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# MICROBIOLOGICAL SUBSTANTIATION OF THE OZONE OILS USAGE FOR THE TREATMENT OF PATIENTS WITH CHRONIC APICAL PERIODONTITIS

***Abstract.*** *The efficacy of endodontic treatment of patients with chronic apical periodontitis determines the rational use of antibacterial medicaments. To elimination of the microflora is used various antibacterial drugs. However, they very quickly became insensitive to the conditionally pathogenic root canal microflora. So relevant is the increase of the effectiveness of antibacterial agents. A promising in this regard is a combination of antibacterial medicaments with ozone. Very important is microbiological assessment of effectiveness ozonotherapy of patients with chronic apical periodontitis. Aim. To determine the microbiological comparative antibacterial activity of non-ozonized and ozonized oil "Eugenol" ("VladMiVa", Russia) on the mixed microflora (aerobic type) from root canal teeth of patients with apical periodontitis. Carrying out a comparative assessment of the quality of root canal treatment after mechanical treatment and application of ozone in chronic periodontitis based on microbiological data. Materials and methods. To determine the sensitivity of microorganisms to the medicaments was used modification of the disk-diffusion method. As a test microorganisms were used reference strains and mixed microflora from the root canal teeth of patients with apical periodontitis. Endodontic treatment was performed on 96 patients (98 teeth) with chronic periodontitis. The material for the background microbiological study was taken immediately after opening of the root canal (before processing the canal). Results. Ozonized oil "Eugenol" showed selective antibacterial properties on mixed microflora (aerobic type) and prolonged duration. After a microbiological research of the root canal content, it was found that root canals with chronic periodontitis in 100 % of cases were infected with various microorganisms, including anaerobic ones. In the initial examination, 125 strains of microorganisms were isolated, and after a thorough chemomechanical treatment of root canals, their number was only 35, the percentage of anaerobic microorganisms remained at the same high level and was 40 %. Conclusion. Ozonized oil "Eugenol" showed selective antibacterial properties and prolonged duration. These microbiological results allow usage this medicaments for clinical use in the treatment of chronic apical periodontitis. Using of ozonotherapy for the treatment of chronic periodontitis in 90.7 % of cases we have been disinfected root canals.*

***Key words:*** *ozonated oil "Eugenol", chronic apical periodontitis, root canals, microorganisms, ozonotherapy.*

**Introduction.** Microbiological studies in the root canals of patients with periodontitis revealed a variety of conditionally pathogenic microflora. Most often, they are representatives of the families Bacteroides, Fuzobacterium, Streptoccocus, Peptostreptoccocus, Lactobacillis, etc. The predominance of anaerobic microorganisms is noted [6, 7, 9].

The main goal of the endodontic treatment of apical periodontitis is to suppress the conditionally pathogenic microflora of the root canal system and prevent its re-infection [13, 27]. Achieving this goal complicates the complex morphology of the root canal system [1, 8] and only mechanical treatment is clearly insufficient [18, 19 ] For the suppression of conditionally

pathogenic microflora, a large number of various antibacterial preparations have been proposed in the form of solutions for irrigation of the root canal [1, 8, 10]. They should have significant antibacterial properties, but on the other hand, do not damage periapical tissues of the patient [10]. An ideal antibacterial drug for irrigation of the root canal should be bactericidal, nontoxic, harmless to periapical tissues, do not interfere with their regeneration, have a long antimicrobial effect [2-4, 23].

Various antiseptic agents are used to irrigate the root anal system [10, 12, 14, 16, 17]. In recent years, promising treatment in many areas of medicine is ozone therapy. Clinical and laboratory studies of the effectiveness of the use of medical ozone in dentistry in the treatment of foci of chronic infection, in particular periodontitis, indicate a pronounced antibacterial effect of ozone [3, 5, 8, 12-14]. Its application allows to achieve a reduction of pain; suppression of bacterial microflora in the root canal; reduction of inflammatory process in periodontium and stimulation of reparative processes in periapical tissues [9, 11, 21, 24].

In dentistry, ozone is used in three basic forms: ozone-oxygen mixture, ozonized solutions and ozonized oils. Ozonized oils are used to temporarily fill the root canal with the treatment of pulpitis and periodontitis [21, 24].

To substantiate the clinical application and determine the antibacterial effectiveness of various ozonized solutions, microbiological studies were performed.

**Objective.** Microbiological determination of antibacterial activity of non ozonized and ozonized oil "Eugenol" on the mixed microflora of the root canal of teeth in patients with chronic periodontitis, comparative assessment of the quality of root canal treatment after mechanical treatment and application of ozone in chronic periodontitis based on microbiological data.

**Material and methods of research.** For the manufacture of ozonized oil, 25 ml of eugenol ("Eugenol" (Vladmiva)) was used which was bubbled (ozonized) with the help of the apparatus "OZON UM-80" (Kharkiv) according to the manufacturer's instructions for 60 minutes at an ozone concentration of 35 mg / Flow 0.5 l / min.

