""Microbiological evaluation of the effectiveness of depot, apex of fluctuorization and electrophoresis in complex treatment of chronic apical periodontitis".

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Abstract:*Treatment of chronic apical periodontitis (CVP) is one of the most important and not fully solved problems of therapeutic dentistry. This is primarily due to the significant prevalence of this disease, the complexity and complexity of medical manipulations, a large percentage of failures and complications in treatment, as well as the lack of stability of the results obtained using known treatment methods. Therefore, the constant search for new and more effective means and methods of treatment of CVP is one of the urgent tasks of modern dentistry.*

Key words: *periodontitis, patients, treatment, microflora, efficiency.*

1. Purpose of research

Improving the effectiveness of treatment of chronic apical periodontitis by improving endodontic treatment of the disease with separate and combined use of new methods of depo – and apex-foresis with joint application of fluktuorization.

2. Research materials and methods

Streptococci and staphylococci were most frequently detected in the study material: Str. sanguis - in 52% of patients, Str. mutans-in 68%, Str. salivarius-in 52%, and St. epidermidis-in 41%. In addition, Peptostreptococcus anaerobius was detected in root canals in 38% of patients, and Clostridium spp was detected in 12% of patients. In the area of rarefaction, there is no drawing of Candida albicans bone beams.

The use of depo – and apex-Forez in the treatment of chronic apical periodontitis leads to a significant ($P<0.05$-0.001) rapid acceleration of the processes of regeneration of periapical tissues in comparison with traditional methods of treatment of the disease. At the same time, the combined use of depo -, apex-foresis and fluktuorization has a 1.3-2.2 times more effective effect on the condition of the periapical tissue of the teeth than using them separately, which is expressed in reducing the number of complications, accelerating the process of bone regeneration in the apical periodontal area and thereby reducing the number of visits to the dental institution.

Treatment of chronic apical periodontitis (CVP) is one of the most important and not fully solved problems of therapeutic dentistry. This is primarily due to the significant prevalence of this disease, the complexity and complexity of medical manipulations, a large percentage of failures and complications in treatment, as well as the lack of stability of the results obtained using known treatment methods. Therefore, the constant search for new and more effective means and methods of treatment of CVP is one of the urgent tasks of modern dentistry.

Meanwhile, in recent years, new methods have been used in the treatment of periodontitis of problem teeth: copper – calcium hydroxide depophoresis and apex-foresis with the joint use of the physiotherapy method of fluktuorization. Depophoresis of copper-calcium hydroxide and apex-foresis has a transcanal effect on the apical part of the tooth root by direct current using a silver-copper conductor, and fluktuorization simultaneously has an analgesic (analgesic) anti-inflammatory; local myostimulating (increases the functional state of muscle tissues); trophic-regenerative (increases the supply of tissues with nutrients, which contributes to their accelerated recovery) effect.

Despite conflicting opinions about these methods, a number of studies and clinical observations indicate their effectiveness as an alternative to impregnation methods. The prospect of using these methods in destructive forms of CVP is due to the fact that they allow you to completely clean additional channels and branches from pulp residues, thoroughly sterilize and obturate them, and also have a pronounced antibacterial activity on the anaerobic microflora of the root canals of teeth, as well as rapid post-pain termination, which can not be achieved using well-known traditional methods.

All patients were examined the microflora of the root canals of the teeth, x – ray examination and electrodontodiagnostics (EDI) from the mouths of the root canals before traditional treatment and treatment with depot, apex-Forez together with the physiotherapy method of fluktuorization. X-ray images were used to record the absence or presence of destructive changes in the periodontium, and EDI was used to determine the state of the root pulp. If the remains of infected pulp were found in the root canals of the teeth (electrical excitability-below 100 mkA), it was devitalized with a devit. The procedures were prescribed after complete devitalization of pulp residues with EDI readings above 100 mkA.

Evaluation of the immediate results of endodontic dental treatment was performed within 7 to 14 days based on patient complaints and objective clinical examination of the tooth and surrounding tissues.

Long-term treatment results were evaluated after 6 and 12 months based on patient complaints, objective clinical examination of the tooth and surrounding tissues, and x-ray examination.

To analyze and compare the results of x-ray examination, we used the periapical index PAI, proposed by D. Qrstavik et al. (1986) and modified by a.m. Solovyova (1999). The periapical index allows not only to detect rarefactions in the bone tissue, but also to assess the structure of bone trabeculae and bone marrow spaces in the periapical region (table 1).

Analysis of radiographs using PAI provides for their high quality, in doubtful cases, radiographs were evaluated with a higher PAI score, and images of multi-root teeth were evaluated by the highest of the detected values.

