DOI:10.33667/2782-4101-2023-1-7-9

REAL–TIME POLYMERASE CHAIN REACTION (PCR) APPLICATION FOR THE DETECTION OF PERIODONTOPATOGENS

E. M. Jafarov[1], A. A. Dolgalev[1], U. B. Edisherashvili[1], M. Z. Choniashvili[2], D. T. Taimazova[3]

[1]Federal State Budgetary Educational Institution of Higher Professional Education «Stavropol State Medical University», Ministry of HealthCare of the Russian Federation
[2]Federal State Budgetary Educational Institution of Higher Professional Education «Kosta Khetagurov North Ossetian State University Ministry of Science and Higher Education of the Russian Federation
[3]City Dental Clinic № 1, Vladikavkaz, Russian Federation

SUMMARY
The ethiological factor of periodontal diseases is the presence of periodontopathogens; in state of imbalance with commensals they begin to affect pathologically. With a decrease in the number of periodontopathogens in the biofilm, it is possible to restore the balance and prevent periodontal diseases or their transition to the stage of remission. Nowadays, the most informative and accessible diagnostic method for determining periodontopathogens is real-time polymerase chain reaction (PCR).

KEYWORDS: *periodontitis, periodontopathogens, polymerase chain reaction (PCR), periodontium, prevention.*

CONFLICT OF INTEREST. *The authors declare no conflict of interest.*

Relevance

Periodontitis is a chronic inflammatory disease of the gums of polymicrobial etiology. Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola are directly associated with this disease and are therefore called periodontopathogens or red complex bacteria [14.15]. These gram-negative obligate anaerobic bacteria exist in the biofilm that forms in subgingival pockets, and Fusobacterium nucleatum serves as a bridge to the supragingival biofilm, which consists mainly of streptococci [16]. The lipopolysaccharide (LPS) of these bacteria acts as an immunostimulant, causing gingival inflammation and activating osteoclasts through Toll-like receptors (TLR 2 or TLR 4), which trigger the expression of various cytokines, which in turn causes alveolar bone resorption [14]. However, recent metagenomic studies indicate that a wide range of microbiota associated with periodontitis may be involved in the disease process, which has to be identified yet [4].

Traditional treatment of periodontitis involves a non-surgical treatment aimed at controlling the pathogenic plaque and calculus biofilm from the crown and root surface through mechanical procedures (scaling) combined with improved personal hygiene, thus reducing inflammation and pocket depth. [10, 19.] In severe cases, antibiotic therapy may be required to hasten resolution of the disease [5]. Such therapeutic approaches are not always associated with success, and the frequent recolonization of treated areas with periodontal pathogens, as well as the emergence of antibiotic resistance, have led to the need to search for new therapeutic approaches for the treatment of periodontal diseases [3].

Among the various procedures used to detect oral bacteria, such as microbial culture, immunological assays, enzymatic methods, and molecular biology, the polymerase chain reaction (PCR) diagnostic method has become a powerful and increasingly popular tool due to its speed, sensitivity, and efficiency [12].

A number of methods have been developed for the detection and quantification of periodontal pathogens, including bacterial cultures, flow cytometry, DNA-DNA hybridization, immunoassays, enzymatic methods, and standard polymerase chain reaction (PCR). However, most of these methods are labor intensive and time consuming. In addition, they all have their own subjective limitations to achieve the desired sensitivity and specificity for accurate quantification of specific bacteria in samples [7, 13]. In recent years, quantitative real-time PCR technology has been developed to quantify bacteria. Real-time PCR with species-specific primers overcomes the limitations of traditional methods and becomes more suitable for bacterial quantification [1, 2, 6, 8, 9, 11, 19, 20, 21, 20]. Over the past 10 years attention has been drawn to the usage of saliva as a diagnostic fluid for periodontal disease [17].

Materials and methods

The survey included 20 patients: 10 men and 10 women who applied to North Caucasus Medical Training Centre LTD.

All patients completed a General and Disease History Questionnaire, Bleeding on probing (BoP), Probing Depth (PD), and Clinical Attachment Loss (CAL) and Plaque Index (PI), and a– radiological bone loss (RBL). The criteria for inclusion of patients in the experimental group was the diagnosis of moderate periodontitis (presence of BOP, PD – ≤ 5 mm, CAL – 3–4 mm, RBL – 15 % – 33 % PI – 1–3). No history of tooth loss due to periodontitis. The control group consisted of patients with healthy periodontal tissues (BOP – <10 %, PD – ≤ 3mm, CAL – no, RBL – no PI – 0–1)

The experimental group included 10 patients (5 men and 5 women) in the control group 10 (5 men and 5 women)

The material was taken in the morning, before the procedure of tooth brushing on an empty stomach. Previously, the tooth was dried with sterile gauze swabs. Samples were taken using

