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Treatment of Periapical Chronic Periodontitis Using Antifungal Drugs

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Abstract

Aim: The aim of the investigation was to study some of the physicochemical properties and clinical trials of a paste based on zinc oxide with eugenol and an antifungal supplements (nistatine, fluconazole and “Narine”-lyophilized lactic-oxide bacteria) for temporary filling of canals in endodontic treatment of chronic apical periodontitis.

Materials and methods: A total of 74 patients were divided into 2 groups: group I included 54 patients with whom the composition of the paste was used as an antibacterial and antifungal agent zinc oxide (ZnO) antifungal preparation (nistatine, fluconazole and “Narine” lyophilized lactic-oxide bacteria, eugenol (clove oil), which was left in the root canal for 7 days, followed by filling with zinc-eugenol paste; group II included 20 patients, whose root canals were treate according to the described method, followed by filling a sealant paste based on zinc-eugenol paste (without an antifungal component).

Results: The obtained results laboratory and clinical studies on show that the antifungal drugsfluconazole, nystatin, "Narine" have antibacterial and antifunga activity against. Taking into account the beneficial effect on the periapical tissues and the obtained data of laboratory studies allowed us to use the above mentioned drugs in the treatment of root canals and subsequent obturation with their introduction into the sealer.

Conclusion: (The results obtained allow us to recommend a temporary endodontic paste based on zinc oxide with eugenol and an antifungal supplements (nistatine, fluconazole and “Narine”-lyophilized lactic-oxide bacteria) for temporary filling of canals in endodontic treatment of chronic apical periodontitis as an innovative method.

Keywords: Chronic forms of apical periodontitis; Endodontic treatment; Antifungal therapeutic temporary; Root filling

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Introduction

Periodontal disease is an infectious disease with a mixed microbial etiology and a pronounced inflammatory response, the therapy is aimed at eliminating the pathogenic microorganisms associated with the disease, and is usually treated by nonsurgical mechanical treatment of the root canal [1]. Microbiological studies using scanning electron microscopy of the contents of the root canals of qualitative composition of which, in fact, remains in the process of medical interventions and in the post-treatment period [2,3].Chronic apical periodontitis usually has no clinical signs or symptoms; however, this chronic condition causes inflammation of the tissue surrounding the teeth and can cause destruction of this tissue, and the periapical tissue is sterile in most cases, even though microbes may be present in the root canal system [4].

The rationale for endodontic treatment is to eradicate the infection, to prevent microorganisms from infecting or re-infecting the root and/or periradicular tissues The facultative microorganisms is of importance in the root canal infection, among Endodontic pathogens that cause primary intraradicular infections, the following are found.

1. Gram negative anaerobic rods include species into two genera: (a) saccharolytic species- Prevotella and (b) asaccharolytic species – Porphyromonas.

2. *Tannerella forsythia* the first periodontal pathogen to be detected in endodontic infection.

3. Asaccharolytic obligately anaerobic Gram negative coccobacilli.

4. Fusobacterium.

5. Spirochetes fall into the genus Treponema

6. Gram positive anaerobic

7. Gram positive cocci.

8. Viruses although the bacterial composition is mainly represented by the coccal flora, yeast fungi are also constantly present, the frequency of their detection according to various sources, is 17%-20% [5-13].

9. Fungi-particularly, Candida spp. (e.g.,) *Candida albicans*, *Candida glabrama*, *Candida guilliermondi*, *Candida inconspicua*, *Candida eotrichum candidum*, etc.

Moreover, these fungi are able to form biofilms even in relatively clean and filled root canals [14]. It should be noted that in apical periodontitis, Candida fungi are also common in pediatric practice and in primary purulent periodontitis of milk teeth [15-17].

The pathogenic properties of endodontic flora are influenced by a complex of factors,

a. Interaction with other microorganisms in the root canal,

b. The ability to interfere with the protection of the owner,

c. The release of Lipopolysaccharides (LPS) and others bacterial moduli,

d. Synthesis of enzymes that damage host tissues.

Periapical periodontitis treatment is a root canal procedure aimed at minimizing inflammation and sterilizing the root canals and the surrounding apical region of the tooth by irrigating the canals with disinfectants or antibacterial solutions. The main function of root filling is to prevent new infection of the root canal system. Successful disinfection and effective sealing of the infected pulp canal ensures success on a par with the removal of vital pulp.

Because bacteria can invade in root periapical tissues, mechanical therapy alone is sometimes ineffective on this basis, the microbial etiology of inflammatory periodontal diseases provides the rationale for using antimicrobial medication in periodontal therapy [18]. Antibiotics are used in the pharmacological treatment of apical periodontitis. Local use of antibiotics in periodontal therapy can further eliminate pathogens and thus enhance the effect of conventional therapy without the side effects of systemically administered antibiotics. Local application of antibiotics and other therapeutic agents in the treatment of intracanal periodontal infections in the root canal has a promising high antimicrobial effect [19-25].

For pathogens usually found inside root canals in peridontitis, an antibiotic paste consisting of metronidazole, ciprofloxacin, and minocycline has been shown to be effective[26,27]. Combinations of corticosteroids and antibiotics have also been used as intracanal drugs due to their anti-inflammatory action, to relieve pain associated with acute apical periodontitis [28]. Calcium hydroxide (Ca(OH)2) is widely used in endodontics as an intracanal drug to destroy the remaining microorganisms after chemical-mechanical treatment [29-31].

