MICROBIOLOGICAL FINDINGS OF ROOT CHANNEL FLORA IN ACUTE AND CHRONIC PERIAPICAL PARODONTITIS

Simonović Dragica, Kocić Branislava, Stanković Nedeljković Nataa, Jovanović Nadica, Gašić Jovanka, Dašić Stefan

Faculty of Medicine Niš – Stomatological Clinic, Institute of Public Health
Clinical Laboratory Department – Health Center Aleksinac

Infective agenses are the most important ethiological factors in Periapical parodontitis. Almost all inhabitans of oral cavity can take place in some phases of progress of periapical lesions. Microbes, some of their degradation products and methabolic products (antigens) are the most common causes of illness. In pathogenesis of Periapical parodontitis, the important role have yeasts of Candida spp, Spirohettae, amebes and even viruses.

Five channel swabs were taken from each patient and they were disseminated in a dentist s office. Immediately, after they were taken, they were disseminated on the medium for anaerobic cultivation, and then on the medium for microaerophil and aerobic cultivation. Solid mediums were disseminated with Miller niddle, whereas liquid mediums were disseminated with chrome wire, 0,4 mm in diameter, which was sterilized together wth liquid medium.

Columbia agar, Saburaud agar and glucose broth were used for aerobic cultivation. Schaedler agar, Schaedler agar with the addition of 29% gall and Schaedler agar with the addition of vancomycin in concentration of 7,5 mg/l were used for anaerobic cultivation. Tyoglycolate broth was used as a liquid base. Chocolate agar was incubated under microaerophil conditions.

Direct microscopic preparations were stained according to Gram and Giemsi.

Mediums for aerobic cultivation were incubated 20 hours in a thermostat at 37^0 C. Microaerophil conditions were achieved by means of GAS-PAC bags for microaerophil cultivation ("Torlak"), and incubation lasted 48 hours. Mediums for anaerobic cultivation were incubated 48 hours under anaerobic conditions achieved in pots for anaerobic cultivation by means of GAS-PAC bags for anaerobic incubation (bioMerieux) /10, 11, 13/. The identification of the isolated bacterial types was performed on the basis of microscopic, cultural, physiological- biochemical and antigenic characteristics.

Anaerobic microorganisms were found in 28 (93.33%) of 30 examinated swabs, from infected root channels in adults, with Parodontitis periapicalis acuta and chronica. The investigated group included: six patients with Parodontitis acuta (P.A.) or 20%, five patients with Parodontitis chronica fibrosa (P.F.) or 17%, nineteen patients with Parodontitis chronica granulomatosa (P.G.) or 63%.

Acording to our investigation, it is obvious, that Prevotella spp. Play very important role in periapical lesions, although, it is difficult to determinate the predominant strain of microorganism in infected root channel.

Key words: periodontitis periapicalis, microbiology

Parodontitis periapicalis could be defined as inflammatory pathological process which take place in tissues of parodontium with location on root apex, oposit to main channel aperture (4).

Infective agenses are the most important ethiological factors in *Periapical parodontitis*. Almost all inhabitans of oral cavity can take place in some phases of progress of periapical lesions. Microbes, some of their degradation products and methabolic products (antigens) are the most common causes of illness. In pathogenesis of *Periapical parodontitis*, the important role have yeasts of *Candida spp*, *Spirohettae*, amebes and even viruses (13).

Periapical parodontitis could be clasified as acute and chronic. Depending on immunological answer of a patient, chronic parodontitis could be defined as fibrous or granulomatous. Unfoturnately, the unique terminology and classification of periapical parodontitis does not exist (1, 2, 3).

Healthy oral cavity represents a complex microsystem, changeable in a number and type of bacteria, fungi, viruses and protozoa, which can be found there as commensals or as a part of flora. In the oral cavity, $4{,}3x10^7$ or $5{,}5x10^9$ bacteria (8, 9) can be found. Almost all the types of bacteria found in the mouth have sufficient pathogenic potential to induce inflammatory processes on teeth and soft tissues.

The causes of periapical inflammation are polymorphic. Their accurate identification can provide a clinican with the guidelines of therapy procedures. The examination of isolated microorganisms can significantly reduce certan therapeutic doubts.

