for the risk of bias (Figure 1). Two studies [6, 9] had a high risk of bias in allocation concealment. The highest proportion of low risk of bias included other bias allocation concealment, blinding of outcomes, selective reporting and incomplete outcome data. Meanwhile blinding of participants and personnel, selective reporting, and allocation concealment were noted as the highest proportion of unclear risk bias.

Figure 1. *Cohraine Collaboration for the risk of bias*





3.3 Characteristics of included studies

2 of the studies were cross-sectional, 2- comparative studies and 3 studies were controllend clinical trials. A total of 1356 patients were included in the studies. Despite the control group, only patients with periodontal diseases were included in the study. Participants were enrolled in either the healthy/gingivitis group or the periodontitis group (in most of the studies). Patients in the healthy/gingivitis group had < 3 mm attachment loss, >4 mm periodontal probe depth (PD), and no radiographic alveolar bone loss. Patients with periodontitis showed at least 4 sites with radiographic bone loss, 4 sites with more than 3 mm attachment loss, and at least 4 sites with more than 4 mm PD.

3.4 Principles of polymerase chain reaction

The polymerase chain reaction (PCR) amplifies a single or a few copies of a piece of DNA through multiple orders of magnitude, producing thousands to millions of copies of a specific DNA sequence [5]. The PCR process consists of three essential steps: denaturation, annealing, and extension [4].

In the first step, the DNA is denatured at high temperatures (90-97 degrees Celsius). Primers anneal to the DNA template strands in step two to prime extension . In step three, the annealed primers are extended to form a complementary copy strand of DNA. [4,5,8].

There are a lot of types of PCR (such as Quantitative polymerase chain reaction, Nested polymerase chain reaction, Real-time polymerase chain reaction, Multiplex polymerase chain reaction, and etc.) but all of them have identical benefits and drawbacks (Figure 2).

3.5 Qualitative synthesis of results

All 7 articles agreed that individual microbial species and total bacterial count in dental plaque samples may be accurately quantified using Q-PCR or real-time PCR. [2, 7-11].

Figure 2. *Advantages and disadvantages of PCR*

Benefits of PCR	Limitations of PCR
Exact identification of bacterial strains with disparate phenotypes	Expensive cost
The simplicity of quantifying	High technical capabilities are required.
Precision	Changing the specificity of PCR product produced
Amplification of DNA or RNA millions of times	Results that are either positive or falsely negative
Rapid examination	Creating a high sterile atmosphere has constraints.
Least contamination	Contamination of DNA
Increased sensitivity	Low detection ability between closely related and highly recombinant species
Reproducibility	Multiplex PCR using various primers has limits.
The capacity to measure several targets in a clinical specimen	The ability to contaminate other reaction vials
Quality assurance	
The capacity to look for several organisms or genes in a single response.	
Identification of microorganisms from bacterial colonies	
Detection of very tiny quantities of samples	
The study of strictly anaerobic infections	
Virus detection and mRNA expression levels	

Porphyromonas gingivalis (Pg), Aggregatibacter actinomycetemcomitans (Aa), Tannerella forsythia (Tf), Prevotella intermedia (Pi), Prevotella nigrescens, Parvimonas micra (Pm), Eubacteria, Campylobacter rectus (Cr), Capnocytophaga sputigena, Capnocytophaga ochracea, and *Capnocytophaga gingivalis* have all been found in subgingival plaque samples [2, 4, 6, 7, 10, 11]. *Pg* and *Aa* levels were comparable in aggressive periodontitis patients and controls, but according to Shahriar Shahi et al only *Aa* was linked to periodontal disease [4].

Some of the articles compared bacterial cultivation (the golden standard) and real-time PCR for the detection of the most common periodontal microbes [6, 7, 10].

P.-M. Jervøe-Storm et al found that polymerase chain reaction showed better results for the detection of *F. nucleatum* (53 % and 73 % respectively), *P. gingivalis* (84 % and 94 % respectively) and *T. forsythensis* (56 % and 93 % respectively). The culture method was better for the detection of *A. actinomycetemcomitans* and *P. intermedia* [7].

Other article used both methods for detection of *P. gingivalis* in subgingival plaque samples. The results revealed that *P. gingivalis* was detected in 111 (43 %) of the 259 subgingival plaque samples by culture and in 138 (53 %) samples by PCR. The sensitivity, specificity, and positive and negative predictive values of the real-time PCR were 100, 94, 94, and 100 %, respectively [6].

In the meantime, Jin Uk Choi et al demonstrated that the amount of salivary *Pg* was more prominent in patients with periodontitis than that in healthy people [8]. On account of PCR, it may be suggested that *Pg* in saliva has the potential to be utilized as a diagnostic marker of periodontitis [8, 11].

Jin UK Choi et al demonstrated that the majority of target bacteria exhibited increased counts as the severity of periodontitis increased. *Pg, Tf, Td, Pm, Cr, and En* were significantly correlated with the severity

of periodontal disease (ρ = 0.530, 0.438, 0.209, 0.276, 0.283, 0.311, respectively) [8].