Studies it was shown that the presence of yeast in the periapical focus significantly changes the bacteria product, which suggests the use of non-standard therapeutic treatment.

Based on experimental data, in root canals, Enterococcus faecalis remains viable for a long time along with other forms of bacteria, in particular Candida fungi. It was also revealed that after endodontic treatment, these two types of microorganisms retain their viability mainly due to the use of serum as a nutrient medium, which is very important in terms of post-treatment inflammatory complications [32-33]. In an effort to achieve the above goals, no drug has been completely predictable or effective, each has its own advantages and disadvantages, and further research is needed to determine which one is best.

There is also another view point stating that in cases of persistent chronic periapical lesions, yeast fungi are often found in the focus, among which, according to cell morphology characteristics, growth and absorption characteristics of hydrocarbons, about 20 strains of candida fungi are distinguished: *C. glabrata*, *C. albicans*, *C. guilliermondii*, *C. inconspicua*, *Geotrichum candidum*, and etc [34-36].

The analysis of the scientific literature shows that Candida fungi are of pathogenic importance in the development of apical inflammatory processes [37]. To reveal the endodontic presence of Candida fungi in the development of subsequent complications and their absence after endodontic treatment, comparative studies were carried out [30]. The results showed that with the development of complications, yeast fungi were found in 36.7% of cases, and in cases when there were no complications, this indicator was 13.3%.

We can conclude that antifungal treatment should be of great importance in endodontic therapy. This dictates to include antifungal agents in the endodontic treatment of these lesions and justifies the relevance of this study.

Materials and Methods

A total of 74 patients (the age was 27 to 62 years, from 2016 to 2021) in age group were selected for the study. All the patients were diagnosed chronic apical periodontitis of pulpal origin and endodontic treatment was performed.

For endodontic treatment of periapical chronic periodontitis in study a paste was used in the composition of which included zinc oxide (ZnO)-2.5 parts, an antifungal drug-1.0 part (nystatin, fluconazole) and the preparation "Narine" - lyophilized lactic-oxide and eugenol. "Narine" probiotic-lyophilized lactic acid (*Cactobacillus acidophilus* Er-2 strain 317/402, RA) is produced from natural pasteurized cow skim milk by fermentation with

Lactobacillus acidophilus MDC 9602. "Narine" normalizes intestinal microbial biocyanosis in a shorter period of time, restores the anaerobic flora of bifido and lactobacilli, inhibits the growth of opportunisti, as well as produce lectolin, lactocylacticin, lactobacilli. The strain Narine suppresses pathogenic microorganisms, as classified by the World Health Organization [38]. Due to its unique biological formula the "Narine" is widely used as a probiotic. The product has won the widest recognition as a therapeutic and preventive agent in various disorders of gastrointestinal tract. It plays an essential role for normalization of the intestinal microflora in dysbacteriosis of different origin and its aftereffects, including secondary immunodeficiencies and chronic fatigue syndrome. It is a mighty antioxidant, which removes radionuclides, toxins and various pathological agents from the human body.

The obtained data of laboratory studies on the use of antifungal compositions (nystatin, fluconazal, "Narine" (*Lactobacillus acidophileus*), zinc oxide with the study of some of their physicochemical properties(time of hardening, disintegration, superficial) made it possible to recommend it as a temporary obturation of root canals in chronic apical periodontitis.