For the microbiological study, a mixed root canal microflora was isolated and cultivated for patients with chronic periodontitis, aged 20-44 years. The material was collected from the root canal by a sterile instrument (pulp extractor), which was placed in a sterile test tube with a semi-rigid tioglycol medium (produced by the FSUE SSCPM, Russia). Tubes with thioglycolic medium were incubated at 37 ° C in a thermostat for 5 days. After the incubation, the relative number of microorganisms was evaluated by the degree of cloudiness of the medium. Subsequently, the isolated blended microflora of the root canal was used similarly to the test strains of microorganisms.

To determine the antimicrobial action of the studied oil samples, the method of "well" [22, 26] was used, which is a kind of disk-diffusion method for determining the sensitivity of microorganisms [20]. The standard test culture of Ecsherihia coli, Staphylococcus aureus, Porphyromonas aeruginosa, Candida albicans was also used for the study.

A standardized inoculum was applied to a petri dish with a nutrient medium (agar containing 5% red blood cells) in a volume of 1-2 ml and evenly distributed, the excess inoculum was removed by pipette. The open cups were dried for 10-15 minutes at room temperature. After that, at the same distance from the edges of the Petri dish, the wells were 6 mm in diameter. For this purpose, steel thin-walled cylinders were installed (internal diameter - 6.0 + 0.1 mm, height - 10.0 + 0.1 mm). After drying, the cylinders were extracted with sterile tweezers and in the resulting wells were ozonized oil "Eugenol" (mark "1") and non-ozonized oil "Eugenol" (mark "2") with a standard loop diameter of 3 mm. Immediately after application, the cups were placed in a thermostat and incubated at 35 ° C. for 24 hours. At the end of the incubation period, the diameter of the growth retardation zone in millimeters was measured. Determination of the diameter of the growth retardation zone (sensitivity of the microflora) was carried out in 1 and 28 days of incubation. Each of the experiments for statistical authenticity was

repeated 5 times.

Statistical analysis of data was carried out in the applied computer programs StatSoftStatistica 10 and MicrosoftOfficeExcel 2010 with the help of variational and one-factor dispersion analyzes [15, 25].

In the clinical part of the study, 96 patients with chronic apical periodontitis were examined and treated. In patients, the material was collected from the root canal for microbiological studies: before the treatment of the canal after the instrumental and medical treatment of the root canal and after the application of ozone.

The results of laboratory and clinical studies were processed by methods of variation statistics [26 Mintser Truhacheva].

**Results of the research and their discussion.** Indicators of the growth retardation zone of microorganisms cultures (in mm) on the nutrient medium are presented in Table 1, 2.

*Table 1*

**Antimicrobial activity of oil samples after 24 hours of incubation**

| Samples of oil | Growth region (in mm) of test strains of microorganisms | | | | |
|---|---|---|---|---|---|
| | Pseudomonas aeruginosa | Escherichia coli | Staphylococcus aureus | Candida albicans | Змішана мікрофлора |
| «Eugenol»ozonized | 12,0±0,34 | 7,0±0,14 | 9,0±0,19 | 13,0±0,42 | 11,9±0,35 |
| «Eugenol» non-ozonized | 9,0±0,44 | 9,0±0,44 | 6,0±0,14 | 15,0±0,45 | 11,0±0,24 |
| p | <0,05 | <0,05 | <0,05 | <0,05 | <0,05 |

*Note: p – the reliability of the difference between the values of different types of oils*

*Table 2*

**Antimicrobial activity of the oil samples after 8 days of incubation**

| Samples of oil | Growth region (in mm) of test strains of microorganisms | | | | |
|---|---|---|---|---|---|
| | Pseudomonas aeruginosa | Escherichia coli | Staphylococcus aureus | Candida albicans | Mixed microflora |
| «Eugenol» ozonized | Zone of growth retardation is absent | 9,0±0,44 | 7,0±0,15 | 6,0±0,14 | 8,5±0,24 |
| «Eugenol» non- ozonized | Zone of growth retardation is absent | 6,0±0,14 | 9,0±0,45 | 6,0±0,14 | 7,6±0,22 |
| p | >0,05 | <0,05 | <0,05 | >0,05 | <0,05 |

*Note: p – the reliability of the difference between the values of different types of oils*

According to the results of the study, Staphilococcus aureus, Escherichia coli, P.aeruginosa, Candida albicans, ozonized and non-ozonized "Evgenol" oils showed a wide range of antimicrobial activity and showed a long antibacterial effect.

Determination of zones of growth retardation of microorganisms after 1 day of incubation showed (Table 1) that the most sensitive to the effect of ozonized oil "Eugenol" were the test strains Candida albicans - a growth retardation zone of 13.0 ± 0.42 mm and Pseudomonas

aeruginosa - the growth retardation zone of which is 12.0 ± 0.34 mm. Less susceptible were strains of Staphylococcus aureus, a growth retardation zone of 9.0 ± 0.19 and Escherichia coli, a growth retardation zone of 7.0 ± 0.14 mm.

