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FACULTY OF DENTAL MEDICINE
Department of „Conservative dental treatment and oral
pathology“

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**Comparison of treatment methods for chronic periapical inflammatory
diseases**

SUMMARY

Of Ph.D. Thesis
for awarding the educational and scientific degree „DOCTOR“

SCIENTIFIC SPECIALTY

Therapeutic dentistry

SCIENTIFIC ADVISORS:

Assoc. Prof. Dr. Tsvetelina Borisova-Papancheva, MD, PhD

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The Ph.D. Thesis was approved and directed for defense at a meeting of the “Department of Conservative Dental Treatment and Oral Pathology” at the Faculty of Dental Medicine of the Medical University of Varna "Prof. Dr. Paraskev Stoyanov”.

The dissertation contains 185 standard pages and is illustrated with 46 tables and 96 figures. The bibliography consists of 199 sources, of which 6 in Cyrillic and 193 in Latin.

The public defense of the Ph.D. Thesis will take place on September 9, 2021 in the Auditorium "Assoc. Dimitar Klisarov ” at the Faculty of Dental Medicine of the Medical University of Varna "Prof. Dr. Paraskev Stoyanov”, in front of a a scientific council consisting of:

Chair:

Prof. Dr. Vladimir Panov – internal member

Members:

Prof. Dr. Anton Djorov, MD, PhD – external member

Assoc. Prof. Dr. Elka Radeva, MD, PhD– external member

Assoc. Prof. Dr. Janet Kirilova, DSc, PhD– external member

Assoc. Prof. Dr. Miglena Balcheva, MD, PhD – internal member

Prof. Dr. Radosveta Andreeva-Borisova, DSc, PhD – reserve internal member

Assoc. Prof. Dr. Georgi Tomov, MD, PhD – reserve external member

The materials on the defense are available in the Scientific Department of MU - Varna and are published on the website of MU - Varna.

Note: The numbers of the figures and tables in the symmary do not correspond to the numbers in the dissertation.

USED ABBREVIATIONS

MO – microorganisms

MTT analysis - colorimetric analysis to assess cellular metabolic activity

NSAIDs – non-steroidal anti-inflammatory drugs

OPG – orthopantomography

HDT – hard dental tissues

CPP – chronic periapical periodontitis

Ca(OH)₂ – Calcium hydroxide

CBCT – conical beam computed tomography

CHX – chlorhexidine

EDTA – Ethylenediaminetetraacetic acid

IPI – iodine potassium iodide

MTAD – a mixture of tetracycline isomer, citric acid and detergent

NaOCl – sodium hypochlorite

NiTi – nickel-titanium

PAI – periapical index

VAS – visual-analogue scale

VDS – verbal descriptor scale

INTRODUCTION

One of the main etiological factors for the development of apical periodontitis is the presence of microorganisms colonizing the root canal system. In order for the endodontic treatment to achieve optimal results, bacterial populations in the root canal must be eliminated or at least significantly reduced to levels compatible with the healing process of the periapical tissues.

The endodontic environment provides a selective medium for the creation of a mixed, predominantly anaerobic flora. The treatment of periodontitis aims to eliminate the etiological factor, i.e. microorganisms and their toxins; to influence the inflammatory process in the periapical tissues and to stimulate the healing process; to provide a hermetic filling of the root canal to isolate the apical periodontium from contact with the saliva in the oral cavity. The removal of the etiological factor is associated with mechanical and chemical treatment of the root canal.

Since most endodontic problems are of microbial origin, their removal is considered the most important step in root canal therapy. The number of visits for treatment of infected root canals is one of the most discussed topics in endodontics.

Endodontic treatment through multiple visits is a traditionally accepted method. However, an alternative protocol involving one visit has been proposed. The concept of treating root canals in one step was described in the 1980s.

AIM AND TASKS

Aim:

To compare the methods of periodontitis treatment and to study the effectiveness of different materials used for their treatment.

Tasks:

To achieve the aim, the following tasks were assigned:

1. A survey among dentists and patients to monitor the postoperative dental pain in chronic periodontitis, depending on the used methodology.
2. To study the effectiveness of the treatment of chronic periodontitis using a one-visit method.
3. To monitor the chronic periodontitis treatment method using a multi-visits method by placing a medication dressing of calcium hydroxide for 7 days.
4. To study the chronic periodontitis treatment using a multi-visits method by placing a sterile cotton swab between visits.
5. To monitor the chronic periodontitis treatment method using a multi-visits method by irrigation with 2% chlorhexidine.

MATERIALS AND METHODS

Selection of patients

A certain number of healthy male and female patients, aged 16 to 69, with no systemic diseases are required to perform the assigned tasks. Each person has one or more teeth on the upper, lower or both jaws, diagnosed with chronic apical periodontitis except for the wisdom teeth. The diagnosis was made based on clinical and paraclinical studies. The teeth are asymptomatic and do not respond to thermal tests. The values reported from electroodontodiagnostics are above 100 μ A. Radiographic diagnostics is one of the main methods to verify the diagnosis of chronic apical periodontitis. Demineralization and destruction of periodontal tissues, cementum and alveolar bone tissue are detected by periapical x-ray. CBCT can be used to confirm the diagnosis

Contraindications for the inclusion of the teeth in the study can be general and local. The systematic criteria for excluding patients from the study are pregnancy, patients who had used antibiotics in the past month, and diabetics. The lack of sufficient structures and the inability to restore the HDT (ferrule effect) for isolation using a rubber dam is one of the most important contraindications for the inclusion of certain teeth in this study.

There are several criteria for teeth with previous endodontic treatment to be included in the study. Firstly, one should be able to fully negotiate the root canals. Secondly, the canal filling should reach the middle third of the canal leaving the apical part not filled. Lastly, the presence of separated instruments in the examined canal is inadmissible.

1. Materials and methods for task 1. A survey among dentists and patients to monitor the postoperative dental pain in chronic periodontitis, depending on the used methodology.

Survey among dentists to establish the method of treatment of teeth with CPP, the medicaments used and the irrigation protocol

A special anonymous questionnaire including 18 questions (Appendix No. 1) was created for the needs of the study. 80 dentist from Varna and Sofia regions participated in it. The survey was conducted during scientific and educational events. With the help of the questionnaire, we will find out what are the methods that dentists use to treat teeth with the respective diagnosis, as well as whether they use a dressing and if so - what is its type when they follow the multi-visits method. The questionnaire includes questions about the duration of the follow-ups of the treated teeth, the technique and materials of filling, the irrigation protocol that the respondents apply. The emphasis is on the means of isolation of the operating field.

Survey among patients in order to monitor the postoperative pain in teeth with chronic periodontitis, depending on the methodology and the medication used

For the needs of the study, a special card to be filled in by the patients has been created (Appendix No. 2). The total number of surveyed patients is 71. 31 of them were treated using a single-visit method and 40 - using a multi-visits method. The participants are divided by gender and age, there are 4 age groups. The first age group includes people aged 16 to 18. The second group - 19 to 35, the third group includes patients aged 36 to 60 and the last group – people over 60 years of age.

The questionnaire includes a record of pain over a period of time after root canal obturation. Visual Analogue Scale (VAS) is used to monitor the level of pain during 5 different time intervals – immediately after the obturation of the root canals, in 6 hours, in 24 hours, in 48 hours and 7 days after filling them (Fig. 1).

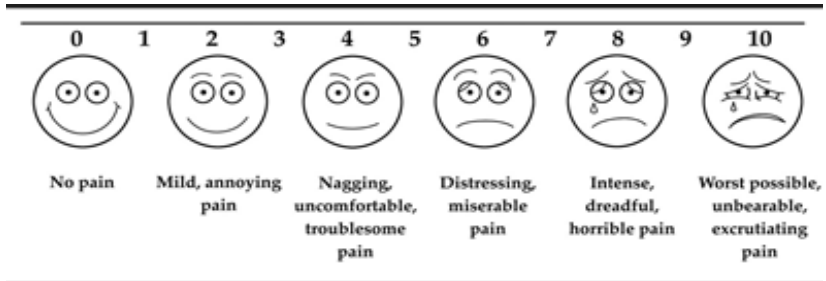


Fig 1. Visual Analogue Scale (VAS)

If a multi-visits method is used to treat chronic apical periodontitis, the data are also reported after the placement of the intra-canal medication dressing. Some additional questions about the type of pain and what provoked it, as well as whether the patient was taking analgesics and how they affected the pain, were also included in the questionnaire.

The aim of the survey is to gather data from the patients about the frequency and the type of postoperative pain after the treatment of chronic apical periodontitis, as well as whether there is a correlation between gender, age and the postoperative pain. The analysis of the experimental data was performed with a specialized statistical analysis package IBM SPSS Statistics 20.

2. Materials and methods for task 2. To study the effectiveness of the treatment of chronic periodontitis using a one-visit method.

Paraclinical methods to confirm the diagnosis

To clarify the diagnosis an X-ray test, which may be a parallel intraoral sector radiography, OPG or CBCT, is performed on the patients during their first visit. Parallel intraoral sector radiography is performed using frontal and lateral holders (KerrHawe, Switzerland) as well as silicone occlusal registration material, which is made of C-silicone (Zataflow,

Zhermack, Germany) for individual positioning in parallel radiographic technique (Fig. 2).

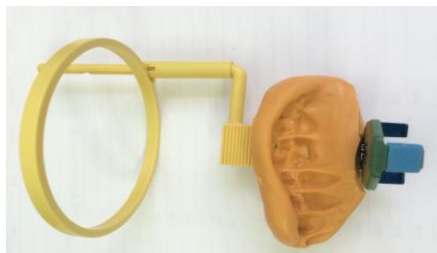


Fig. 2. Silicone occlusal registration material

Planmeca ProMax 2D S3 unit Helsinki, Finland (Fig. 3) is used to take digital orthopantomographs.



Fig. 3. Planmeca ProMax 2D S3 Unit Helsinki, Finland

CBST can be used to confirm the diagnosis. A Planmeca ProMax 3D Max camera is used to capture 3D images. The following parameters are registered:

1. The number of the examined tooth in the dentition.
2. The area of periapical inflammatory lesion of endodontic origin in mm^2 .
3. The bone density in the area of periapical inflammation.
4. The volume of periapical inflammatory lesion, measured in cm^3 .
5. The bone density in the area of healthy bone.