In order to study the effect of treatment on the root canal microflora, a bacteriological study was performed twice-before and after the endodontic treatment (before filling the root canal). To do this, using a sterile paper absorber, the material was taken from the root canal of the tooth and stirred into a semi-liquid Ames culture medium for subsequent transportation. Further bacteriological research was carried out in accordance with the generally accepted rules of clinical anaerobic Microbiology: quantitative sectoral seeding was performed on media intended for the cultivation of oral bacteria in aerobic and anaerobic conditions. Pure cultures of obligate and facultative anaerobic bacteria under anaerobic conditions were obtained using 5% hemagar prepared on the basis of Brain-Heart Infusion from Difco with the addition of gemin (5 mg/l) and menadion (0.1 mg/l) with the mandatory placement of crops in anaerostats with an oxygen-free gas mixture containing 80% nitrogen, 10% hydrogen, 10% carbon dioxide. A palladium catalyst was used to reduce oxygen residues. The type of isolated bacteria was determined using a complex of morphological, cultural, and biochemical characteristics. Biochemical identification of pure cultures of anaerobic bacteria, streptococci, staphylococci, and gram-negative bacteria was performed using API (France) and Roche (Germany) test systems.

Clinical strains of facultative anaerobic bacteria obtained from the root canals of teeth, namely: Staphilococcus epidermidis, Streptococcus sanguis, Streptococcus mutans, Streptococcus salivarius, Candida Krusei, Escherichia coli, Clostridium spp., were used to determine the optimal parameters of apex-Forez dosage that provide a pronounced antibacterial effect. For growing Staphylococcus, streptococci and Clostridium, 5% blood agar was used, Escherichia coli-meat-peptone agar, and for Candida Krusei-Saburo medium.

In accordance with the existing recommendations, the study was carried out as follows: cultures of microorganisms at a concentration of 1 million CL/ml (according to the optical turbidity standard) were sown on the surface of freshly prepared agar in Petri dishes using a "lawn" method, evenly distributing them over the agar surface using a sterile spatula. Then the Petri dish was divided into sectors. In one sector, a silver-copper electrode was placed, which was connected to the plus of the current source, in the other - a similar electrode connected to the minus. Only the active working part of the electrodes was immersed in agar. The Potok-1 and Elfor-prof devices were used as a direct current source. The procedures were dosed according to the amount of electricity (mA min). To determine the optimal parameters, 3 doses of exposure were studied: 1.5 mA min, 2.5 mA min and 5 mA min. After the procedure was completed, the electrodes were removed from the agar, and Petri dishes were placed in anaerostats with an oxygen-free gas mixture containing 80% nitrogen, 10% hydrogen, and 10% carbon dioxide. A palladium catalyst was used to reduce oxygen residues. The results were recorded after 7 days of incubation of Petri dishes in an anaerostat at 37oC. Accounting was performed by measuring the diameter of the bacterial colony growth delay zone (in millimeters) around the hole left by the electrode on the agar. Depending on the diameter of the growth retardation zone, the antibacterial effect was evaluated as weak (with a diameter of less than 5 mm), medium (with a diameter of 5-10 mm) and high (with a diameter of more than 10 mm).

To study the effect of apex-phoresis on difficult-to-cultivate pathogenic anaerobic bacteria, a test system based on polymerase chain reaction (PCR) was used . The microflora of the root canal contents was determined using PCR twice: the first time - before conducting transcanal direct current treatment (after mechanical treatment of the root canal for patency without irrigation with antiseptics), the second time - 2 weeks after completing the course of electrical procedures. PCR was performed using the Multident-5 test produced by Genlab (Russia). The method of sampling for PCR diagnostics from root canals was as follows:

- introduction of a sterile paper endodontic pin (point) into the root canal so as to exclude contact with the mucous membrane, enamel surface or crown of the tooth;

- transfer the paper pin to Eppendorf tubes and transport it to the laboratory.

Samples of the test material taken from root canals using a sterile paper point were delivered within 1 hour to the laboratory of the Department of Microbiology, immunology and Virology of the Moscow state medical University, which conducts PCR diagnostics. For DNA isolation, the method of accelerated sample preparation using a set of reagents for DNA isolation from clinical material (Genlab) was used.