Laboratory methods

For the sampling of material for bacteriological research, sterile paper points was introduced into the tooth root in a period of 3-5 minutes. The resulting material was transferred into sterile tubes. After numbering, they were transported in a portable household refrigerator of the "BoMaNN" company to the bacteriological laboratory for 1-2 hours, where inoculations were carried out on nutrient media to determine the characteristics of the microbial landscape. For the evaluating the effectiveness comparative aspect, antibacterial and antifungal activity of some antifungal drugs (fluconazole, nystatin and "Narine"-lactobacillus acidophilicus) the contents into the tooth rootwere transferred into a Petri dish with an appropriate nutrient medium, afterwards they were taken to a thermostat and incubated at 37°C. The obtained material was inoculated on yolk-salt agar (YSA) and endo medium, after which the dishes were incubated in a thermostat for 18-20 hours at a temperature of 37°C. On the next day, smears were prepared from the grown colonies, stained according to Gram, examined under a microscope and rolled onto a slant of agar to obtain a pure culture and identification. The test material was inoculated on milk-salt agar, and the next day, in the light at room temperature, lecitovitelase activity and pigmentation were detected. After 48 hours, the character and growth were visually determined according to the following parameters: no growth, single growth (10-25 colonies), poor growth (60-80-100), abundant growth (500-1000 colonies) and confluent growth (more than 1000 colonies). Colonies of the same type and test tubes were placed in a thermostat at 37 °C for one day. For 3 days, the purity of the isolated culture of staphylococci was checked. To study the properties of pure cultures, elective and differential diagnostic media were used. The pathogenicity of staphylococcus strains was established by the presence of plasma coagulase, lecithinase, fibrinolysis, hemotoxin and pigmentation. Streptococcus strains were differentiated depending on growth in blood agar, resistance to 40% bile, and reduction of 0.1% methylene blue. Identification of other, facultatively anaerobic species (gram-negative bacteria, bacilli) was carried out on the basis of morphological and cultural properties. The Smirnova-McClean technique was applied, in accordance with the instructions of the Institute of Epidemiology and Microbiology. The sensitivity of cultures to antibiotics was determined by the method of paper disks according to the standard technique. The sensitivity to antibiotics was determined for all patients under observation (the main group and the comparison group). If the culture of staphylococcus had lasma-coagulating and lecitovitelase activity, then the isolated strains of staphylococci were considered golden. If the culture possessed one of the indicated characteristics and positive fermentation of mannitol under aerobic conditions, then such staphylococci were also mistaken for golden staphylococci. If only the mannitol fermentation reaction was positive, then staphylococci were taken for saprophytic ones. If all the reactions were negative, then it was considered that Staphylococcus epidermidis was isolated. Of the gram-negative bacilli, Escherichia coli was the most common, identified by plating on three-sugar agar, where the decomposition of carbohydrates and the formation of gas took place. When determining the plasma-coagulating activity, we used rabbit blood plasma at a dilution of 1:5, in an amount of 0.5 ml. the diluted plasma was poured into sterile 0.5 ml centrifuge tubes. and one drop of 18-20 hour agar culture of staphylococcus was added to each one. To exclude spontaneous coagulation of plasma, control of uninoculated plasma was also set. The tubes were placed after 3 hours, then they were removed and left at room temperature for 18-20 hours. The final registration of the reaction was carried out the next day. To determine the reaction of mannitol under anaerobic conditions, a medium prepared from dry agar with Andrede's indicator, containing 0.3% mannitol with the addition of sterile petroleum jelly, was used. The test culture of staphylococcus was inoculated with an injection to the bottom. The tubes were placed in a thermostat at 37°C for 5 days. In the case of decomposition of mannitol, the medium turns blue. To identify *Pseudomonas aeruginosa*, paper indicators with oxidase were used. After applying the culture *Pseudomonas aeruginosa* on test paper, a dark blue color appeared. Yeast-like fungi of the genus Candida and streptococci were determined by morphology under a microscope. To detect fungi, an enrichment medium is added to the resulting material. Before that, sowing is carried out on Saburo's Wednesday, since the mushrooms grow for 5 days. Repeated sowing on Saburo's Wednesday is also carried out daily. There is a CAN-2 index in the laboratory, which represents a special European ready-made environment. With the help of a Petri dish, within 24 hours after incubation, the mushrooms begin to grow in different colors, by which you can determine their genus. Incubation takes place in a thermostat. After the corresponding bacterial growth, a standard amount of tested pastes was placed in them and the antibacterial activity was determined after 1, 5, 10, 20 and 30 minutes.

Clinical methods

Preclinical testing of the studied pastes with the determination

of antibacterial activity (the effect of the proposed pastes on the incubated nutrient medium with the growth of the corresponding bacterial colonies in a Petri dish) proved its resistance to suppressing the growth of microorganisms isolated from root canals and the expediency of their use in clinical practice. Treatment was carried out taking into account intraoral and extraoral examination of patients. We focused on intraoral examination methods: the level of oral hygiene, palpation, periodontal examination, tests for pulp sensitivity, radiographs, occlusive features. For all patients filled out outpatient cards, where complaints, diagnostics, and treatment plan were registered. A diagnostic radiograph was examined prior to treatment **(Figures 1 and 2)**. A standardized protocol was used for treatment. Endodontic treatment was carried out after anesthesia determining the working length using apex locators. After applying the rubber dam, conventional endodontic therapy was performed using mechanical Ni-Ti in instrumentation. The prepared tooth canal should be tapered from the crown to the apex. After thorough mechanical treatment of the root canals (average expansion from 25-35 mm), opening the apical foramen by step-by-step evacuation of the root canal contents and putrid masses (in order to prevent its penetration behind the apex), constant antiseptic treatment of root canals on a thin root needle was carried out. Subsequently, drying was carried out with sterile paper, temporary filling of the root canals with a therapeutic paste with antifungal drug (for 7 days), followed by their filling with zinc-oxy- eugenol paste (X-ray control required).

Patients were divided into 2 groups: group I included 54 patients with whom the composition of the paste was used as an antibacterial and antifungal agent zinc oxide (ZnO)-2, 5 parts, antifungal preparation-1.0 part (nistatine, fluconazole and “Narine”-lyophilized lactic-oxide) filling with zinc-eugenol paste; group II included 20 patients, whose root canals were treated according to the described method, followed by filling a sealant paste based on zinc-eugenol paste (without an antifungal component). Post-treatment clinical observation was carried out on all the patients in course of 3 months. To avoid re-infection, great attention was paid to the final filling, for the restoration were used composite materials of the latest generation, taking into account the group belonging of the teeth using a rubberdam. The quality of endodontic treatment was carried out according to the protocol of the European Society of Endodontology.

Statistical analysis

All analyses were performed in the software SPSS version 24 and p values<0.05 were considered statistically significant. Statistical processing of the obtained data was carried out using the Student's criterion (t), Statistica software package. A difference of more than 95% (P<0.05) was considered significant.

Results

The indicators of the sensitivity of root canal microorganisms to chlorhexidine, nystatin, fluconazole, "Narine" are shown in **Table 1**.

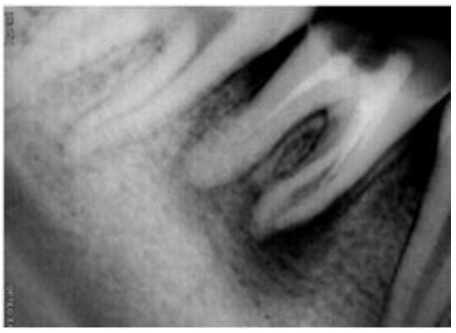

Figure 1 Radiovisiographic examination of the 36 tooth; the diagnosis - chronic granulomatous periodontitis, due to incomplete obturation of the canals with endodontic material; destructive changes in the apex area of the mesial and distal roots, expansion of the periodontal gap.