Out of all patients that participated in the study, only one was scheduled for CBCT imaging. Four of his teeth met the requirements for inclusion in the study (Fig.4). All intraoral images were taken using Planmeca ProX Unit, Helsinki, Finland (Fig. 5)

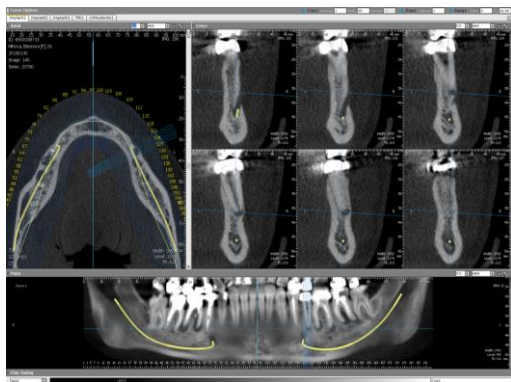


Fig.4. CBCT to confirm the diagnosis



Fig. 5. Planmeca ProX Unit, Helsinki, Finland

Methodology

Thirty one patients were studied and all of them signed consent forms. If necessary, build up was performed. Root canal treatment begins with isolation of the operative field with a rubber dam (Fig. 6). An endodontic cavity is prepared using a diamond cylindrical bur, an Endo Z bur (Dentsply Maillefer) (Fig. 8) and a Gates Glidden (Fig. 7). If present, the old canal filling is removed with retreatment files - Protaper Retreatment D1, D2 and D3 (Dentsply maillefer) (Fig. 9). The working length is determined by electrometric method using an apexlocator IPexII (NSK, Japan) (Fig. 10). An initial microbiological sample is taken at this stage of the treatment to prove the presence of pathogenic microorganisms in the root canals. The surface layer of dentin from the root canal is scraped with a sterile K-file. The material is taken with a sterile paper point that stays in contact for 10 seconds. It is transported to the laboratory in Amies liquid transport medium. The apical part of the root canal is processed manually to No. 20 K-file (Fig. 11), and the subsequent expansion of the canal is achieved by rotary

instrumentation using Protaper Next X1 and X2 (Fig. 12). In the beginning of the root canal treatment, a 2 ml solution of 5.25% NaOCl is applied for 30 to 60 seconds per canal. Distilled water is used to neutralize its effect. Then they are retreated with a 2 ml 5.25% NaOCl solution using ultrasonic activation at a wavelength of 45 kHz (Ultra X, Eighteeth). A distance from the tip of the tool to the full working length must be provided within a minimum of 5 mm to avoid the release of irrigation solution outside the root canal into the periodontal space and the surrounding bone. This is followed by rinsing with distilled water and washing with 17% EDTA solution (Gusiyska A., Dyulgerova E.) (2009). At the end of the preparation, all irrigation materials must be neutralized. Drying of the root canals using sterile paper points for Protaper Next follows (Fig. 13). Following is the second microbiological test. The material is taken with a sterile paper point that stays for 10 seconds. It is transported to the laboratory in an Amies transport medium. The root canals are obturated with thermoplastic gutta-percha using a Soft-Core Heater (CMS Dental) (Fig. 14). Initially, a small amount of AH Plus Jet sealer was introduced (Dentsply Sirona, Germany) (Fig. 15). The heated shutter is then inserted slowly with a constant stroke without turning to the full working length. We wait for 1-2 minutes for the gutta-percha to harden. Then the handle is removed. Control parallel sector radiography is prescribed.



**Fig. 6. Rubber dam set
MEDESY**



Fig. 7. Gates Gidden



**Fig. 8. Endo Z bur
Dentsply**



Fig. 9. Protaper Retreatment Dentsply



Fig. 10. Apexlocator IPexII, NSK



Fig. 11. No.20 K-file



Fig.12. Rotary files Protaper Next

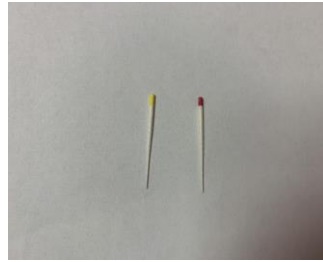


Fig. 13. Sterile paper points



Fig. 14. Soft-Core Heater, CMS Dental



Fig. 15. AH Plus Jet, Dentsply Sirona

One year after treatment and obturation of the root canals, the patients are scheduled for a second radiographic examination. It is performed in the same way as the first radiography. The measurement of the results is performed on the parallel intraoral radiographs in two stages. Periapical changes are reported in mm² on the first radiograph, immediately before endodontic treatment and on control graph, 6 months or one year after therapy. Using a computer program (PS CS5 EXTENDED), measurements are made in the vertical and horizontal directions (Fig. 16). The vertical size

is equal to the distance from the apex of the root apex to the highest point of the periapical lesion, and the horizontal one corresponds to a line perpendicular to the vertical, formed by connecting the two furthest points from the lesion. The obtained results are recorded in tables and are subject to statistical analysis with a specialized package IBM SPSS Statistics 20. Another sign of the healing process is the presence of newly-formed bone seams in the area of the periapical lesion. This is done using the Periapical Index (PAI) (Orsatavik 1986), (Gusiyska A. 2013) (Table 1). The structure of the newly formed bone may differ from the normal one.



Fig. 16. Measurements in vertical and horizontal direction using a computer program PS CS5 EXTENDED

Table 1. Periapical index (PAI)

Periapical index PAI	1	2	3	4	5
Condition of periapical tissues	Normal periapical structures	Slight changes in bone structure	Changes in bone structure with loss of mineral substance	Periodontitis with clear radiological boundaries	Periodontitis with evidence of exacerbation

Laboratory methods to confirm pathogenic microorganisms in the root canals

Colombia blood agar (Fig. 17) or a chromogenic identification medium (Fig. 18) is used for the cultivation of the microorganisms. Cultivation was performed at 37° C. After 24 h, grayish colonies 1-2 mm in size were observed on the blood agar, showing an α hemolytic zone, weak β hemolysis or non-hemolytic at all. They form small lactose-positive colonies on the intestinal media.



Fig 18. Chromogenic identification medium



Fig. 17. Columbia blood agar

Method for identification of streptococci and enterococci: In a drop of distilled water on a glass slide, dilute one yoze of the test culture, then add a drop of 3% hydrogen peroxide. With a positive reaction, bubbles of varying intensity appear after about 1 minute.

Esculin hydrolysis: Esculin hydrolysis (bile-esculin test) and growth in broth with 6.5% NaCl is used to identify the Enterococcus genus, which are able to hydrolyze esculin in the presence of bile. Enterococci and certain streptococci hydrolyze glucoside, esculin to esculetin and dextrose.

Test procedure: Stroke culture is carried out using a calibrated loop. The tube is incubated at $35 \pm 2^\circ$ C in an aerobic atmosphere. The tubes are checked after 18 - 24 and 42 - 48 h. for growth. If more than half of the beveled glass has blackened after 24 to 48 hours, the test is considered positive (Fig. 19).



Fig. 19. Bile-esculin test

**Conglutination and latex agglutination reaction – reagent and kit
Crystal BD Crystal BD (Becton Dickinson)**

The BBL Crystal system uses modified conventional, fluorogenic and chromogenic substrates to identify gram-positive bacteria. It is designed to identify frequently isolated anaerobic gram-positive bacteria. (Fig. 20)



Fig. 20. Kit Crystal BD (Becton Dickinson)

3. Materials and methods for task 3, 4, 5. To monitor the method of treatment of chronic periodontitis using multi-visits method by placing a calcium hydroxide dressing for 7 days, a dry sterile swab or irrigation with 2% chlorhexidine.

Twenty three patients were studied and all of them signed a consent form. During their first visit, the method for treatment of the root canals from task 2 was applied. In some of the studied patients a microbiological examination was applied to detect pathogenic microorganisms in the infected root canals. During their first visit two microbiological samples were taken

before the treatment of the root canals and immediately after their preparation. The teeth are treated using a multi-visits method by placing an intracanal calcium hydroxide dressing or a dry sterile swab between visits. In task 5 an additional medication for irrigation - 2% chlorhexidine was used. The teeth were hermetically sealed with a temporary filling for a period of 7 days. After this period, the teeth were isolated with a rubber dam, opened and irrigated with 5.25% NaOCl. The last microbiological test was carried out. The material was taken with a sterile paper point that stays in contact for 10 seconds. It was transported to the laboratory in Amies liquid transport medium. This test would prove the effectiveness of the medication used as a dressing. The final irrigation started with 5.25% NaOCl, which was subjected to ultrasonic activation for 20 seconds. The next step was rinsing with distilled water and then with 17% of EDTA solution. The final irrigation was done using distilled water - 2 ml per canal. The root canal was obturated according to the already used method. Control parallel sector radiography was prescribed.

A year after the treatment and obturation of the root canals of the patients, a control X-ray examination was prescribed. It was done in the same way as in the previous task.

RESULTS

Results from task 1.

Results obtained from the survey among dentists

After analyzing the survey carried out among 80 dentists, we came to the following conclusions:

1. A relatively large percentage (83.75%) of the dentists apply a multi-visits method of treatment of asymptomatic apical periodontitis.
2. 98.51% of the dentists prescribe calcium hydroxide alone or in combination with another medication as an intracanal medication dressing.
3. A relatively large part of the dentists apply modern methods and means for obturation of the root canal system.
4. Despite the proven benefits and needs for good isolation, achieved through adequate application of a rubber dam and preendodontic build up, relatively large number of dentists report that they do not apply these methods.
5. The majority of dentists monitor the results from the treatment from the third month to the second year.

From the answers to the first question concerning the method of treatment, the survey among dentists shows that the majority of them (83.75%) treat teeth with asymptomatic apical periodontitis using a multi-visits method (Fig. 21). This could be due to the fact that they rely on the antimicrobial activity of the medication used for temporary dressing.

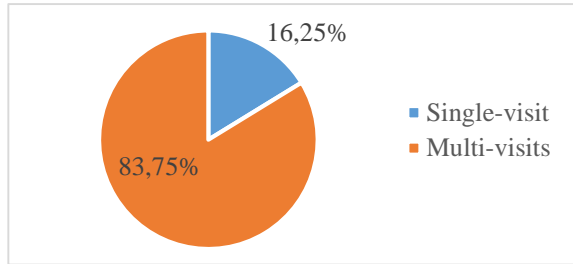


Fig 21. Distribution of answers to question No 1: "How do you treat teeth diagnosed with chronic asymptomatic apical periodontitis?"