To amplify the DNA of pathogenic bacteria Actinobacillus actinomycetemcomitans, Prevotella intermedia, Bacteroides forsythus, Treponema denticola, and Porphyromonas gingivalis, a multiplex PCR method was used to simultaneously identify several pathogens. PCR was performed in a Tertsik MS-2 amplifier (manufacturer-DNA technology, Moscow). The reaction took place in the Matrix temperature control mode according to the following program: denaturation at 95 ° C for 120 s (1 cycle); denaturation at 95 ° C for 30 s; annealing at 60 ° C for 30 s; synthesis at 72 oC for 40 s (33 cycles); final stage synthesis at 72 oC for 240 s (1 cycle). The incubation mixture with a final volume of 25 µl contained 19 µl of SUPERMIX, 1 u / µl of polymerase, and 5 µl of DNA isolated from the root canals of teeth. To prevent evaporation of the reaction sample, 25 µl of mineral oil was layered on top of the mixture. The resulting DNA products were determined by electrophoresis in 1.6% agarose gel.

3. **Results**

As shown by the results of microbiological studies, the material taken before the start of various types of treatment from the root canals of teeth revealed a variety of microbial landscape in the form of obligate and facultative anaerobic bacteria. Streptococci and staphylococci were most frequently detected in the study material: Str. sanguis - in 52% of patients, Str. mutans-in 68%, Str. salivarius-in 52%, and St. epidermidis-in 41%. In addition, Peptostreptococcus anaerobius was detected in the root canals in 38% of patients, Clostridium spp was detected in 12%, and Candida albicans bone beams were absent in 14% of the fungi of the genus in the area of rarefaction.

The study of anaerobic bacterial strains obtained from the root canals of teeth before treatment had (table.1), that in traditional therapy of chronic apical periodontitis in all strains of facultative anaerobic bacteria, the growth retardation zones were less than 5.1 (3.7-5.0 mm), and in depophoresis with copper-calcium hydroxide at a current strength of 1.5 mA x min – 4.9 (3.5-4.8 mm). In accordance with the existing criteria for evaluating antibacterial activity, such values of inhibition of growth of test cultures can be regarded as a weak antibacterial effect of traditional treatment and depoforesis at a dose of 1.5 mA x min.

In cases where the amount of electricity during depoforesis was 2.5 mA x min, the diameter of the growth retardation zones corresponded to a moderate antibacterial effect (growth retardation zones-6.8-9.3 mm). The most pronounced antibacterial effect appeared in cases when the dose of depoforesis was 5 mA x min - the diameter of the zones of growth delays of colonies of all studied bacteria was more than 15.1 mm (15.1-21.8 mm). Therefore, the optimal doses of depoforesis that have an antibacterial effect are 2.5-5 mA x min.

When treating chronic apical periodontitis with apex-Forez using a silver-copper electrode, the growth retardation zones of all strains of the studied facultative anaerobic bacteria were 5.1 mm (3.9-5.0 mm), at a dose of 1.5 mA x min, which is regarded as a weak antibacterial effect of this dose of apex-Forez.

While the current increases during the procedure to 2.5 mA x min, the diameter of the growth delay zones is 8.6-9.6 mm, which corresponds to a moderate antibacterial effect. The most pronounced antibacterial effect was detected at a dose of apex-Forez 5 mA x min, that is, the diameter of the zones of growth delays of colonies of the studied bacteria was more than 15.4 mm (15.4-22.4 mm).

Thus, the optimal doses of apex-Forez that have an antibacterial effect are also 2.5-5 mA x min.

In the combined endodontic treatment of chronic apical periodontitis with the use of depo – and apex-Forez together with the method of fluktuorization, an even more pronounced antibacterial effect is observed than using them separately (table 1). Thus, the diameter of the growth retardation zones of the studied anaerobic bacteria strains with the combined use of depo -, apex-Forez and the fluktuorization method is on average 20.4 mm (17.8-24.5 mm), which is 4.8 times more antibacterial than traditional treatment (4.2 mm, respectively), 1.6 times more than depoforez (12.8 mm, respectively) and 1.5 times more than apex-Forez (13.2 mm, respectively).

Thus, the combined endodontic treatment of chronic apical periodontitis with the use of copper-calcium hydroxide depoforesis, silver-copper electrode apex-foresis and physiotherapy method of fluktuorization has the most pronounced antibacterial, anti-inflammatory and analgesic effect than the use of these methods of treatment separately.

It should be noted that the detection of only one form of bacteria (multiinfection) in the root canals of teeth

was detected only in 6 (7.4%) persons with chronic granulating periodontitis out of 81 examined, while in most cases (92.6%) associations of pathogens were observed, including from 2 to 6 types of microbes. For example, the largest spectrum of microflora was isolated from the material obtained from patients with chronic granulomatous periodontitis, and monoinfection was not observed at all in patients with chronic granulomatous periodontitis. In all forms of the disease, Streptococcus and Candida fungi were present in patients before treatment, with streptococcal microflora dominating the associations.