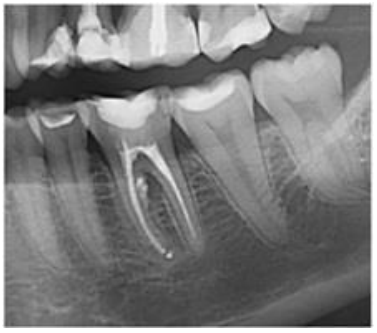

Figure 2 Radiovisiographic examination of the 36th tooth after 12 months. Endodontic treatment with obturation of root canals in the periapical tissues was carried out, significant positive changes are noted, almost complete restoration of the tissue structure in the periapical area.

Table 1: Comparative assessment of root canal microorganisms resistance to chlorhexidine, nystatin, fluconazole, Narine.

Exposure time in minutes	*Stahylococcus aureus* (ATCC 2913)	*E.Coli* (11230)	Streptococcus	Diphtheroids	*Acidophilus bacillus*	Pseudomonas (Pseudomonas aeruginosa)	Drug name
1	-	-	-	-	-	-	Chlorhexidine 0.2%
5	+	-	-	+	-	-	
10	+	+	+	-	-	-	
20	+	+	+	-	-	-	
30	+	+	+	-	+	-	
1	-	-	-	-	-	-	Nystatin+ZnO (paste)
5	+	-	-	-	-	-	
10	+	-	+	-	+	-	
20	+	+	-	-	-	-	
30	+	+	+	-	+	-	
1	+	-	-	+	-	-	Fluconazole+ZnO (paste)
5	+	-	-	-	+	+	
10	+	+	+	-	+	+	
20	+	+	+	-	-		
30	+	+	+	-	+	+	
1	+	-	-	-		+	"Narine" (lactobacillus acidophilicus)+ZnO (paste)
5	+	+	-	-	-	-	
10	-	+	+	-	-	-	
20	+	+	+	-	-	-	
30	+	-	+	-	+	+	

The obtained results show that the antifungal drugs fluconazole, nystatin, "Narine" have antibacterial activityagainst *Staphylolococcus aureus*, Escherichia coli, Streptococcus pseudomonas. Preclinical testing of the studied pastes with the determination of antibacterial activity (the effect of the proposed pastes on the incubated nutrient medium with the growth of the corresponding bacterial colonies in a Petri dish) proved its resistance to suppressing the growth of microorganisms isolated from root canals and the expediency of their use in clinical practice. Taking into account the beneficial effect on the periapical tissues and the obtained data of laboratory studies allowed us to use the above mentioned drugs in the treatment of root canals and subsequent obturation with their introduction into the sealer.

The resulting complications after endodontic treatment are shown in **Table 2**. As it can be seen in the table the most common causes of periapical inflammatory complications after endodontic treatment were insufficient filling or re-filling of root canals, which make up about 63% of all possible causes. Immediately after the constant filling of the root canals, some patients of the both groups experienced unpleasant feeling of discomfort in the area of the treated teeth. Comparative percussion showed weak sensitivity in 7 cases of group I (12.96%) and in 3 cases in group II (15.0%), which disappeared within 3-5 days of the post-treatment period. Indicators of collection from root canals Candida yeast for microbiological examination, before treatment in patients of group I was (35.1%) group II patients had (35.3%).

Indicators of collection from root canals Candida yeast for microbiological examination 7 days after preliminary endodontic treatment, in patients of group I was (7%,4%) in patients of group II had (25.3%).

The comparative assessment of the obtained data indicate the beneficial effect of antifungal drugs in the composition of sealers used for obturation of root canals on periapical tissues.

Dynamic observation patients of both groups based on the data of Electrodontodiagnosis (EDI), the results of which are displayed in **Table 3**, show the following comparison: it should be noted that before treatment, the average Electrodontodiagnosis (EDI) values in the groups did not differ significantly (the coefficient of reliability t<2).

We canobserve significant differenceof the Electrodontodiagnosis (EDI) indicator between the average values of I group, and in II group, by this time (3 months) a slight decrease in tissue electrical excitability was observed when comparing the average Electrodontodiagnosis (EDI) indicators in this group.

X-ray examination of the treatment results also show the positive use of temporary endodontic paste with the prefungal drug "Narine", namely, in group I, (3 months after the treatment), in all cases, there was either stabilization of the pathological process in the area of the tops of the teeth (42.59%), or some restoration of periapical tissues (57.41%) **(Figures 3 and 4)**. In group II, 4 patients (20%) had an X-ray deterioration of the pathological process (an increase in the volume of destructive changes in the area of the apex of the teeth), and in other cases (80%) only stabilization of the pathological process was observed.

Table 2: Reasons for the development of inflammatory complications in the periapical area.

The reason for the development of complications after treatment	Occurrence frequency (amount /%)
Incomplete root pulp removal	3/4,05
Root wall perforation	7/9,46
Anatomical constriction destruction	7/9,46
Endodontic instrument fracture	5/6,77
Insufficient canal filling	27/36,49
Removal of material beyond the apical foramen	19/25,67
Unknown cause (presence of undiagnosed additional tubules)	6/8,10
Total number of patients	74

Table 3: Dynamic indicators of electrical excitability in the tested groups.