The main material used by the majority of dental specialists (68.66%) for the dressing is (question 2) calcium hydroxide (Fig. 22). Due to its biological and therapeutic properties, calcium hydroxide is often preferred in endodontics. It has a wide range of antimicrobial effects against common endodontic pathogens, but is less effective against *Enterococcus faecalis*.

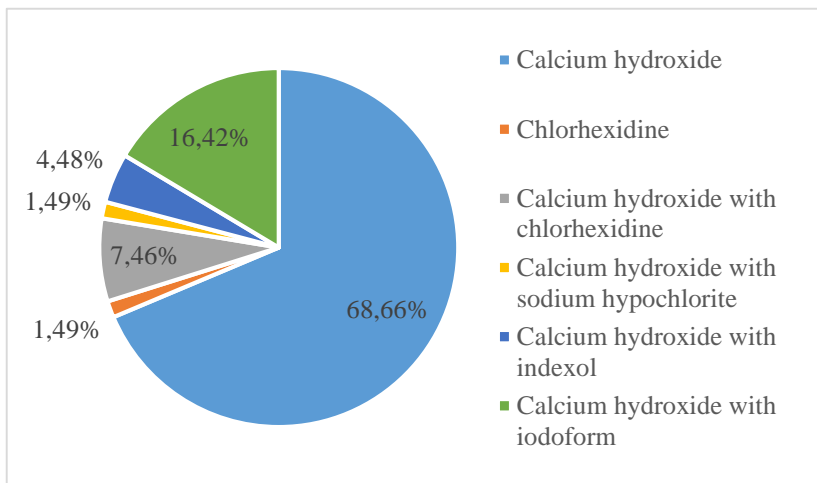


Fig. 22. Distribution of answers to question No 2: "If you treat teeth using a multi-visits method, what type of insert do you use?"

The next question concerns the exposure time of the intracanal medication dressings. The results show that it is relatively similar. 27 people

answered that they put the dressing for a period of 7-14 days (40.30%) and 22 people (32.84%) for a period of 5-7 days (Fig. 23).

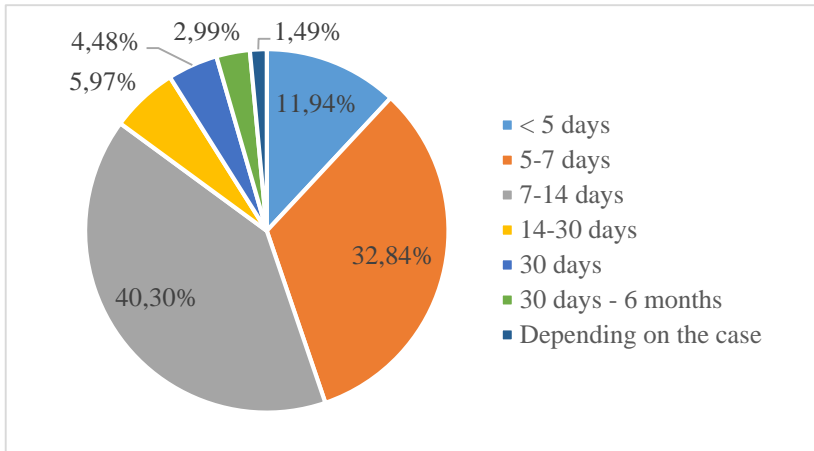


Fig. 23. Distribution of answers to question No. 3: "For how long do you put the dressing?"

Question No. 4 refers to the irrigation protocol used by dentists in the treatment of teeth with CPP. After the survey, we came to the conclusion that the main irrigation protocol used by almost half of the people (42.5%) is sodium hypochlorite, citric acid, EDTA and saline (Fig. 24). 25 people (31.25%) add chlorhexidine to their protocol. It has been proven that chlorhexidine has a major role in the reduction of the main pathogen – *Enterococcus faecalis*.

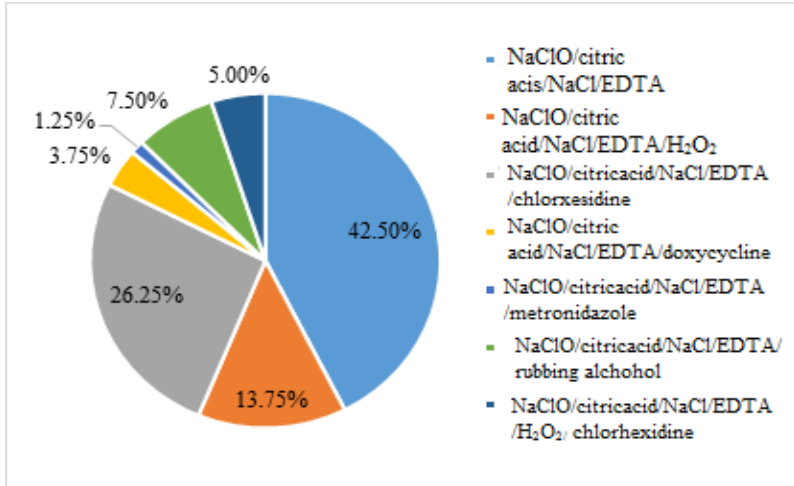


Fig. 24. Distribution of answers to question No 4: "What is your irrigation protocol with teeth with CPP?"

63.75% of the dentists answer question No. 5 – "Do you prescribe analgesics to patients with CPP?", with a "no", and 12 of them (15%) answered that they always prescribe analgesics after treatment. The rest of the dentists prescribe analgesics if it is necessary (21.25%) (Fig. 25).

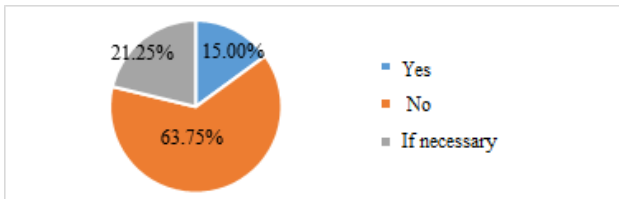


Fig. 25. Distribution of answers to question No. 5: "Do you prescribe analgesics to your patients diagnosed with CPP?"

For question 6 – "How often do you observe complications?" – 59 people (73.75%) reported that they rarely (10-20%) observe complications (Fig. 26).

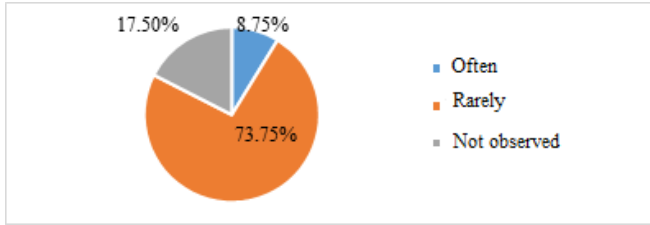


Fig. 26. Distribution of answers to question No. 6: "How often do you observe complications?"

The main complications reported by the participants in the survey are pain and exacerbation (38.75%). Nine of the dentists report that they do not observe a healing process (11.25%) (Fig. 27).

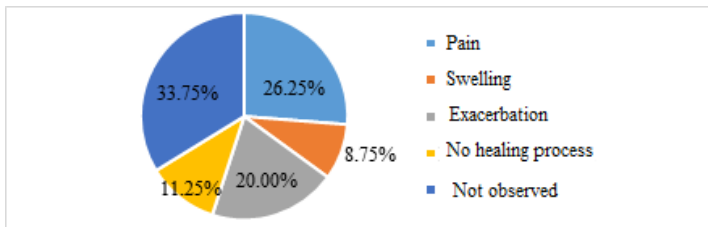


Fig. 27. Distribution of answers to question No. 7: "What type of complications do you observe?"

Taking a microbiological sample from the root canal system to identify the available microorganisms (6.25%) rarely happens. 80% out of the five dentists who perform biochemical identification report that *Enterococcus faecalis* is isolated (Fig. 28).

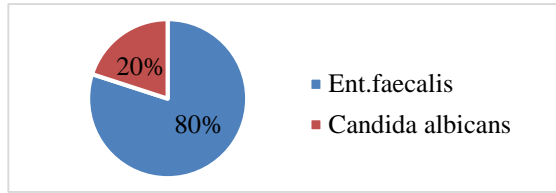


Fig. 28. Distribution of answers to question No 9: "Which MO is most often isolated?"

Comparing the frequency of chronic apical periodontitis in intact teeth or after endodontic treatment, the second group of teeth prevails (92.5%) (Fig. 29).

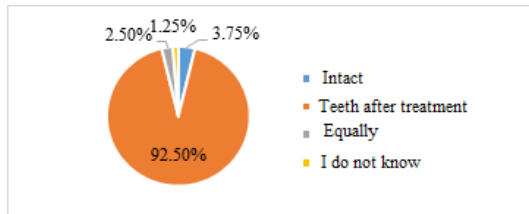


Fig. 29. Distribution of the answers to question No. 10: "Do you observe CPP more often in intact teeth or in teeth after endodontic treatment?"

With regard to the materials used for obturation of the root canals (question 11), the largest percentage (63.75%) rely on sealers based on epoxy resins (Fig. 30). This is explained by the fact that the material is easily accessible, inexpensive, hermetically sealed and has excellent biocompatibility and good X-ray contrast.

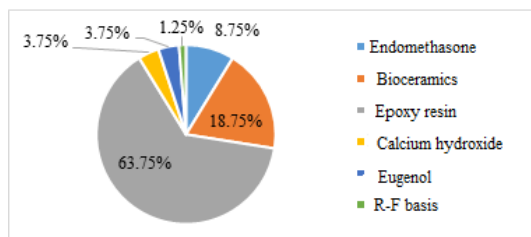


Fig. 30. Distribution of answers to question No. 11: "What type of canal fillers do you use for teeth with CPP?"

The fact that sizeable number of dentists (26.25%) skip one of the main stages in endodontic treatment, which is adequate isolation when placing a rubber dam, is worrying (Fig. 31).

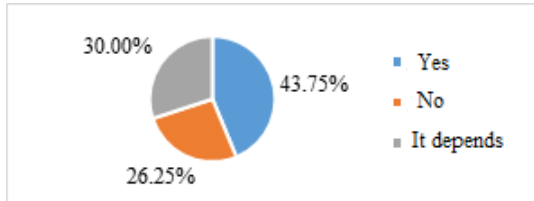


Fig. 31. Distribution of answers to question No 12: "Do you place a rubber dam when treating teeth with CPP?"