The aim of this investigation is:

- Isolation and identification of prevalent microorganisms from channel swabs (*Gram negative anaerobic bacilli, Streptococcus spp, Staphylococcus spp, Gram positive anaerobic bacilli, spiral bacteria, Candida spp*).
- The analysis of prevalent microorganisms in the channel swabs in a paticular parodontitis type.

Material and methods

The examination was performed at Stomatological and Clinical laboratory department of Health Center in Aleksinac and at the Institute of Public Health in Niš. The examination involved 30 adults.

Five channel swabs were taken from each patient and they were disseminated in a dentist' s office. Immediately, after they were taken, they were disseminated on the medium for anaerobic cultivation, and then on the medium for microaerophil and aerobic cultivation. Solid mediums were disseminated with Miller niddle, whereas liquid mediums were disseminated with chrome wire, 0,4 mm in diameter, which was sterilized together wth liquid medium (17).

Isolation and identification of microorganisms

Columbia agar, Saburaud agar and glucose broth were used for aerobic cultivation. Schaedler agar, Schaedler agar with the addition of 29% gall and Schaedler agar with the addition of vancomycin in concentration of 7.5 mg/l were used for anaerobic cultivation. Tyoglycolate broth was used as a liquid base. Chocolate agar was incubated under microaerophil conditions.

Direct microscopic preparations were stained according to Gram and Giemsi.

Mediums for aerobic cultivation were incubated 20 hours in a thermostat at 37^0 C. Microaerophil conditions were achieved by means of GAS-PAC bags for microaerophil cultivation ("Torlak"), and incubation lasted 48 hours. Mediums for anaerobic cultivation were incubated 48 hours under anaerobic conditions achieved in pots

for anaerobic cultivation by means of GAS-PAC bags for anaerobic incubation (bioMerieux) /10, 11, 13/. The identification of the isolated bacterial types was performed on the basis of microscopic, cultural, physiological- biochemical and antigenic characteristics (12, 8, 14, 15).

Members of *Streptococcus* species were identified by the following methods: API-STREPTO system (bioMerieux), SLIDEX STREPTO-Kit (bioMerieux) and by the following tests: optohin, bacitracin, CAMP. According to need, some additional examinations of biochemical and physiological characteristics were used.

Bacteria of *Neisseria* species were identified on the basis of cultural characteristics, microscopic preparations, biochemical features, and growth capacity on a nutritious agar, growth capacity at the room temperature and oxidase test.

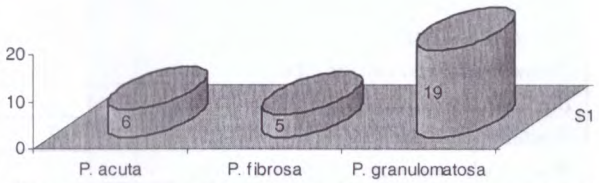
Gram negative bacteria from *Enterobacteriacea* family were identified on the basis of cultural characteristics, microscopic preparations and physiological properties, the examination of which is performed in a regular laboratory work.

Anaerobic bacteria were identified on the basis of cultural characteristics (for example dark pigmentation of bacterial colonies of *Prevotella* and *Porphyromonas* species), microscopic properties, aerotolerance test, growth capacity on Schaedler agar with the addition of 20% gall, growth capacity on Schaedler agar with vancomycine. In a final identification, API 20 A (bioMerieux) was used.

Yeasts of *Candida* species were identified after incubation of 7 days, at the temperature of 37^0 C primarily from Sabourad base (with the addition of maltose and 0.8% chloramphenicol) on the basis of cultural, biochemical and microscopic characteristics.

Results

Anaerobic microorganisms were found in 28 (93.33%) of 30 examinated swabs, from infected root channels ni adults, with *Parodontitis periapicalis acuta and chronica*. The investigated group included: the six patients with *Parodontitis acuta* (P. A.) or 20%, the five patients with *Parodontitis chronica fibrosa* (P. F.) or 17%, the nineteen patients with *Parodontitis chronica granulomatosa* (P. G.) or 63%.