Patient groups and forms of periodontitis	EDI (mA)		t
	Before	After 3 months	
I group (n=54)	145,02 ± 1,56	140,28 ± 1,47	2,21
II group (n=20)	145,13 ± 2,19	145,33 ± 2,44	0,06
t	0,04	1,77	-

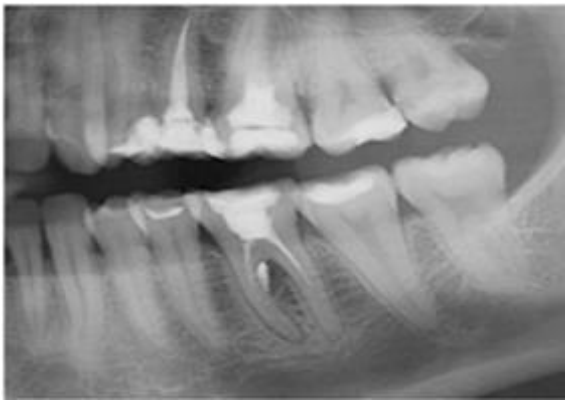

Figure 3 Radiovisiographic examination of the 36th tooth before treatment. DS: Exacerbation of chronic apical periodontitis as the filling material went behind the apex, destructive changes in the apical area.

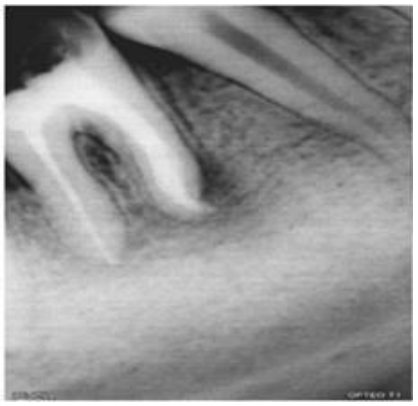

Figure 4 Intraoral targeted X-ray of the 36 tooth after 3 months; endodontic treatment with root canal obturation was performed; significant positive changes are noted in the periapical tissues - almost complete restoration of the X-ray structure of tissues in the distal root area and their partial restoration in the mesial root area.

Discussion

Apical periodontitis is an inflammatory process of the periapical region caused by an infection of the dental root canal system. An important and fundamental goal of root canal treatment is to eliminate bacteria from the root canal and prevent infection, since bacteria or their products are considered the main etiological factors of periapical lesions. In most cases, chemo-mechanical preparation of the root canal and local treatment with calcium hydroxide followed by filling the root canal with gutta-percha and sealant will eliminate the infection and heal the lesion [39]. Sometimes apical periodontitis is difficult to treat root canals, and periapical inflammation caused by a root canal infection can persist for months or even years despite treatment.

Several factors can contribute to the unsuccessful treatment of resistant cases. Most often, these factors are associated with difficulties in the chemo-mechanical preparation of root canals. Apical periodontitis is a polymicrobial infection dominated by obligate anaerobes. The characteristics of microbial associations in the oral cavity and dental cavities are quite diverse and are represented by obligate-anaerobic and anaerobic, as well as obligate-aerobic bacteria.

Necrotic root canals create harsh environmental conditions for microorganisms compared to other parts of the oral cavity. The influence of these conditions on the selection of yeast strains was studied in a recent study comparing the phenotypes and genotypes of *C. albicans* isolates from root canals and periodontal fissures [40].

For teeth with apical periodontitis, none of the anaerobic bacteria species is considered as the main pathogen, among pathogenic microbes, yeast fungi like *Candida albicans* are almost always found [41]. According to the findings of various studies. The most common fungus in apical periodontitis is *Candida albicans* in the range of 7%-18%.

They are found either in pure cultures or together with bacteria. Almost all isolated yeasts are of the genus Candida, with *C. albicans* being the predominant species. *C. albicans* expresses several virulence factors that are capable of infecting the dentin-pulp complex, including the dentinal tubules. This consequently induces an inflammatory response around the root apex, suggesting a pathogenic role for this organism in apical periodontitis. Yeast is especially associated with persistent root canal infections that do not respond to conservative root canal therapy. This may be due to the resistance of all oral Candida species to the commonly used topical drug calcium hydroxide. However, other antimicrobial agents may offer alternative therapeutic approaches and improve the management of these persistent cases of apical periodontitis.

Systemic or topical antifungal agents may be considered in some acute or persistent cases after microbiological diagnosis of a root canal infection with yeast.

Endodontic treatment to identify the role and presence of fungi of the genus Candida, comparative studies have been carried out and the authors obtained indicate that with the development of periapical periodonts, fungi are found in 36%, 8% of cases [42,43]. The authors conclude that antifungal treatment is of paramount importance and come to the conclusion that the question of the pathogenetic participation of Candida yeasts in the development of peri-apical inflammatory pathology still remains uncertain and requires further research in this direction.

Study Baumgartner et al. had evaluated the presence of *Candida albicans* from the aspirates of abscesses and cellulitis of endodontic origin and infected root canals by using PCR method concluded the study by finding the presence of *Candida albicans* in 5 of 24 samples which were taken from root canals i.e; 21% [44].