More satisfactory answers to question No.13 compared to the previous question about the isolation (Fig. 32) are given. A large percentage of the dentists (67.5%) report that they always perform preendodontic build up, which is a prerequisite for isolating the cavum pulpae from sulcus fluid and saliva, as well as for the hermetic sealing of the endodontic cavity between patients' visits.

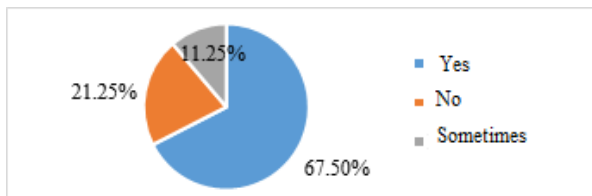


Fig. 32. Distribution of answers to question No. 13: "If it is necessary, do you first do preendodontic build up?"

Questions 14-16 in the survey are related to the irrigation solutions and their concentration. Answers to question 14 include 60% use of sodium hypochlorite at a concentration of 2.5% or less, 32.5% rely on a higher concentration – 5.25% and the remaining 7.5% use sodium hypochlorite in a concentration in between the previous two (Fig. 33).

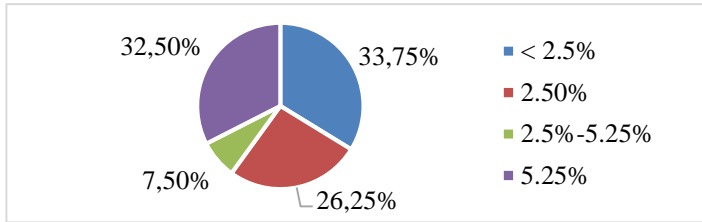


Fig. 33. Distribution of answers to question No. 14: "What percentage of hypochlorite do you use?"

Regarding the amount of solutions, seventeen dentists (42.5%) use less than 5 mm³, 26 respondents (32.5%) use between 5 and 10 mm³. The percentage (17.5%) of dentists who rely on a larger amount of irrigation solutions is small (Fig. 34).

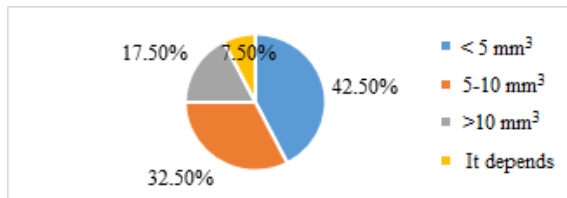


Fig. 34. Distribution of answers to question No. 15: "How many mm³ of solution do you use per canal?"

About the activation of irrigation medicaments, a high percentage (70%) reports that they activate the chemical agents introduced into the root canal (Fig. 35).

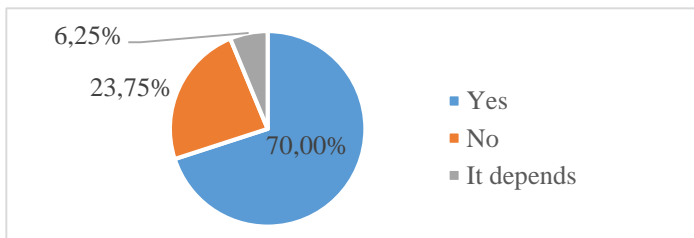


Fig. 35. Distribution of answers to question No 16: "Do you activate the solutions?"

Regarding the tracing of the results (question 17), it is clear that 63.75% of the dentists prescribe control radiography between 6 and 12 months after the end of the treatment. The remaining 48.75% prescribe it before the sixth month (Fig. 36).

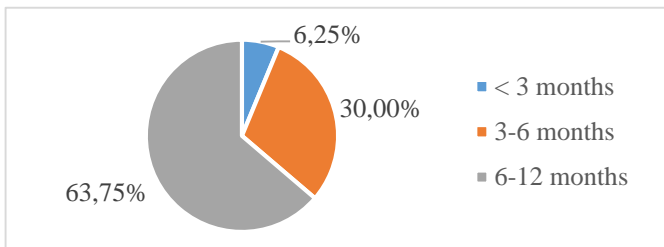


Fig. 36. Distribution of answers to question No. 17: "When do you prescribe control radiography?"

When asked about the period of time they follow their cases, 46 of the dentists (57.5%) report that they follow them for a period of 2 years, and 33.75% - for a period of up to a year. The percentage (8.75%) that follows their cases for a longer than two-year period is small (Fig. 37).

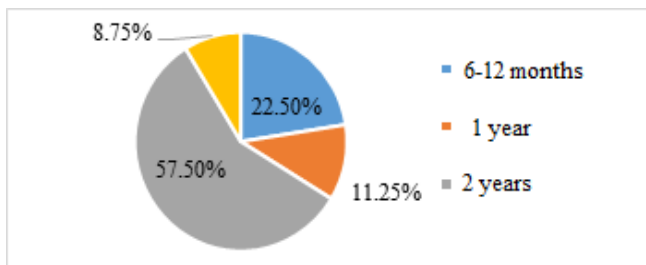


Fig. 37. Distribution of answers to question No. 18: "For how long do you monitor teeth with CPP?"

Discussion of the results obtained from the survey among dentists.

After finalizing the survey and reviewing the results, we conclude that the majority of dentists rely on the generally accepted protocol for treatment of teeth with CPP using a multi-visits method. According to us, this is due to the fact that they want to prevent a possible exacerbation of inflammation resulting from the chemo-mechanical treatment of root canals, as well as from the fact that the treatment opens access to aerobic microorganisms, which would result in mixed infection.

Based on the survey, it is clear that a large number of dentists report the application of modern irrigation techniques and root canal obturation, which a number of authors (Ahmed F. et al. 2016), (Gusiyska A. et al. 2009) described as a basic requirement for adequate endodontic treatment.

The main problem we find is the neglect of one of the main steps for adequate endodontic treatment, namely, adequate isolation. We attribute it to the fact that most of the dentists have a busy work schedule. Another reason for the low percentage relying on adequate isolation is the need for additional practical training to work with a rubber dam for doctors who completed their training years ago. Therefore, we can recommend dentists to improve their quality of work through modern techniques for isolation of the operative field, because, as it turns out, they have an impact on the microbial count. This has been confirmed in another study (Borisova-Papancheva Tsv. et al. 2016), which comments on the low quality of endodontic treatment in Bulgaria.

Results obtained from the survey of patients with CPP, to whom one of the two studied treatment methods was used.

Study of the pain after treatment of teeth diagnosed with CPP using one-visit and multi-visits method. The frequency of complications and the need for analgesics have been analyzed. The total number of surveyed patients is 71. 31 of them were treated using a single-visit method, and the remaining 40 – using a multi-visits method with placement of a temporary dressing or sterile swab.

After analyzing the surveys conducted among 71 patients the following main points have been identified:

1. A relatively large portion (70%) of the patients have reported mild pain immediately after filling the root canal.
2. The majority of the patients (67.7%) have reported lack of pain 24 hours after root canal filling.
3. A relatively large portion (90.3%) of the patients have reported lack of pain a week after filling the root canal.
4. The more common symptoms have been observed in cases treated using a multi-visits method, after application of a temporary dressings.
5. The patients who received analgesics have been treated using a multi-visits method.

Results from a survey of patients treated using a single-visit method.

We have divided the patients into two groups according to their gender: men –13 (41.9%) and women – 18 (58.1%). All patients were divided into four subgroups according to their age. No patients were registered in the first subgroup (16 -18). In the second subgroup, 19-35 years of age, there were 6 men and 10 women. In the third subgroup - 36-60 years of age, there were 7 men and 8 women. The fourth subgroup included patients over 60 years of age. There are no registered patients in this group (Fig. 38).

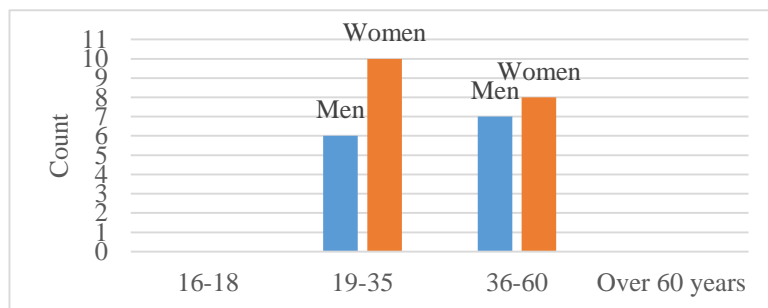


Fig.38. Distribution of patients treated using one-stage method according to their age

Based on the data collected from the patients, it has been found that in twelve male patients (92.3%) symptoms of pain and discomfort were present immediately after filling. Six patients (46.2%) reported persistent pain 48 hours after root canal filling. All were men, three from each of the two groups, 23.1% - 19-35 years old and 36-60 years old. Three patients (23.1%) have reported pain one week after the treatment, as well as percussion pain. Only one female patient (5.6%) from the age group 36-60 years has reported persistent mild pain 24 hours after root canal filling, which has been absent during the next period of reporting (Fig. 39).

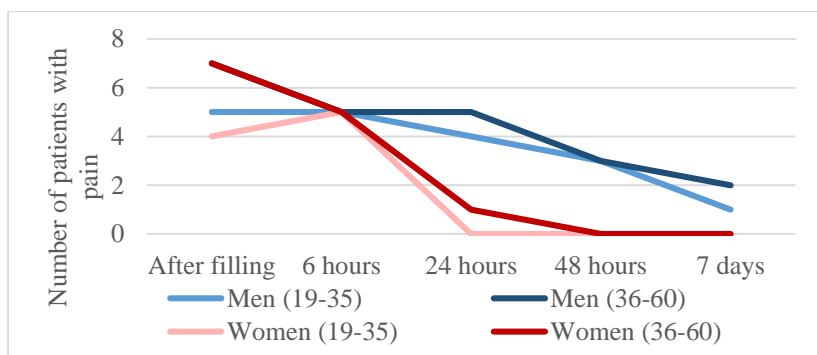


Fig. 39. Reporting pain according to the gender and age of the patients

Taking into account the average value of pain in men and women between the different periods of reporting, we have found (Fig. 40):

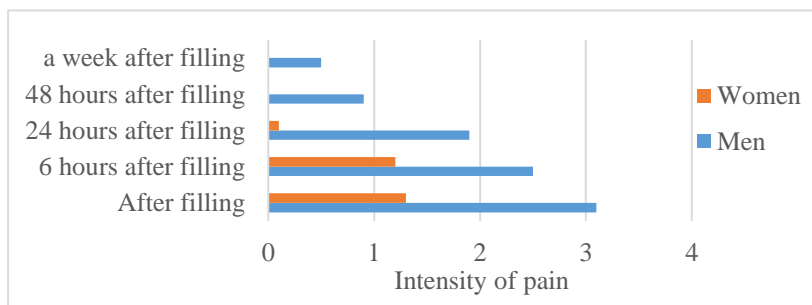


Fig. 40. Average pain values in patients in different periods, divided according to their gender

Pain symptoms in men at each stage of the treatment have been more pronounced. We studied the pain symptoms in men and women in the different age groups. There were no registered patients in the 16-18 age group. The group we will further discuss includes patients 19-35 years of age (Fig. 41).