Table 1

Results of the examination of the control group of patients

No.	Sex	Age	Tooth	Actinobacillus actinomycetemcomitans (Lg)	Porphyromorans gingivalis (lg)	Prevotella intermedia (lg)	Tannerella forsythesis (lg)	Treponema denticola (lg)
	M	31	4.6				2.5	
	M	26	4.6					
	F	30	4.6				2.1	
	F	34	3.6		2.1	0.9	2.4	2.2
	F	28	3.6					
	M	27	3.6				2.1	
	F	30	4.6			2	2.9	
	M	24	4.6				2.6	1.6
	M	25	2.6				2.6	
	F	29	2.6				1.7	

Table 2

Results of the examination of the experimental group of patients

No.	Sex	Age	Zone	Actinobacillus actinomycetemcomitans (Lg)	Porphyromorans gingivalis (lg)	Prevotella intermedia (lg)	Tannerella forsythesis (lg)	Treponema denticola (lg)
	F	48	3.3		4.6	4.5	3.8	3.4
	M	60	4.6	2	5.3	4.2	3.8	4
	F	53	1.5		1.4			2.5
	M	61	3.6		4.5		3.4	2.6
	M	54	2.6		4.2			3.3
	F	64	4.4		2.5		0.8	
	M	61	2.5		5.2		4.3	4
	F	61	3.5		1.9			
	M	56	2.2		3.5	2.9	3.4	
	F	44	3.4		4.1	3.1	3.4	3.9

sterile paper endodontic pins of size No. 25, by immersion in the deepest periodontal pocket (patients of the experimental group) or in the gingival sulcus (patients of the control group) for 10 s. Then the paper point was removed and placed in a plastic test tube of the Eppendorf type with the DNA-Express. Detection of five periodontopathogenic microorganisms: Actinobacillus (Aggregatibacter) actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), Tannerella forsythensis (Tf) and Treponema denticola (Td) was performed by quantitative PCR with real-time detection of results using Dentoscreen set.

Research results

Tannerella forsythensis was found in eight patients in the control group, Treponema denticola was found in 6 patients, which may indicate an increased risk of developing periodontal diseases. According to the literature, Tannerella forsythia and Treponema denticola are included in the «red complex» of periodontopathogens (Socransky et al., 1998). Bacteria included in this group prevent the colonisation of commensal bacteria and are considered the most pathogenic periodontal complex, found in significant numbers in active and progressive periodontitis (Thurnheer et al., 2014; Holt & Ebersole, 2005). The results of the control group are presented in *Table 1.*

In 100 % of patients of the experimental group, Porphyromorans gingivalis (42.9 %), Tannerella forsythensis (66.7 %) and Treponema denticola (33.3 %) are found mostly.

The most rarely detected Actinobacillus actinomycetemcomitans (4.8 %) and Prevotella intermedia (14.3 %). The results of the experimental group are presented in *Table 2.*

Conclusion

The results of the study provide an understanding of the qualitative and quantitative presence of periodontopathogens in individuals with moderate periodontitis, as well as in clinically healthy patients. Microorganisms are the etiological cause of inflammatory diseases of periodontal tissues, and their detection at an early or preclinical stage determines the success of treatment, allowing to reduce the microbial load. The PCR method is not widely used in clinical practice, although it has several advantages:

- control of the microbial landscape before treatment, as well as during the stages;
- prevention of periodontal disease at an objective level;
- earlier detection of periodontal disease.