During the recent years' new combinations of healing substances have been worked out and offered for clinical use, as well as directed for eliminating pathogenic yeast fungi in the root and dentinal tubules. Particularly, the laboratory investigations have revealed a rather high antibacterial activity of the combination of 2% chlorhexidine and silver nanoparticle (AgNP) against such common forms of intratubular pathogens as *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Candida albicans* [45]. On the whole, many authors come to a conclusion that adding antimycotic substances (for example, ketokonazole and fluconazole) into the composition of root filling materials enhances their antimicrobial effect without any changes of their physical properties [46]. Microbiological studies have shown that during inflammation of the periodontal complex, pathogenic and opportunistic microorganisms are released, both in monoculture and in associations. Considering the diversity of the microflora of root canals during endododetic treatment and the ineffectiveness of many proposed treatment regimens, the search for new therapeutic agents for the conservative treatment of chronic periodontal disease is an urgent task of endodontics.

Since in many studies, fungal associations are encountered in the diversity of microflora found in the root canal, antifungal drugs must also be included in the complex drug treatment of periodontitis. For this reason, an experimental-clinical approbation was also carried out by using substances on the base of herbal plants. An expressed inhibiting influence on the mentioned micro-organisms is revealed in such irrigants of the root canals as QmiX and extract of the guava leaves mixtures of extracts of guava leaves and apple cashew, mixture of methanol extract *Azadirachta Indica* (Neem), *Mimusops elengi* (Bakul) and chlogexidine, combination of chitozane and ozonized olive oil,etc [47-49]. The concept of using probiotics for the treatment of endodontic diseases is new and insufficiently studied [50]. Currently, there are several methods for identifying microbes, including culture methods and non-culture-based methods. Traditionally, microorganisms in endodontic specimens have been identified using a variety of culture procedures that rely on isolation, growth, and laboratory identification.

The inclusion of antimycotic drugs and probiotics in the composition of root paste is a very promising area of antibacterial endodontic therapy. The obtained data on the physicochemical properties of the paste developed by us and laboratory data obtained in our research allowed the use of the above drugs in the

treatment of root canals and subsequent obturation with their introduction into the sealer. As probiotics of microorganisms, the authors of this study tested a paste containing the drug "Narine" probiotic-lyophilized lactic acid bacteria (*Cactobacillus acidophilus* Er-2), which in vitro showed high efficiency against *E. faecalis* and *C. albicans*. *L. acidophilus n.v.* The Er2 317/402 Narine strain is a functional probiotic culture and suppresses pathogenic microorganisms [50]. The available literature lacks data on the use of "Narine" in endodontic practice.

Preclinical tests of the studied pastes with the determination of antibacterial activity (the effect of the proposed pastes on the incubated nutrient medium with the growth of the corresponding bacterial colonies in a Petri dish) proved their resistance to the suppression of the growth of microorganisms isolated from root canals, and the expediency of their use in clinical practice. Taking into account the beneficial effect on the periapical tissues and the obtained data of laboratory studies allowed us to use the above mentioned drugs in the treatment of root canals and subsequent obturation with their introduction into the sealer. According to the results of microbiological (suppression of fungal growth), Electrodontometric (lowering the threshold of electrical excitability of tissues and X-ray (restoration of periapical tissues) studies, it can be concluded that inclusions (nystatin, fluconazal, "Narine" (*Lactobacillus acidophileus*) in the paste for temporary filling of root canals) gives positive results in the treatment of chronic forms of periodontitis, which leads to the stabilization of the pathological process and some restoration of the tissues of the periapical region. The obtained data of laboratory studies on the use of antifungal compositions (nystatin, fluconazal, "Narine" (*Lactobacillus acidophileus*), zinc oxide with the study of some of them) physicochemical properties, made it possible to recommend it as a temporary obturation of root canals in chronic apical periodontitis (hardening time, decay, superficial).

Conclusion

The results obtained allow us to recommend a temporary endodontic paste based on zinc oxide with eugenol and an antifungal supplements (nistatine, fluconazole and “Narine”-lyophilized lactic-oxide bacteria) for temporary filling of canals in endodontic treatment of chronic apical periodontitis as an innovative method.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors Contributions

KJ, HS, TL, TL, KG conceived and designed the study, conducted research, provided research materials, and collected and organized data. HG, KJ, AH analyzed and interpreted data. HG, TL wrote initial and final draft of article, and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Ethical Approval

Research protocol was approved by the local Ethical Committee (Approval number N14, Date 17.11.19).

Informed Consent

Informed consent was obtained from all individual participants inclincluded in the study.