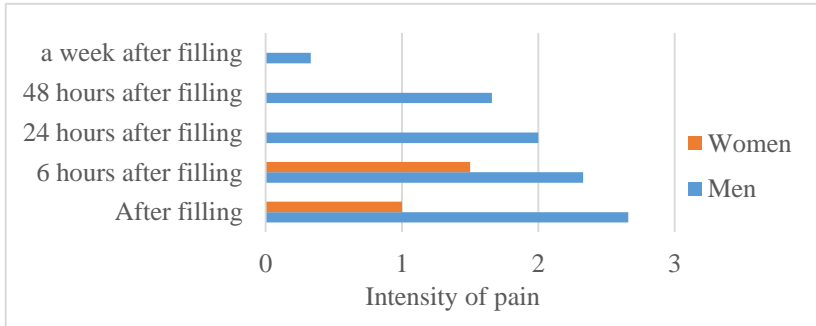


Fig. 41. Average pain values in patients in the 19-35 age group

After averaging the values, we have concluded that more frequent pain symptoms were present in men in the 19-35 age group.

The next age group includes patients between 36 and 60 years of age (Fig. 42).

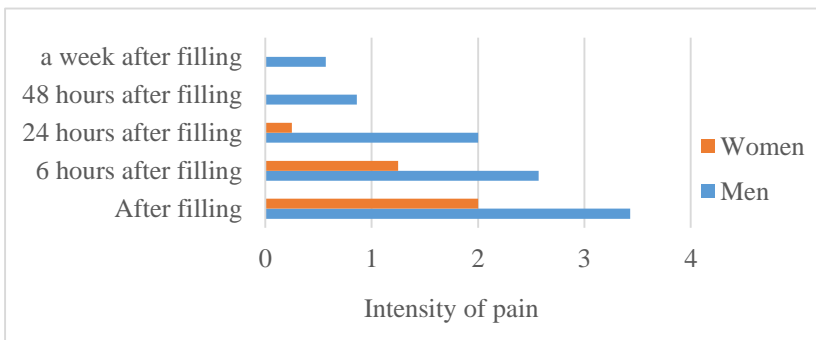


Fig. 42. Average pain values in patients in the 36-60 age group

The collected data has shown once again that more pronounced symptoms were present in male patients.

We have studied men and women separately and compared the data provided to us according to the different age groups (Fig. 43) (Fig. 44).

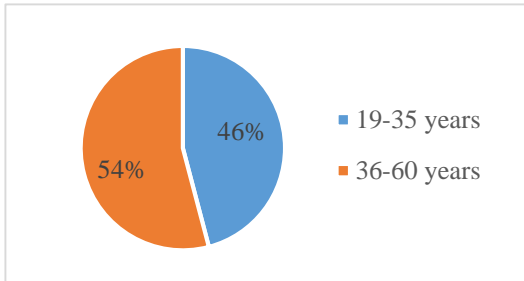


Fig. 43. Average pain values in men from both age groups

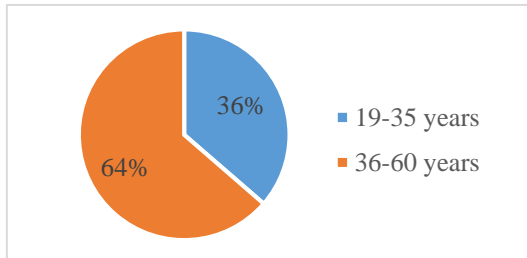


Fig. 44. Average pain values in women from both age groups

According to the results, a conclusion was made that patients aged 36-60 reported more pain on average compared to the 19-35 age group.

The results have shown that women between the age of 36 and 60 had more pronounced symptoms than the ones from the previous group.

Statistical analysis of the results obtained from a survey of patients treated using a single-visit method.

The analysis of the experimental data from subtask 4.2. has been carried out with IBM SPSS Statistics 20 package, specialized for statistical analysis (Table 2).

Hypotheses based on pain at different time intervals following one-visit treatment:

Table 2. T-test for two dependent samples based on the surveyed patients treated using one-visit method

		Paired Samples Test							
		Paired Differences			95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Pain after obturation - Pain 6h. after obt.	.323	1.045	.188	-.061	.706	1.718	30	.096
Pair 2	Pain after obturation - Pain 24h after obt.	1.226	.990	.178	.863	1.589	6.892	30	.000
Pair 3	Pain after obturation - Pain 48h after obt.	1.677	1.469	.264	1.138	2.216	6.356	30	.000
Pair 4	Pain after obturation - Pain one week after	1.871	1.455	.261	1.337	2.405	7.161	30	.000

The table presents the average difference between the two samples, as well as the statistical significance of the test (Sig.). The table has represented a two-tailed test to test the null hypothesis. We have modified the two-tailed test into one-tailed test with the following values:

- p-value = 0.048 (comparing the pain after filling and 6 hours after obturation of the root canals). Therefore, we have rejected H₀ in favor of H₁ at each significance level $\alpha = 5\%$ or $\alpha = 10\%$. With 95% or 90% certainty, we can say that there has been a statistically significant difference between the results before and after the treatment based on the sample, i.e. the treatment has helped to reduce the pain 6 hours after root canal obturation.
- p-value = 0.0000 (comparing the pain after filling and 24 hours after obturation of the root canals); p-value = 0.0000 (comparing the pain after filling and 48 hours after root canal obturation); p-value = 0.0000 (comparing the pain after filling and 1 week after root canal obturation) => We have rejected H₀ in favor of H₁ at each significance level $\alpha = 1\%$, $\alpha = 5\%$ or $\alpha = 10\%$, => With 99%, 95% or 90% certainty, we can say that there has been a statistically significant difference between the results before and after the treatment based on the sample, i.e. the

treatment has helped to reduce pain 24 hours, 48 hours and 1 week after root canal obturation was performed.

A statistically significant difference between the results before and after the treatment has been established.

Discussion of results obtained from the survey of the patients who were treated by one-visit method.

Based on the data obtained from the surveyed patients, it has become clear that men had more pronounced symptoms during different periods of the study, which differs from the results obtained by other authors (Nair M. et al. 2017).

When dividing patients by age groups, we have found that those in the 36-60 age group were more likely to report pain symptoms, which may be related to changes in humoral and cell-mediated immunity due to aging.

Based on the statistical analysis, we have found a statistically significant difference (p -value = 0.0000) between the first period of pain reporting (immediately after filling the root canal) and the remaining reporting periods. This may be due to the fact that complications were less common in teeth treated using a single-visit method due to shorter exposure to irrigation solutions and medications that could induce an immune response, which has also been confirmed by other authors (Yingying Su et al. 2010), (Kalhor FA et al. 2009), (Yousaf O. et al. 2016)

Results from patients treated using a multi-visits method.

We divided the patients into two groups according to their gender – men 18 (45%) and women 22 (65%). All patients were divided into four subgroups according to their age (Fig. 45).

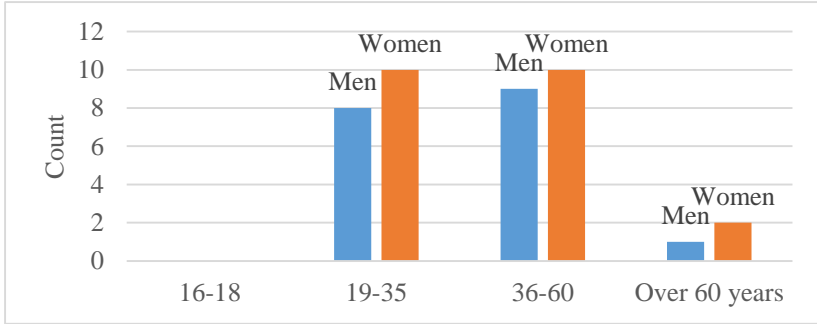


Fig. 45. Distribution of patients, treated using a multi-visits method, by age

The surveyed 40 patients were divided into three groups according to the dressing used in their treatment and their gender (Figs. 56, 57). The first group included 20 people (50%) treated with a calcium hydroxide dressing. The second group was treated by applying a sterile cotton swab, and for the third group a 2% chlorhexidine for irrigation was used (Fig. 46). 10 people were studied in the second group, as well as in the third one.

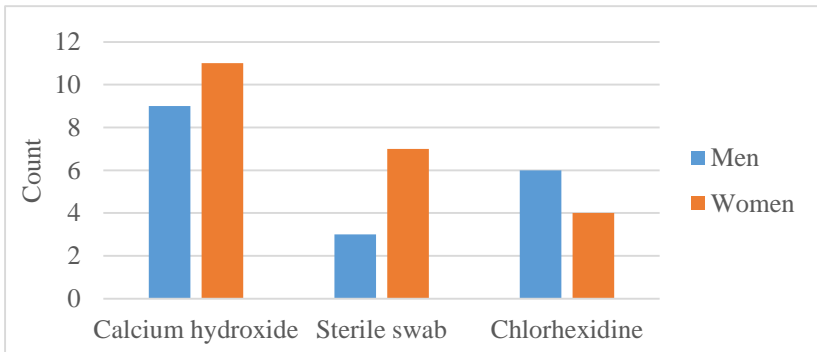


Fig. 46. Distribution of patients by gender according to the medication applied

Based on the data collected from the studied patients, it was established that 14 male patients (77.8%) reported symptoms during the first period. After filling the root canal, this percentage increased to 88.9%. These were two more patients from the 36-60 age group. Five patients (27.8%)

reported persistent pain 7 days after root canal filling. Four of them were in the 36-60 age group and one in the 19-35 age group.