Graph 1. The particular parodontitis type in group of examinated adults

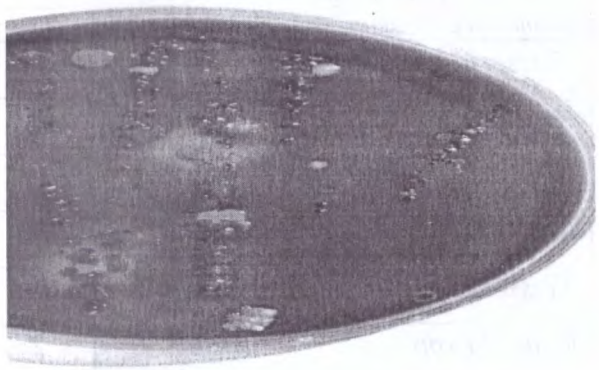
Gram negative anaerobic bacills

Gram negative bacills, *Prevotella-Porphyromonas spp*, were the most common isolated bacterial types. They were found in 24 (80%) of samples, out of 30 examinated subjects.

a) *Porphyromonas gingivalis*

The most common species of microorganism in infected root channels of adults was *Porphyromonas gingivalis*, which had been found in eighteen or 60% of swabs.

Porphyromonas gingivalis was isolated from two swabs of channels in patients with *Parodontitis acuta* and from sixteen swabs with *Parodontitis chronica* (statisticaly significant differens, X square 4,72 and C 0,44).



Picture 1. Porphyromonas gingivalis – colonies

Table 1. The most common species of microorganisms in different form of parodontitis

Strain	P. A.		P. F.		P. G.	
	N	%	N	%	N	%
Porphyromonas gingivalis	2	6.66	2	6.66	14	46.6
Prevotella intermedia	2	6.66	2	6.66	12	40
Prevotella oralis	2	6.66	1	3.33	2	6.66
Prevotella dentalis	2	6.66	1	3.33	2	6,66
Prevotella melaninogenica	2	6.66	1	3,33	2	6,66
Bacteroides ovatus	2	6.66	1	3,33	3	10
Bacteroides fragilis	2	6.66	-	-	-	-
Bacterodes rumnicola ruminocola	2	6.66	-	-	-	-
Streptococcus salivarius	-	-	-	-	2	6.66
Streptococcus sanguis II	4	13.33	-	-	2	6.66
Streptococcus cremoris	1	3.33	2	6.66	5	16.66
Streptococcus mutans	-	-	1	3.33	2	6.66
Streptococcus mitis	-	-	1	3.33	3	10
Peptostreptococcus spp.	1	3.33	2	6.66	1	3.33
Gemella morbilorum	-	-	4	8.88	3	10
Streptococcus intermedius	3	10	3	1o	2	6.66
Steptococcus constelans	1	3.33	-	-	-	-
Streptococcus asaccharolyticus	1	3.33	-	-	1	3.33
Staphylococcus aureus	-	-	2	6.66	3	1o
Staphylococcus epidermidis	1	3.33	-	-	8	6.66
Staphylococcus saprophyticus	-	-	-	-	2	6.66
Lactobacillus fermentum	-	-	1	3.33	3	1o
Lactobacilus acidophylus	-	-	1	3.33	1	3.33
Lactobacillus minutus	-	-	-	-	2	6.66
Lactobacillus odontolyticus	1	3.33	1	3.33	1	3.33
Actinomyces viscosus	2	6.66	1	3.33	2	6.66
Actinomyces odontolyticus	-	-	1	3.33	3	10
Actinomyces neaslundi	1	3.33	-	-	-	-
Actinomyces israeli	1	3.33	-	-	-	-
Neisseria mucosa	1	3,33	1	3,33	3	10
Neisseria sicca	4	13,33	2	6,66	4	3,33
Neisseria catarrhalis	1	3,33	2	6,66	-	-
Neisseria flavescens	1	3,33	1	3,33	3	10
Neisseria perflava	-	-	-	-	2	6,66
Candida spp.	2	6,66	-	-	6	20

b) *Prevotella intermedia*

Prevotella intermedia was isolated from sixteen channels or 53,33% swabs.

Prevotella intermedia was significantly present et lavel of statistic signification of p<0,01 (X square 2,8 and C 0,26).