Some articles compared the prevalence of periopathogens in healthy individuals and patients with periodontitis [10, 11]

Renato R. R. Braga et al [11] compared the quantification of five putative periodontal pathogens (*A. actinomycetemcomitans*, *E. corrodens*, *F. nucleatum*, *P. gingivalis* and *P. intermedia*). Oral specimens from all individuals tested positive for *A. actinomycetemcomitans*, *E. corrodens*, and *F. nucleatum*. Besides, *P. gingivalis* was detected in 70.0 % and 46.6% and *P. intermedia* in 90.0 % and 80.0 % of periodontal patients and healthy subjects, respectively. However, only *P. gingivalis*, which was found in greater quantities in specimens from individuals with chronic periodontitis, showed a statistically significant difference ($p = 5.2 \times 10^{-3}$) [11].

The other study investigated that the prevalence of *P. gingivalis* was low in healthy people (9.9 % by RT-PCR) but rose to 45.5 % in periodontitis patients. *T. forsythia* was detected in 33.2 % of healthy people and had a prevalence of 89.2 % in patients when tested with RT-PCR. *P. intermedia* exhibited a significant difference in healthy individuals: 23.2 %, whereas it increased to 83 % in periodontitis patients [10].

Also, all of the studies (that compared people with periodontitis and healthy humans) indicate higher rate of the *A. actinomycetemcomitans* and *P. gingivalis* in periodontal patients than healthy individuals [2,4,10,11].

None of the articles found a specific amount of pathogens that determines the onset of periopathology.

4. Discussion

PCR was first developed in the mid-80s and is well-known for its sensitivity and specificity to determine various microbiome [5]. While the polymerase chain reaction initially stood out as a novel molecular diagnostic method for different fields of medicine, over time it became increasingly popular in the field of dentistry, particularly periodontology.

Although the cost of using PCR for diagnosing periodontal disease is high and not yet widely available in many dental clinics, a PCR as a diagnostic tool will eventually become the golden standard for minimally invasive periodontal treatment [17]. Exploring the pathological pathways that lead to the development, progression, and management of periodontal diseases could be highly beneficial and has some potential to provide proactive strategies for prevention and treatment, as well as reducing the risk for relevant systemic conditions [4, 8, 10, 11, 15-23]. The upcoming publication of the PCR will be beneficial in developing a more comprehensive understanding, from the identification of disease-promoting agents in the periodontium to effective treatment strategies.

The high sensitivity and specificity of PCR allow it to be a precise, efficient, and rapid method for the detection, identification, and quantification of microorganisms [4, 5, 26-28]. Several potential periodontal pathogens such as *Porphyromonas gingivalis (Pg), Aggregatibacter actinomycetecomitans (Aa), Tannerella forsythia (Tf), Prevotella intermedia (Pi), Prevotella nigrescens, Parvimonas micra (Pm), Eubacteria, Campylobacter rectus (Cr), Capnocytophaga sputigena, Capnocytophaga ochracea*, and *Capnocytophaga gingivalis* have been identified in samples of subgingival plaque (as referenced in the sources) [16].

This method also plays a crucial role in identifying bacteria responsible for periimplantitis before implant placement, thereby mitigating the risk of

periimplantitis [12, 13, 18, 25]. Quantitative PCR (Q-PCR) has identified opportunistic pathogens like *E. faecalis* within the peri-implant environment of diseased implants. This discovery suggests that removing the prosthesis and regularly decontaminating the implant surface and implant abutment connection may be necessary [12, 25].

Moreover, PCR is used for the detection of *Mycobacterium tuberculosis* in cases of osteomyelitis and hypertrophic gingivitis [4, 16]. Open-ended PCR/sequencing techniques are utilized to identify Gram-positive bacteria, including *Peptostreptococcus*, *Filifactor*, *Desulfobulbus*, *Deferribacteres*, *Atopobium*, *Treponema*, *Megasphaera*, *Dialister*, *Eubacterium*, *Selenomonas*, *Catonella*, *Streptococcus*, *Tannerella*, and *Campylobacter* in such situations [16].

Unfortunately, the use of PCR has some limitations. The most prominent of these is the cost of equipment and tests, as well as the potential for false positives and false negatives [4, 6-11, 24, 26, 28]. Additionally, PCR has a limited capacity to detect closely related and highly recombinant species [4, 5].

5. Conclusions

Investigating the pathological processes that contribute to the formation, progression, and management of periodontal diseases offers the potential to give proactive preventive and treatment techniques, as well as reduce the risk for associated systemic disorders. PCR as a diagnostic tool upgrades the diagnostic field of periodontology and allows dentists to recognize periopathology in the early stage and select the approprate treatment.

Declaration of interests

There are no conflicts of interest to declare.

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