Based on the data collected from the studied patients, it was found that 16 female patients (72.8%) reported symptoms which included pain and discomfort during the first period. After filling the root canal, this percentage decreased to 63.6%. Only one female patient from the 19-35 age group and one from the 36-60 age group reported persistent mild pain 7 days after root canal filling (Fig. 47).

A total of three people (7.5%) reported severe pain - 6 (from VAS) during all reporting periods. All of them were male patients from the 36-60 age group.

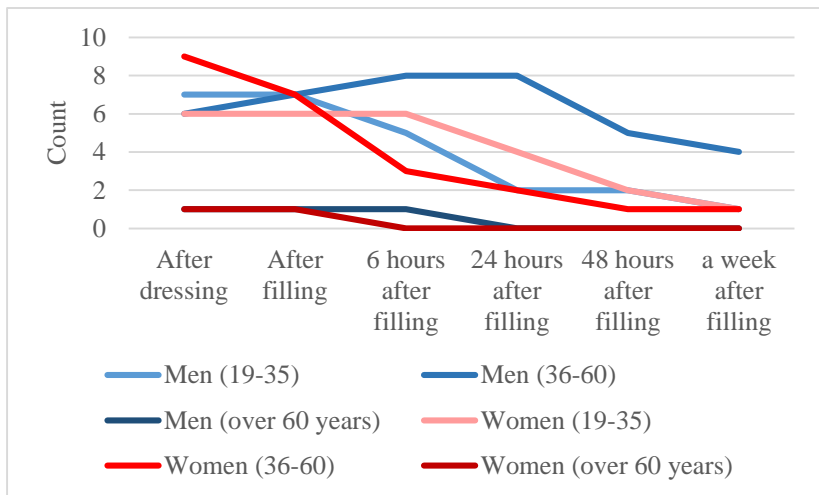


Fig. 47. Intensity of pain according to the gender and age of the patients

After analyzing the data obtained from the patients, we have found that all the women (100%) treated with calcium hydroxide dressing reported pain after application of the medicaments. Eight men (88.9%) reported pain after the first stage of the survey. In the next stage of the study, all men (100%) reported the presence of symptoms and these values stayed the same in the next stage of the study. Nine women (81.8%) reported pain

immediately after root canal filling. The data has shown that 7 days after treatment 5 men (55.6%) and one woman experienced persisting pain (Fig. 48).

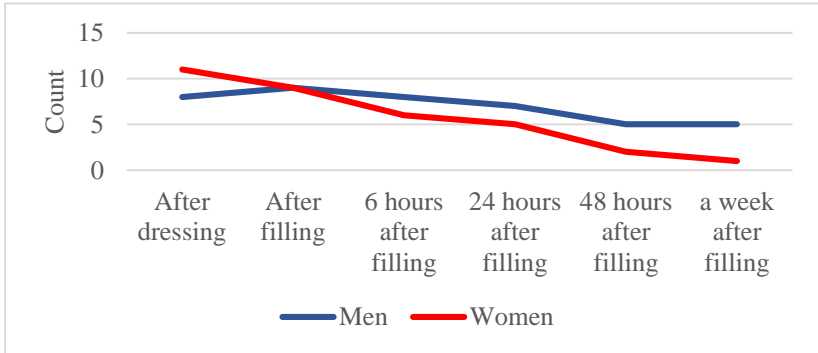


Fig. 48. Intensity of pain in multi-visits treatment after application of calcium hydroxide

After analyzing the data obtained from the patients, we found the same values in the first two stages of the survey – after placing a dry sterile swab and immediately after filling the root canal - two of the surveyed men (66.6%) and 4 of the women (66.6%) reported pain. The data has shown that in one woman (14.28%) pain persisted 7 days after treatment, (Fig. 49).

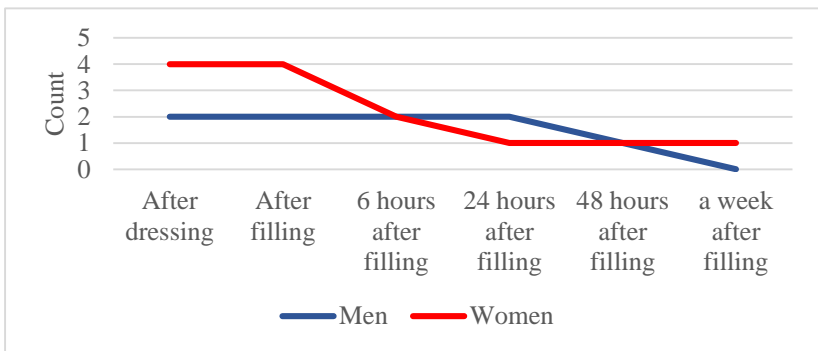


Fig. 49. Intensity of pain in multi-visits treatment without a dressing

After analyzing the data obtained from patients, we have found that one woman (25%) out of the patients treated with chlorhexidine reported pain after it had been applied. 4 men (66.7%) reported pain after the first stage of the survey. In the next stage of the study, the number of men with symptoms increased to 5 (83.3%), and these values decreased in the following survey periods. Only one woman (25%) reported pain immediately after root canal filling. The data has shown that none of the surveyed men and women reported pain and discomfort 7 days after treatment (Fig. 50).

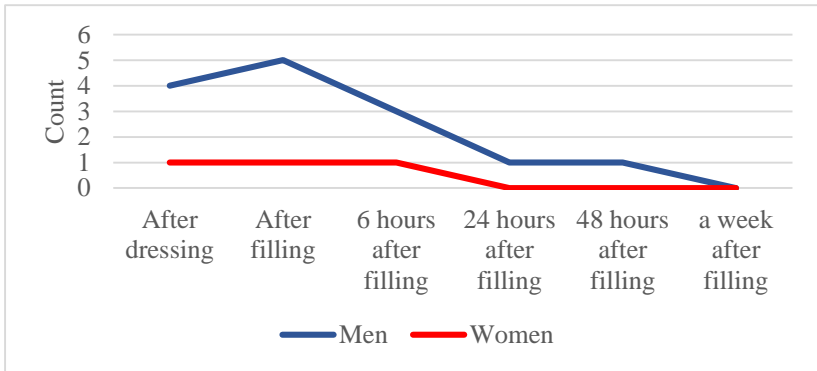


Fig. 50. Intensity of pain in multi-visits treatment after CHX

Taking into account the average pain values in men and women and between the different periods of reporting pain, we found out the following (Fig. 51):

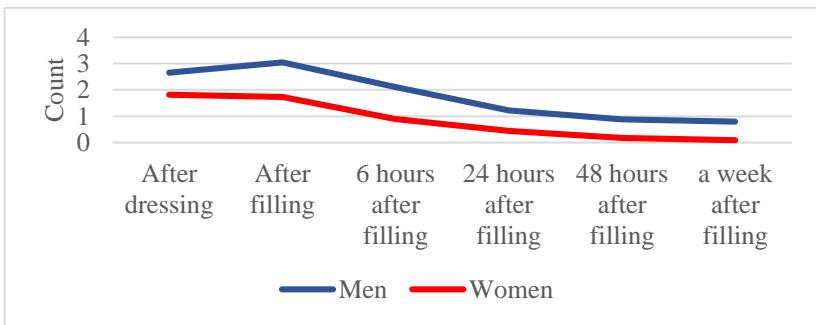


Fig. 51. Average pain values in different periods in patients divided by their gender

At each stage of their treatment, we have observed that men experienced more pronounced pain.

We examined the pain symptoms in men and women from the different age groups. There were no patients in the 16-18 age group. The group we discussed included patients from the 19-35 age group (Fig. 52).

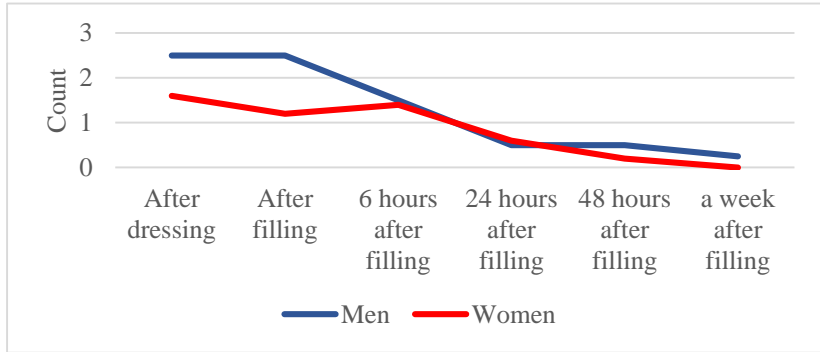


Fig. 52. Average pain value in patients from the 19-35 age group

After averaging the values, we have observed more frequent pain symptoms in men from the 19-35 age group, except in the 24 hours after filling the root canals period.

The next age group included patients in the 36 - 60 age group (Fig. 53).

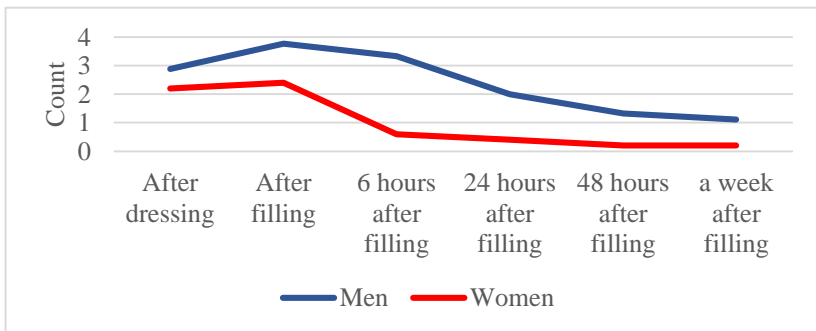


Fig. 53. Average pain values in patients from the 36-60 age group

The collected data have once again demonstrated that male patients experienced more pronounced symptoms.

The last group with registered patients is the over 60 years group (Fig. 54).