References

1. Manuilova EV, Mikhalchenko VF, Mikhalchenko DV, Zhidovinov AV, Filyuk EA (2014) The use of additional research methods to assess dynamics of the treatment of chronic apical periodontitis, Modern problems of science and education. 6:10-20
2. Smolyanko LYe (1992) Temperature evaluation treatment of chronic periodontitis in the stage of exacerbation with the preparation of immobilized proteinaz profezim Dentistry. Stomatologiia J 1:18-19.
3. Khachatryan ZhR, Ambartsumyan SG, Mikaelyan AR, Tatintsyan VG, Andriasyan LG (2021) Some physicochemical properties of medicinal pastes based on zinc oxide with eugenol and antifungal additives. Bulle Denti Maxillofa Surg 18:4-9.
4. Al Nazhan S, Al-Sulaiman A, Al-Rasheed F, Alnajjar F, Al-Abdulwahab B (2014) Microorganism penetration in dentinal tubules of instrumented and retreated root canal walls In vitro SEM study. Restor Dent Endod 39:258-264.
5. Ashraf H, Samiee M, Eslami G, Ghodse Hosseini M (2007) Presence of *Candida Albicans* in Root Canal System of Teeth Requiring Endodontic Retreatment with and without Periapical Lesions. Iran Endod J 2:24-28.
6. Bernal-Trevino A, Gonzalez-Amaro A, Mendez Gonzalez V, Pozos-Guillen (2018) Frequency of Candida in root canals of teeth with primary and persistent endodontic infections. Rev Iberoam Micol 35:78-82.
7. Dumani A, Yoldas O, Yilmaz S, Koksal F, Kayar B, et al. (2012) Polymerase chain reaction of enterococcus faecalis and *Candida albicans* in apical periodontitis from Turkish patients. J Clin Exp Dent 4:34-39.
8. Gao Y, Jiang X, Lin D, Chen Y, Tong Z (2016) The Starvation Resistance and Biofilm Formation of Enterococcus faecalis in Coexistence with Candida albicans, Streptococcus gordonii, Actinomyces viscosus, or Lactobacillus acidophilus. J Endod 42:1233-1238.
9. Kovac J, Kovac D, Slobodnikova L, Kotulova D (2013) Enterococcus faecalis and Candida albicans in the dental root canal and periapical infections. Bratisl Lek Listy 114: 716-720.
10. Kumar J, Sharma R, Sharma M, Prabhavathi V, Paul J, et al. (2015) Presence of Candida albicans in Root Canals of Teeth with Apical Periodontitis and Evaluation of their Possible Role in Failure of Endodontic Treatment. J Int Oral Health 7:42-45.

11. Lana M, Ribeiro-Sobrinho A, Stehling R, Garcia G, Silva B, et al. (2001) Microorganisms isolated from root canals presenting necrotic pulp and their drug susceptibility in vitro. II Oral Microbiol Immunol 16:100-105.

12. Ledezma-Rasillo G, Flores-Reyes H, Gonzalez-Amaro A, Garrocho-Rangel A, RuizRodriguez Mdel S, et al. (2010) Identification of cultivable microorganisms from primary teeth with necrotic pulps. J Clin Pediatr Dent 34:329-333.

13. Ohshima T, Ikawa S, Kitano K, Maeda N (2018) A Proposal of Remedies for Oral Diseases Caused by Candida: A Mini Review. Front Microbiol 9:1-7.

14. Richards D, Davies J, Figdor D (2010) Starvation survival and recovery in serum of Candida albicans compared with Enterococcus faecalis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 110:125-130.

15. Sen B, Safavi K, Spangberg L (1997) Growth patterns of Candida albicans in relation to radicular dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 84:68-73.

16. Persoon I, Buijs M, Ozok A, Crielaard W, Krom B, et al. (2017) The mycobiome of root canal infections is correlated to the bacteriome. Clin Oral Investig 21:1871-1881.

17. Shrestha S, Mala K (2013) Evaluation of sealing ability of a root canal sealer with various antibiotic additives: An in vitro study. J Interdiscip Dent 3:21-24.

18. Zaruba MTW, Filli T, Rechenberg DK, Thurnheer T, Attin T, at al. (2015) Intracanal Antibiotic Medication for Sustained Root Surface Disinfection–A Laboratory Evaluation. Open Dent J 9:396-401.

19. Attia DA, Farag AM, Afifi IK, Darrag AM (2015) Antimicrobial effect of different intracanal medications on various microorganisms. Tanta Dental J 12:41-47.

20. 21. Sato I, Ando-Kurihara N, Kota K, Iwaku M, Hoshino E (1996) Sterilisazation of infected rootcanal dentine by topical application of a mixture of ciproflaxin, metronidazole and minocycline in situ. Int Endod J 29:118-124.

21. Vijayaraghavan R, Mathian VM, Sundaram AM, Karunakaran R, Vinodh S (2012) Triple antibiotic paste in root canal therapy. J Pharm Bioallied Sci 4:230-233.

22. Kim D, Kim E (2015) Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: a literature reviewpart II. In vivo studies. Restor Dent Endod 40:97-103.

23. Ramta Bansal, Aditya Jain (2014) Overview on the Current Antibiotic Containing Agents Used in Endodontics. N Am J Med Sci 6:351-358.

24. Vijay R, Suman M, Shashikala K (2010) Evaluation of antimicrobial efficacy of tetracycline gutta percha and calcium hydroxide impregnated gutta percha against enterococcus faecalis- an in vitro study. IJDA 2:248-252.

25. Michel Goldberg (2020) Antibiotics and Antibacterial Medications for Endodontic Treatments. JSM Dent 8:1-8.

26. Mohammadi Z, Abbott PV (2009) On the local applications of antibiotics and antibiotic-based agents in endodontics and dental traumatology. Int Endod J 42:555-567.

27. Shrestha S, Mala K (2013) Evaluation of sealing ability of a root canal sealer with various antibiotic additives: An in vitro study. J Interdiscip Dent 3:21-24.

28. Rangasamy Vijayaraghavan (2012) Triple antibiotic paste in root canal therapy. J Pharm Bioallied Sci 4:230-233.

29. Mohammadi Z (2009) Antibiotics as intracanal medicaments: a review. J Calif Dent Assoc 37:98-108.

30. Sharma D, Grover R, Pinnameneni PS, Dey S, Raju PR (2014) Evaluation of efficacy of combinations of five endodontic sealers with five antibiotics against Enterococcus faecalis - An in-vitro study. J Inter Oral Heal JIOH 6:90-95.