4. Discussion

The problem of treatment of chronic apical periodontitis is one of the important, not fully solved and promising tasks of therapeutic dentistry. In the treatment of destructive forms of periodontitis, accumulated clinical experience makes it necessary to optimize the reparative regeneration of the periodontal and bone tissue of the alveolar arches of the jaws in order to achieve a stable positive treatment result.

It is important to include in the complex endodontic treatment of CVP physical factors that can actively influence the main links in the pathogenesis of the parotid pathological process, the elimination of inflammatory and destructive foci and tissue regeneration .

Some of these are depoforez of copper hydroxide-calcium, apex-electrophoresis using silver-copper and physiotherapy method of fluctuorization.

Therefore, we set a goal-to increase the effectiveness of treatment of chronic apical periodontitis by improving endodontic treatment of the disease with separate and combined use of new methods of depot–, apex-foresis together with the physiotherapy method of fluktuorization..

The scientific novelty of the work is that as a result of the research, new data were obtained on the effect of copper-calcium hydroxide depophoresis, apex-foresis using a silver-copper conductor and physiotherapy method of fluktuorization, both individually and in combination, on microbial contamination and clinical and radiological condition of periodontal tissues in patients with chronic apical periodontitis with impassable root canals of teeth. For the first time, the high antibacterial and anti – inflammatory effectiveness of separate application of these methods in comparison with the traditional method of treatment of the disease was revealed, and even high efficiency-with their combined use. It was found that combined endodontic treatment using depot–, apex-foresis and physiotherapy method of fluktuorization increases the effectiveness of traditional treatment of periodontitis, which is expressed in reducing the number of complications and accelerating the process of bone regeneration in the area of apical periodontitis. This effect may be due to the fact that these methods contribute to the purification of the root canal system from pulp residues, endodontic sterilization, and obturation of branches from the macrochannel. In addition, depo–, apex-Forez and physiotherapy method of fluktuorization, provide an opportunity to reduce the number of visits of patients to the dental institution. Of course, the implementation of this method requires considerable time, but, from our point of view, they are fully justified by the final result. This is especially important in the treatment of periodontitis with difficult to pass curved root canals of teeth, when they are used as support for orthopedic structures, especially expensive ones. In connection with the above, it is established that the immediate and long-term results of endodontic treatment of periodontitis using depot–, apex-foresis and physiotherapy method of fluktuorization in our modification can be evaluated as positive and recommend the method for practical use.

The practical value of the work consists in the fact that it is proved the feasibility of complex endodontic treatment of chronic apical periodontitis using new methods of depoforesis of copper-calcium hydroxide, apex-foresis using a silver-copper conductor and physiotherapy method of fluktuorization.A unified scheme of application has been developed and medical tactics for complex endodontic treatment of chronic apical periodontitis using depot-, apex – foresis and physiotherapy method of fluktuorization has been justified..

The proposed improved method of treatment of chronic apical periodontitis can improve the quality and effectiveness of treatment, reduce the number of complications in the near future after filling the tooth channels and get favorable clinical and radiological results in a short time. When using depo–, apex-Forez and physiotherapy method of fluktuorization, it is possible to increase the effectiveness of conservative endodontic treatment of CVP in the conditions of outpatient dental reception of General practice.

The results of the work are implemented in the practice of dental clinics of the Republic and are used in the educational process of the dental faculty of the Bukhara State medical Institute when giving lectures and conducting practical classes with students.

5. Conclusions

1. the use of copper-calcium hydroxide depophoresis and apex-foresis of the silver-copper conductor of the root canal of teeth in the complex endodontic treatment of chronic apical periodontitis leads to a 2.0-3.3 one-time better reduction of facultative anaerobic bacteria than traditional treatment. At the same time, the most pronounced (1.5-2.5 times more) antibacterial effect is the combined use of depot -, apex – Forez and physiotherapy method of fluktuorization, than the use of them separately.

2. the use of depo - and apex-Forez in the treatment of chronic apical periodontitis leads to a significant (P<0.05-0.001) rapid acceleration of the processes of regeneration of periapical tissues in comparison with traditional methods of treatment of the disease. At the same time, the combined use of depo–, apex-foresis and physiotherapy method of fluktuorization has a 1.3-2.2 times more effective effect on the condition of the periapical tissue of the teeth than using them separately, which is expressed in reducing the number of complications, accelerating the process of bone regeneration in the apical periodontal area and thereby reducing the number of visits to the dental institution.

3. it was Found that the immediate and long-term results of endodontic treatment of chronic apical periodontitis using depot–, apex-foresis and physiotherapy method of fluktuorization in our modification can be evaluated as positive and recommend their use in clinical dentistry.

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