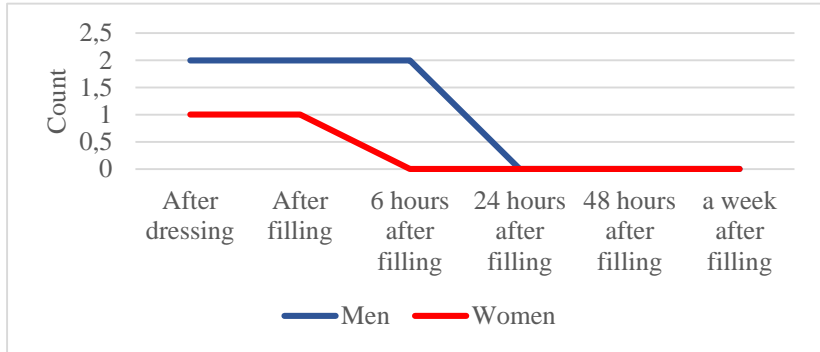


Fig. 54. Average pain values in patients over 60 years old

We studied men and women separately and compared the data from the different age groups. We calculated the average pain values in regard to its intensity for the total survey period and found out the following (Figs. 55, 56):

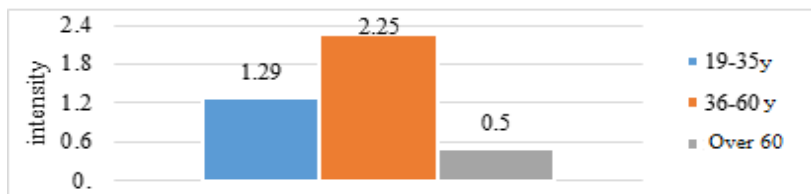


Fig. 55. Average pain values in men from different age groups

With patients from the middle group there were more pronounced symptoms compared to the other two groups.

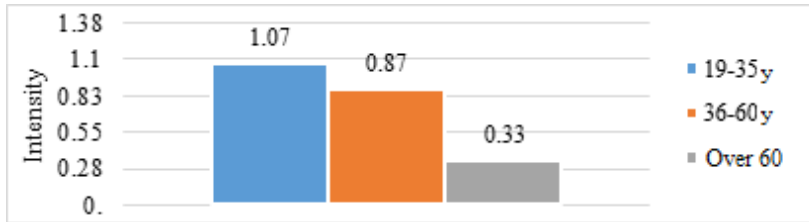


Fig. 56. Average pain values in women from different age groups

Patients between 19 and 35 years had more pronounced symptoms compared to the other two groups.

Discussion of the results from patients treated using a multi-visits method

The present study has demonstrated that 100% of women treated with a calcium hydroxide dressing experienced pain after medication was applied. This may be due to the tendency to develop postoperative pain as they are more prone to psychosomatic disorders (Ng YI et al. 2004), (Colameco S. et al. 1983). Another study explains the more pronounced symptoms in women with variable levels of the hormones serotonin and estrogen (Marcus DA. 1995) (Dao TTT. 1998) (Mathew Th. 2015).

Changes in female hormones during menstruation, hormone replacement therapy, and oral contraceptives may alter serotonin and norepinephrine levels, thus contributing to a reduced pain threshold (Fillingim RB. et al., 1995), (Mathew ST. et al., 2015).

A larger number of the interviewed reported persisting symptoms after multi-visits treatment. This may be due to the fact that they were exposed to medication and irrigants for a longer period of time, which resulted in an immune response following irritation of the periapical space.

When dividing patients in groups according to their age, we have found out that the male patients aged 36-60 are more likely to report pain symptoms, which may be related to changes in humoral and cell-mediated

immunity with aging (Nair M. et al. 2017). These results are similar to the study of pain in patients treated using a single-visit method.

Hypotheses for comparing pain in single-visit and multi-visits treatment:

Independent samples have been used within this statistical analysis.

Table. 3. T-test for independent samples obtained from patients, reporting the pain at different time intervals, according to the used methodology

		Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper	
Pain after obluration	Equal variances assumed	1.634	.205	-.696	69	.489	-.285	.410	-1.104	.533
	Equal variances not assumed			-.707	67.814	.482	-.285	.404	-1.091	.520
Pain 6h. after obt.	Equal variances assumed	1.077	.303	.372	69	.711	.142	.382	-.619	.903
	Equal variances not assumed			.380	68.487	.705	.142	.373	-.603	.887
Pain 24h after obt.	Equal variances assumed	.439	.510	-.123	69	.902	-.036	.295	-.624	.551
	Equal variances not assumed			-.121	58.615	.904	-.036	.301	-.639	.566
Pain 48h after obt.	Equal variances assumed	3.210	.078	-.821	69	.414	-.188	.229	-.645	.269
	Equal variances not assumed			-.850	68.978	.398	-.188	.221	-.629	.253
Pain one week after	Equal variances assumed	4.998	.029	-1.075	69	.286	-.206	.192	-.590	.177
	Equal variances not assumed			-1.133	67.089	.261	-.206	.182	-.570	.157

The table shows the average difference between the two samples as well as the statistical significance of the test (Sig.). It consists of 2 parts: Levene's Test for Equality of Variances and t-test for Equality of Means. In the Levene's test, the variances have been calculated and the significance of their equality has been assessed. In this table Sig. is greater than 0.05, i.e. the statement about the inequality of variances is insignificant. In all samples, the p-value is greater than 0.05. We have no reason to reject the null hypothesis in favor of the alternative at significance level $\alpha = 1\%$, $\alpha = 5\%$ or $\alpha = 10\%$, => We have not discovered enough evidence to claim that the difference single-visit treatment - multi-visits treatment is positive or the values from the one-visit treatment are higher than the multi-visits at a significance level of $\alpha = 1\%$, $\alpha = 5\%$ or $\alpha = 10\%$.

From a statistical point of view, we have no reason to give preference to either of the two CPP treatment techniques (p-value > 0.05).

Discussion of the results obtained from the patients treated with both methods

In our study, we found that patients treated with the multi-visits method reported more frequent persistent symptoms after treatment with the multi-visits method than with the single-visit method. Our results have been confirmed by other authors who compare pain following the two treatment methods (Yingying Su et al. 2010), (Kalhor FA et al. 2009), (Yousaf O. et al. 2016), (Riaz A. et al. . 2018). This may be due to the fact that they were exposed to medication and irrigants for a longer period of time which resulted in an immune response due to irritation of the periapical space. This has also been confirmed by Fonzar F et al. (2017), who found more frequent use of analgesics after a multi-visits method of treatment. They recommend the use of one-visit therapy.

From a statistical point of view, we have no reason to give preference to either of the two CPP treatment techniques (p-value> 0.05).

Results from task 2

Results based on clinical research

This task included 31 patients who were diagnosed with CPP through clinical and paraclinical methods. The effect of the applied one-visit treatment has been taken into account by collecting data from clinical and paraclinical studies, as well as from the information given to us by patients. 13 (41.9%) of these patients were male and the remaining 18 (58.1%) female

All patients were divided into four subgroups according to their age. There are no registered patients in the first age subgroup 16-18. In the second subgroup - 19-35 years of age there were 6 men and 10 women. The third subgroup included patients aged 36-60. There were 7 men and 8 women in this group. The fourth subgroup included patients over 60 years of age. There were no registered patients in this group.

Based on the data collected from the patients, it was found out that 9 male patients (69.23%) experienced symptoms, which included pain and discomfort but resolved within 48 hours after filling the root canal. Four of these patients (30.76%) were in the 19-35 age group. The other five (38.46%) were in the 36- 60 age subgroup. Three patients (23.07%) reported persistent pain one week after treatment as well as percussion pain. Two of them (15.38%) were into the 36-60 age subgroup. Only one female patient (5.56%) aged 36-60 reported pain after root canal filling, which resolved within 48 hours of treatment. All patients returned a week after the treatment.

Results based on microbiological research

From the analysis of the clinical laboratory results of thirty one patients, we have concluded that in 19 of the samples (61.3%) taken before root canal treatment only one MO was isolated, namely, *Enterococcus faecalis* (Fig. 57). Quantitative testing in most samples has revealed a large number of the tested microorganisms $\rightarrow 10^5$. In two of the samples, MO have been observed in the amount between 10^4 and 10^5 . In two of the cases (6.5%), a mixed infection has been found, which included *Enterococcus faecalis*, *E. coli* and *Staphylococcus CNS* (coagulase-negative staphylococcus). *Candida albicans* infection has been observed in 4 (12.9%) of the samples. In one of the samples (3.2%), *Pseudomonas aeruginosa* has been isolated in large quantities 10^5 . In 5 (16.1%) of the microbiological samples no infection has been observed in the root canal system. In 25 of the studied patients (96.1%), complete removal of microorganisms has been observed after mechanical and chemical treatment. Only in one patient (3.85%), the persistence of some of the microorganisms has been detected in the follow-up microbiological examination (Fig. 58). The initial sample revealed a polymicrobial infection caused by *Enterococcus faecalis* and *Staphylococcus CNS* (coagulase-negative staphylococcus). The follow-up microbiological examination revealed the presence of only *Staphylococcus CNS* (coagulase-negative staphylococcus).

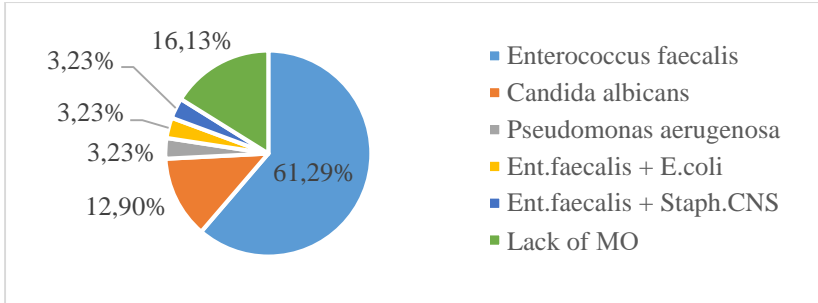


Fig. 57. Isolated microorganisms before medication treatment in teeth treated using a single-visit method

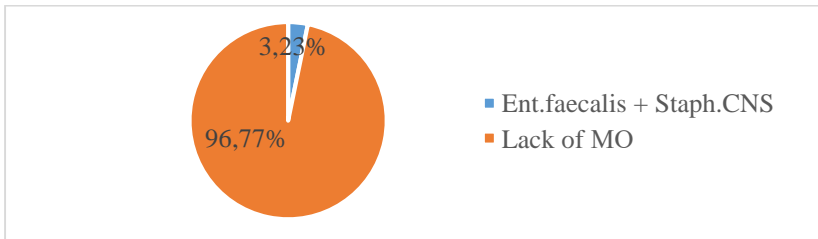


Fig. 58. Isolated microorganisms after medication treatment in teeth treated using a single-visit method

Results based on paraclinical research

Thirty out of all thirty one people examined returned for a follow-up radiography.