31. Chu FCS, Leung WK, Tsang PCS, Chow TW, Samaranayake LP (2006) Microorganisms from root canals with apical periodontitis following two-visit endodontic treatment with antibiotics/steroid or calcium hydroxide dressings. J Endod 32:17-23.

32. Linlin Wang, Chen Hu, Longquan Shao (2017) The antimicrobial activity of nanoparticles: present situation and prospects for the futureInt. J Nanomedicine 12:1227-1249.

33. Athanassiadis B, Abbott PV, Walsh LJ (2007) The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. Aust Dent J 52:64-82.

34. Delgado RJ, Gasparoto TH, Sipert CR, Pinheiro CR, de Moraes IG, et al. (2013) Antimicrobial activity of calcium hydroxide and chlorhexidine on intratubular Candida albicans. Inter J Oral Sci 5:32-36.

35. Vasavada K, Kapoor S (2020) Evaluation of ozonized calcium hydroxide as an effective intracanal medicament during root canal procedures: an in vitro observational study. Med Gas Res 10:122-124.

36. Athanassiadis PV Abbott, LJ Walsh (2007) The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. Aust Den J Supple 52:64-82.

37. Mkrtchyan H, Gibbons S, Heidelberger S, Zloh M, Limaki KH (2010) Purification, characterisation and identification of acidocin LCHV, an antimicrobial peptide produced by Lactobacillus acidophilus n.v. Er 317/402 strain Narine. Int J Antimicrob Agents 35:255-260.

38. Waltimo TMT, Sen BH, Meurman JH (2003) Yeasts in Apical Periodontitis. Criti Rev in Oral Biol Med 14:128-137.

39. Waltimo TMT, Dasanayake RS, Ørstavik D, Haapasalo MPP, Samaranayake LP (2001) Phenotypes and randomly amplified polymorphic DNA (RAPD) profiles of Candida albicans isolates from root canal infections in a Finnish population. Oral Microbiol Immunol 16:106-112.

40. Dohyun Kim, Euiseong Kim (2014) Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: a literature review - Part I. In vitro studies.Restor Dent Endod 39:241-252.

41. Ilona F Persoon, Mark J Buijs, Ahmet R Özok, Wim Crielaard, Bastiaan P Krom, et al.(2017) The mycobiome of root canal infections is correlated to the bacteriome. Clin Oral Investig 21:1871-1881.

42. Kovac J, Kovac D, Slobodnikova L, Kotulova D (2013) Enterococcus faecalis and Candida albicans in the dental root canal and periapical infections. Bratisl Lekárske Listy 114:716-720.

43. Egan MW, Spratt DA, Ng Y-L, Lam JM, Moles DR, et al. (2002) Prevalence of yeasts in saliva and root canals of teeth associated with apical periodontitis. Int Endod J 35:321-329.

44. Baumgartner JC, Watts CM, Xia T (2000) Occurrence of Candida albicans in infections of endodontic origin. J Endod 26:695-698.

45. Charannya S, Duraivel D, Padminee K, Poorni S, Nishanthine C, et al. (2018) Comparative Evaluation of Antimicrobial Efficacy of Silver Nanoparticles and 2% Chlorhexidine Gluconate When Used Alone and in Combination Assessed Using Agar Diffusion Method: An In vitro Study. Contemp Clin Dent 9:204-209.

46. Weckwerth P, Lima F, Greatti V, Duarte M, Vivan R (2015) Effects of the association of antifungal drugs on the antimicrobial action of endodontic sealers. Braz Oral Res 29:1-7.

47. Jose J, Krishnamma S, Peedikayil F, Aman S, Tomy N, et al. (2016) Comparative Evaluation of Antimicrobial Activity of QMiX, 2.5% Sodium Hypochlorite, 2% Chlorhexidine, Guava Leaf Extract and Aloevera Extract Against Enterococcus faecalis and Candida albicans - An in-vitro Study. J Clin Diagn Res 10:20-23.

48. Noushad M, Balan B, Basheer S, Usman S, Muhammed Askar M (2018) Antimicrobial Efficacy of Different Natural Extracts Against Persistent Root Canal Pathogens: An In vitro Study. Contemp Clin Dent 9:177-181.

49. Mistry K, Sanghvi Z, Parmar G, Shah S, Pushpalatha K (2015) Antibacterial efficacy of Azadirachta indica, Mimusops elengi and 2% CHX on multispecies dentinal biofilm. J Conserv Dent 18:461-466.

50. Elshinawy M, Al-Madboly L, Ghoneim W, El-Deeb N (2018) Synergistic Effect of Newly Introduced Root Canal Medicaments; Ozonated Olive Oil and Chitosan Nanoparticles, Against Persistent Endodontic Pathogens. Front Microbiol 3:1-11.

51. Bohora A, Kokate S (2017) Evaluation of the Role of Probiotics in Endodontic Treatment: A Preliminary Study. J Int Soc Prev Community Dent 7:46-51.

52. Haykuhi Charchoghlyan, Heejun Kwon, Dong-Ju Hwang, Jong Suk Lee1, Junsoo Lee, Myunghee Kim (2016) Inhibition of Cronobacter sakazakii by Lactobacillus acidophilus n.v. Er2 317/402. Korean J Food Sci An 36:635-640.