Prevotella melaninogenica, Prevotella dentalis and *Prevotella oralis* were not found in statistically significant number.

Bacteriodes species

The most common isolated microbe, in genus of *Bacteroides spp*, was *Bacteroides ovatus* and with higher frequency in *Parodontitis chronica. Bacteroides fragilis* and *Bacteroides ruminocola ruminocola* were isolated in *Parodontitis acuta*, only.

Streptococcus species

a) *Nonchemolitic and viridans species of Streptococci*

Nonchemolitic and viridans species of *Streptococci* were found in 23 (77,3%) of examinated samples of swabs. Nonchemolitic and viridans species of *Streptococci* were more frequently found in *Parodontitis granulomatosa.*

b) *Gram positiv anaerobic and micro-aerophilic Streptococci*

Gram positive anaerobic and microaerophilic *Streptococci* were very often isolated from swabs of infected root channel, and seemed very pathogenic.

Streptococcus intermedius was found in eight swabs (26,6%) and was the most common isolated strain of *Streptococcus* species.

Staphylococcus spp

Microorganisms of *Staphylococcus spp* were isolated from sixteen (53,33%) swabs. The most common isolated microbe was *Staphylococcus epidermidis*, which was found in nine swabs of investigated channels.

Gram positive anaerobic bacills

Lactobacillus spp

Species of *Lactobacillus* were often found in periapical parodontitis in adults. They were identified in eleven swabs of infected channels (36.66% of samples). *Lactobacillus fermentum* was the most common strain and was found in four (13.3%) samples.

Species of *Lactobacilli*, most frequently, were isolated from channels in patients with *Parodontitis chronica granulomatosa.*

Actinomyces spp

Actinomyces spp were isolated from infected root channels in eleven (36,66%) swabs.

Neisseiae spp

Neiseriae spp were found in 25 (83,33%) swabs. *Neisseria sicca* was isilated from ten (33,33%) samples.

Candidae spp.

Candida yeast was isolated from eight (26,66%) swabs. In *Parodontitis chronica granulomatosa*, was the most frequent.

Discusion

The results of our investigation confirm polymicrobial ethiology of periapical inflammation in *Parodontitis acuta and chronica*. Different anaerobic, aerobic and microaerophilic strains of microbe, *Candida spp* and spiral forms, were isolated, frequently. It was difficult to find prevalent type of microbe.

Prevotella-Porphiromonas groups of microorganisms (pigmented Gram negative anaerobic

bacillus) were found in 80% of investigated patients. It was the most common isolated bacterial group with relevant statistical significance (p<0,01). *Prevotella intermedia* and *Porphiromonas gingivalis* were frequently identified in swabs of *Parodontitis chronica granulomatosa* (p 0-0,5). Gram negative anaerobic bacills are the usual inhabitans of oral cavity. Most of them can be found in a supra and subgingival plaque. The significant presence of them in infected root channels could be explained by communication between oral environment and root channels. That confirms their role in ethiology of periapical processes.

Nonchemolitic and viridans Streptococci are normal inhabitans of mouth cavity. They were found in 76,66% of examinated channels. Anaerobic Streptococci were present in 73,33% examples. The quantity of all *Streptococci spp* had statistically significant level of p<0,01, and they, also, seemed very pathogenic.

Staphilococci spp can be part of permanent or running flora in oral cavity. They were isolated in 53,33% of examinated channels. *Lactobacillus spp, Actinomyces spp.* are usualy, the part of flora mouth. In our investigation they vere frequently found in infected channels.

The common presence of *Candida spp.* could be explained as disturbance of immunological system of patients (68,78,80).

Gomes et al (16) analysed channel flora in 70 patientes with *Periapical parodontitis*. The 37 patients had toothache, 49 had positive percusion sign, 23 had swelling, 6 purulent exudate and 57 had wet channel. Statistical significant conection was noted between:

a. tootache and presence of *Prevotella spp.* or *Peptostreptococcus spp.* (p<0,01)
b. positive percusion and *Prevotella spp.* (p<0,01) or anaerobic bacteria (p<0,05)
c. swelling and *Eubacterium spp.* (p<0,01)
d. purulent exudate and *Fusobacterium necrophorum* (p<0.01)
e. wet channel and facultative anaerobs (p<0.01).