Reference

1. *Choi EJ, Lee SH, Kim YJ (2009) Quantitative real-time polymerase chain reaction for Streptococcus mutans and Streptococcus sobrinus in dental plaque samples and its association with early childhood caries. Int J Paediatr Dent 19:141–147*
2. *Colombo AV, Barbosa GM, Higashi D, di Micheli G, Rodrigues PH, Lorenzetti Simionato MR (2013 Jun 25) Quantitative detection of Staphylococcus aureus, Enterococcus faecalis and Pseudomonas aeruginosa in human oral epithelial cells from subjects with periodontitis and periodontal health. J Med Microbiol 62(Pt 10):1592–1600.*
3. *Gupta G. Probiotics and periodontal health. J Med Life 2011; 4(4): 387–394.*
4. *Hajishengallis G, Darveau RP, Curtis MA. The keystone pathogen hypothesis. Nat Rev Microbiol 2012; 10(10): 717–725.*
5. *Hung HC, Douglass CW. Meta-analysis of the effect of scaling and root planning, surgical treatment and antibiotic therapies on periodontal probing depth and attachment loss. J Clin Periodontol 2002; 29(11): 975–986*
6. *Hyvärinen K, Laitinen S, Paju S et al (2009) Detection and quantification of five major periodontal pathogens by single copy genebased real-time PCR. Innate Immunol 15:195–204*
7. *Loomer PM (2004) Microbiological diagnostic testing in the treatment of periodontal diseases. Periodontol 2000 34: 49–56,*
8. *Maeda H, Fujimoto C, Haruki Y et al (2003) Quantitative real-time PCR using TaqMan and SYBR Green for Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, tetQ gene and total bacteria. FEMS Immunol Med Microbiol 39:81–86*
9. *Morillo JM, Lau L, Sanz M, Herrera D, Martín C, Silva A (2004) Quantitative real-time polymerase chain reaction based on single copy gene sequence for detection of periodontal pathogens. J Clin Periodontol 31:1054–1060*
10. *Pihlstrom BL, Michalowicz BS, Johnson NW. periodontal diseases. Lancet 2005; 366(9499): 1809–1820.,*
11. *Price RR, Viscount HB, Stanley MC, Leung KP (2007) Targeted profiling of oral bacteria in human saliva and in vitro biofilms with quantitative real-time PCR. Biofouling 23:203–213*
12. *Rodu B. The polymerase chain reaction: the revolution within. Am J Med Sci 1990; 299: 210–216*
13. *Sanz M, Lau L, Herrera D, Morillo JM, Silva A (2004) Methods of detection of Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis and Tannerella forsythensis in periodontal microbiology, with special emphasis on advanced molecular techniques: a review. J Clin Periodontol 31:1034–1047*
14. *Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. J Clin Periodontol. 1998 Feb;25(2):134–44. doi: 10.1111/j.1600–051x.1998.tb02419.x. PMID: 9495612*
15. *Socransky, S.S. and Haffajee, A.D. (2002) Dental Biofilms: Difficult Therapeutic Targets. Periodontology 2000, 28, 12–55. http://dx.doi.org/10.1034/j.1600–0757.2002.280102.x;*
16. *Socransky, SS and Haffajee, AD (2005) Periodontal Microbial Ecology. Periodontology 2000, 38, 135–187. https://doi.org/10.1111/j.1600–0757.2005.00107.x*
17. *Spielmann N, Wong DT (2011) Saliva: diagnostics and therapeutic perspectives. Oral Dis 17:345–354*
18. *Suvan JE. Effectiveness of mechanical nonsurgical pocket therapy. Periodontol 20002005; 37:48–71.*
19. *Suzuki N, Yoshida A, Nakano Y (2005) Quantitative analysis of multi-species oral biofilms by TaqMan real-time PCR. Clin Med Res 3:176–185*
20. *Yano A, Kaneko N, Ida H, Yamaguchi T, Hanada N (2002) Real-time PCR for quantification of Streptococcus mutans. FEMS Microbiol Lett 217:23–30*
21. *Yoshida A, Suzuki N, Nakano Y, Oho T, Kawada M, Koga T (2003) Development of a 5' fluorogenic nuclease-based real-time PCR assay for quantitative detection of Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis. J Clin Microbiol 41:863–866*

Received 11.04.2023
Revised 24.04.2023
Accepted 30.04.2023

INFORMATION ABOUT AUTHORS

***Jafarov Elnur Matlab oglu**, postgraduate student of the Department of general practice dentistry and pediatric dentistry, Stavropol State Medical University, Ministry of Healthcare of the Russian Federation. ORCID ID: 0000-0001-9995-3749. Tel. +7-962-400-77-32, email: elnur985@mail.ru*

***Dolgalev Alexander Alexandrovich**, MD, Head of the Center for Innovation and Technology Transfer, Professor of the Department of General Practice Dentistry and Pediatric Dentistry of the Stavropol State Medical University of the Ministry of Health of the Russian Federation, Professor of the Department of Clinical Dentistry with a course of OS and MFS of the Pyatigorsk Medical and Pharmaceutical as Institute-branch of the Volgograd State Medical University Stavropol, Russian Federation. ORCID: https://orcid.org/0000-0002-6352-6750.
Tel. +7-962-440-48-61, email: dolgalev@dolgalev.pro*

***Edisherashvili Ushangi Besikovich**, postgraduate student of the Department of General Practice Dentistry and Pediatric Dentistry, Stavropol State Medical University, Ministry of Healthcare of the Russian Federation. ORCID ID: 0000-0002-1711-1415.
Tel. +7-921-535-35-85. email: ushangi.master@mail.ru*

***Choniashvili Marina Zurabovna**, Senior Assistant of Therapeutic, Surgical and Pediatric Dentistry with a Course of Gerontology and Oral Diseases, Kosta Levanovitch Khetagurov North Ossetian State University, Ministry of Science and Higher Education of Russian Federation. Ministry of Science and Higher Education of the Russian Federation. Tel. +7-928-495-16-61, email: Choniashvil.marina@mail.ru*

***Taimazova Dilyara Timurovna**, Dentist-therapist, «City Dental Clinic № 1», Vladikavkaz, Russian Federation.
Tel. +7-989-039-43-41, email: dilya.04@mail.ru*