Interestingly, the last strain of test microorganisms was more sensitive to neozonized oil "Eugenol" - the growth retardation zone was 9.0 ± 0.44 mm. In all other cases, ozonized oil "Eugenol" produced a significantly better ($p < 0.05$) antibacterial effect compared to non-ozonized oil. The received sizes of zones of growth retardation of microorganisms can be explained by the fact that the oils (in comparison with aqueous solutions) are not sufficiently actively diffused into the nutrient medium. The mixed microflora of the root canal was moderately sensitive to the action of the "Eugenol" oil. The zone of growth retardation of microorganisms under the influence of ozonized oil "Eugenol" was 11,9 ± 0,35 mm, neonozonovoy - 11,0 ± 0,24 mm. The difference is statistically significant (<0.05).

A microbiological study has shown that ozonized oil "Eugenol" has sufficient selective activity in relation to test strains of microorganisms and mixed microflora of the root canal.

Microbiological studies conducted in patients have shown that root canals in patients with chronic periodontitis in 100% of cases are sown with various microorganisms, including anaerobic. In addition, in 50% of cases, the microflora was sown in associations. The results of the study are presented in Table. 3

The associations of streptococci and staphylococci with anaerobic cocci were most often identified. As a rule, in chronic periodontitis in the root canal of teeth are formed such conditions, under which the access to oxygen is sharply limited. Therefore, it is quite obvious that the percentage of allocation of obligate anaerobes is very high. In our study, their number was 34%.

Repeated study of the root canal content in patients was performed after instrumental and drug treatment. The results are presented in table 4.

*Table 3*

**Species composition of microorganisms isolated from the root canal in the initial examination of patients**

| Types of isolated microorganisms | Number of selected strains | |
|---|---|---|
| | abs. | % (relative) |
| Streptococci | 19 | 15,2 |
| Staphylococci | 29 | 23,2 |
| Lactobacillus | 26 | 20,8 |
| Enterococci | 8 | 6,4 |
| Anaerobic species | 43 | 34,4 |
| Number of selected strains | 125 | 100 |
| Lack of growth of microorganisms | - | - |
| Number of observations | 43 | 100 |
| Microbial associations | 38 | 88,4 |
| Monoculture | 5 | 11,6 |

It should be noted that after the chemo-mechanical treatment of the root canal, the picture of the microbial landscape has undergone significant changes. Sharply decreased the total number of isolated strains. If in the initial study, 125 strains (100%) were isolated, then after chemomechanical treatment their number was only 35 (28%), which is 3.5 times less.

We managed to achieve disinfection by 72%.

The third definition of the microflora of the root canal was carried out after the use of ozonotherapy. These data are presented in Table. 5.

Microbial growth was obtained in only four

*Table 4*

**Species composition of microorganisms isolated from the root canal after chemomechanical treatment**

| Types of isolated microorganisms | Number of selected strains | |
|---|---|---|
| | abs. | % (relative) |
| Streptococci | 6 | 17,1 |
| Staphylococci | 7 | 20,0 |
| Lactobacillus | 4 | 11,4 |
| Enterococci | 4 | П,4 |
| Anaerobic species | 14 | 40.0 |
| Number of selected strains | 35 | 100 |
| Lack of growth of microorganisms | 4 | 9.3 |
| Number of observations | 43 | 100 |
| Microbial associations | 19 | 54.2 |
| Monoculture | 12 | 34,3 |

*Table 5*

**Species composition of microorganisms isolated from the root canal after ozonotherapy**

| Types of isolated microorganisms | Number of selected strains | |
|---|---|---|
| | abs. | % (relative) |
| Streptococci | 0 | 0 |
| Staphylococci | 1 | 25,0 |
| Lactobacillus | 0 | 0 |
| Enterococci | 0 | 0 |
| Anaerobic species | 3 | 75,0 |
| Number of selected strains | 4 | 100 |
| Lack of growth of microorganisms | 39 | 90,7 |
| Number of observations | 43 | 100 |
| Microbial associations | 0 | 0 |
| Monoculture | 4 | 100 |

cases (4.17%). In three cases (3,12%), the growth of anaerobic species of microorganisms and in one (1,04%) staphylococci were observed. The results of our studies indicate a fairly high ability of ozonotherapy to disinfect the canals. In 90.7% of cases, root canals were disinfected.

**Conclusions.** Ozonized oil "Eugenol" showed selective antibacterial activity in the aerobic microflora of the root canal, and prolonged duration of action. The obtained microbiological results allow the use of this drug in the treatment of patients with chronic periodontitis.

In chronic periodontitis in the root canal of teeth, associations of streptococci and staphylococci with anaerobic cocci were most often found. In the initial survey 125 strains of microorganisms were isolated, and after a thorough chemomechanical treatment of root canals, their number was only 35, the percentage of anaerobic representatives practically remained at the same high level and was 40%. When using ozonotherapy for the treatment of chronic periodontitis, in 90.7% of cases root canals were disinfected.

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