According to these findings and our investigation, it is obvious, that *Prevotella spp.* Play very important role in periapical lesions, although, it is difficult to determine the predominant strain of microorganism in infected root channel.

Conclusion

1. The polymorphic anaerobic, microaerophilic and aerobic flora and Candida, were isolated from swabs of examinated root channels.
2. The prevalent flora was anaerobic (93,33%).
3. The most common type of *Parodontitis periapicalis* was *Parodontitis chronica granulomatosa* (60%).
4. The most common species of microorganisms were Prevotella-Porphiromonas groups, which were found in 80% of samples (statistical significans of p<0,01).
5. The most common isolated microbe was *Porphiromonas gingivalis*, which was found in 60% of swabs.
6. The presence of *Streptococci spp*, also, had statistically significant level of p<0,01.
7. The frequently presence of Candida should be further investigated.
8. Becouse of polimorphic flora, the culturing of swabs, sometimes should be necessary, in therapy of parodontitis.

References

1. Albandar IM., Brown L.J., Genco, R.J., Loe, H.(1997): Clinical classification of periodontitis in adolescents and young adults. J Periodontol.,68 (6), 545-55.

2. Dibert S.(1997): Children, adolescents and periodontal diseases. J Dent., 25 (2), 79-89.

3. Takahashi K. (1998): Microbiological, Pathological, Inflammatory, Immunological and molecular biological aspects of periradicular disease. Int Endodont J., 31 (5) 311-25.

4. Pavlović V. (1998): Klinika endodoncija. Univerzitet u Nišu, Medicinski fakultet, Niš.

5. Lamster I.B., Grbic J.T.,Mitchell A., Lewis, D.A., Begg MD.(1998) New concepts regarding the pathogenesis of periodontal diseases in HIV infection. Ann Periodontol., 3 (1) 62-75.

6. Maeda N., Okamoto M., Kondo, K., Shikawa H., Osada,P., Tsurumoto, A., Fujita, H. (1998). Incidence of Prevotella intermedia and Prevotella nigrescens in periodontal health and disease Microbiol Immunol., 42 (9), 538-9

7. Matsushida K., Tajima T., Tomita K., Abeyana K., Marmayama J., Takada,H., Nagaoka,S. (1998): Inflammatory cytocikle production and specific antibody responses against possible causativa bacteria in patients with multilesial periapical periodontitis J Endodont., 24 (12), 817-21:

8. Karakašević B. (1997): Mikrobiologija i parazitologija, Medicinska knjiga, Beograd-Zagreb.

9. Kotev-Penev Lj., Filipović, S. (1989): Mikroorganizmi usne duplje. Univerzitet u Nišu, Niš.

10. Doan N., Contreras A., Flynn J., Morrison J. (1999): Proficiences of tree anaerobic culture systems for recovering periodontal pathogenic bacteria. J Clin Microbiol., 37 (1), 171-4.

11. Holt J., Krieg N., Sneath P., Staley J., Williams S. (1994): Bergeys Manual of determine bacteriology. Williams and wilkins. Baltimore. Philadelphia. Hong kong. London. Munich. Sydney. Tokyo.

12. Jawetz E., Melnick J., Adelberg E. (1998): Medicinska mikrobiologija, Savremena administracija, Beograd.

13. Parra B., Slots J. (1996): Detection of human viruses in periodontal pockets using polymerase chain reaction, Oral Microbial Immunol., 11 (5), 289-93.

14. Narikawa S., Suzuky Y., Takahashy m. (1995): Streptococcus oralis previously identified as uncommon Streptococcus sanguis in Bechets disease. Arch Oral biol, 40 (8) 685-690.

15. States D., Stobo J., Wells J.V. (1991): Klinička imunologija, Savremena administracija, Beograd.

16. Gomes B.,Lilley J.D., Drucker D.B. (1996): Clinical significance of dental root canal microflora, J Dent. 24 (1-2), 45-55.

17. Stanković Nedeljković, N.: Mikrobiološki agensi u etio patogenezi akutnog i hroničnog parodontitisa, 2002, Magistarski rad, Niš.