Fig.59. OPG before treatment of tooth 15



Fig.60. Control radiography

Using the PAI-index (Fig.61), we have found out the following:

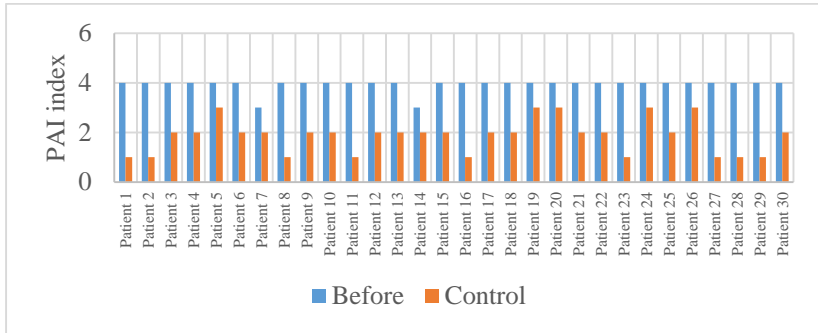


Fig. 61. PAI – before and control

From the results obtained for the average size of the lesion on the PAI scale before and in the sixth postoperative month after completion of treatment, it has been established that its size decreased by an average of 2.1 times. The analysis of the experimental data was performed with a specialized statistical analysis package SPSS.

Hypotheses for reduction of PAI values in teeth treated using one-visit method:

Dependent samples were used in this statistical analysis.

Table. 4. T-test for two dependent samples based on the data obtained during the monitoring of the healing process

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	PAI_index_before - PAI_index_after	2.067	.740	.135	1.790	2.343	15.303	29	.000

The table shows the average difference between the two samples as well as the significance of the test (Sig.). The table uses a two-tailed test to test the null hypothesis. We modified the two-tailed test into one-tailed test and got the following values:

- p-value = 0.0000 - This shows strong evidence against the null hypothesis, as there is less than a 5% probability that it is true. Therefore, we can reject the null hypothesis and accept the alternative. We have registered a statistically significant difference between the results before and after treatment.

There is a statistically significant difference between the results before and after treatment based on the periapical lesion, measured by PAI, in teeth treated with the single-visit method.

Discussion of results from task 2

After analyzing the healing process and reviewing the paraclinical and clinical examinations after filling the root canals and the information obtained from patients, we have concluded that the applied one-visit treatment has proved 95% successful. The cases were considered successful if the tooth showed no clinical symptoms. Judging by postoperative sensitivity, unsatisfactory results from the one-visit treatment have been observed only in patients with persistent pain (9.68%). In the short term we can conclude that the one-visit treatment has given good results.

Our study has proven that the adequate isolation of the operative field, the correct mechanical and chemical treatment, including only EDTA, sodium hypochlorite 5.25% and saline (distilled water), were sufficient to control the infection and reduce microorganisms (*Ent. faecalis*, *C. albicans*, *E. coli*, *Pseudomonas aeruginosa*) in the root canal system (Bhullar KK et al. 2020), (Bukhary C. Et al. 2017). From a microbiological perspective, we have arrived at the conclusion that the treatment of root canals in one visit created favorable environmental conditions for periapical recovery, which has been confirmed by other authors as well (Weiger R. et al. 2000), (Ayhan H. et al. 1999). One of the main advantages of the single-visit treatment is the prevention of reinfection of the root canals between visits, which might occur during the multi-visits treatment.

Our results have not been confirmed by the results obtained from another study (Nair P.N.R. et al. 2005), which used the same irrigation

protocol, but observed residual infection in 87.5% of the teeth included in the study.

By applying paraclinical and statistical methods, we determined that there is a statistically significant difference in the data obtained before treatment and the follow-up X-rays (Joseph J. et al. 1993), (Pekruhn RB et al. 1986), (Maity I. et al. 2014). The results may be due to the fact that all the requirements for a satisfactory endodontic treatment were strictly applied during the procedures. This includes the proper isolation of the operative field, the sufficient amount and time of exposure to the used medications for irrigation and removal of the root canal contents, the three-dimensional obturation of the root canals and hermetic definitive restoration (Ahmed F. et al. 2016), (Borisova-Papancheva Ts. Et al. 2016).

On the basis of the data obtained from this task, it has become clear that adequate isolation and adherence to the simplified, generally accepted irrigation protocol can prove to be sufficient for good recovery results. This thesis has been confirmed by other authors (Ahmed F. et al. 2016), (Eyuboglu TF. Et al. 2017), (Kalhor FA et al. 2009), who presented good recovery results during the follow-up period after one-visit treatment of teeth with chronic apical periodontitis.

The one-visit treatment of teeth with chronic asymptomatic apical periodontitis provides a good option in the already adopted treatment protocol which also includes the multi-visits treatment with application of additional medications such as calcium hydroxide and chlorhexidine (Zaneva-Hristova D. et al. 2017). The application of the single-visit method reduces the treatment time, the number of visits and the potential discomfort of the patients during repeated dental procedures. It also eliminates the risk of reinfection between the individual visits caused by microleakage through the temporary filling. Therefore, it would not be surprising that dentists would prefer the single-visit method for the treatment of teeth with CPP.

Results from task 3

Results from the multi-visits treatment of patients by application of calcium hydroxide

Results from clinical research

To determine the effect of the applied multi-visits treatment with insertion of calcium hydroxide medication for one week we collected data from clinical and paraclinical studies and information obtained from the patients. The male patients were 11 (47.83%) while the remaining 12 (52.17%) were female patients.

All patients were divided into four subgroups according to their age. No patients were registered in the first subgroup - 16-18-years. In the second subgroup - 19-35-years, there were two men and five women. In the third subgroup, which included patients aged 36-60, there were eight men and seven women. The fourth subgroup included patients over 60 years of age. Only one male patient was registered in this group.

The data collected from the patients illustrated that in seven male patients (63.63%) postoperative sensitivity was present, which lasted up to 48 hours after the filling of the root canal. Eight of all men included in the survey (72.72%) reported pain immediately after the application of the medication. Nine men (81.81%) reported pain symptoms after the definitive filling of the root canals. Five men (45.45%) reported persistent pain one week after the treatment, as well as percussion pain. One of them belonged to the 19-35 age subgroup while the other four – to the 36-60 one. In five out of twelve women (41.67%), postoperative sensitivity was observed which lasted up to 48 hours after the filling of the root canals. Eleven of all women included in the survey (91.67%) reported pain immediately after the application of the medication. Nine women (75%) reported pain symptoms after the definitive root canal filling. Only one female patient (8.33%) from the 36-60 age group reported pain, which decreased in intensity but persisted for a week after the treatment. All patients were present for the follow-up examination one week after the treatment. Having analyzed the recovery

process, examined the X-rays performed after filling the root canals, the clinical data and the feedback from the patients, we have arrived at the conclusion that the success rate of the applied multi-visits treatment was 50%. The treatment of the individual cases was considered successful if the tooth did not have any clinical symptoms. In five male and one female patient we observed persistence of pain one week after treatment.

Results based on microbiological research

The conclusion from the analysis of the clinical laboratory tests of twenty patients is that in 15 of the samples (75%) taken before root canal treatment *Enterococcus faecalis* was isolated in large quantities $\rightarrow 10^5$ (Fig. 62). In three of the cases (15%) infection caused by *S. aureus* was detected. In one (5%) of the samples, infection caused by *M. catarrhalis* was observed. No infection in the root canal system was observed in 5% of the microbiological samples.

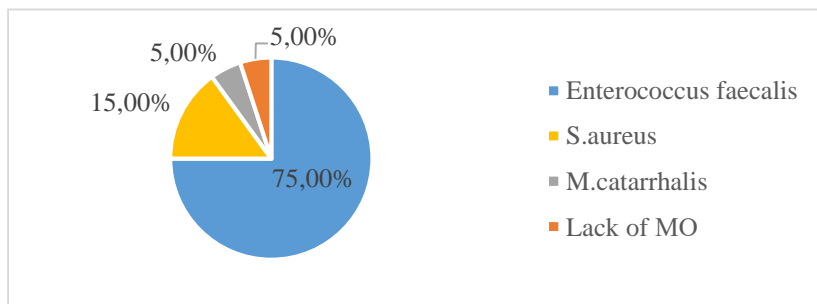


Fig. 62. Isolated microorganisms before the medication treatment of teeth treated by multi-visits procedure and application of calcium hydroxide

In all examined patients (100%), complete removal of microorganisms was observed after the mechanical and chemical treatment. None of the samples taken after the medication dressing showed the presence of microorganisms in the root canals.

Results based on paraclinical research

Twenty of all investigated 23 patients performed a follow-up X-ray (Fig. 63, 64).



Fig.63. Initial X-ray



Fig.64. Follow-up X-ray

Using PAI-index (Fig.65), we have established:

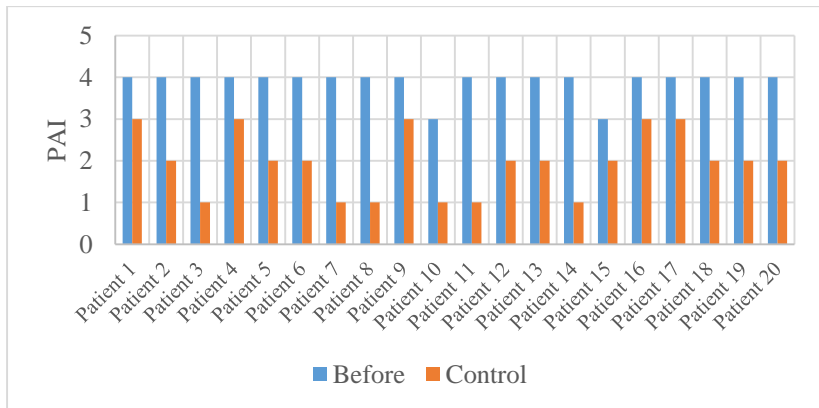


Fig. 65. PAI-before and control after treatment with calcium hydroxide

The data gathered about the average size of the lesion on the PAI scale before the treatment and during the sixth postoperative month demonstrate that the size decreased twice on average.

Discussion of the results from task 3

The study has made it clear that the multi-visits treatment of CPP has not produced satisfactory results in terms of postoperative sensitivity. The same results were established in another study, which compared the two treatment methods. (Risso P.A. et al. 2008). This may be due to the possible cytotoxic effect of calcium hydroxide after the extrusion into the periapical tissues. While following the healing process of the teeth from this task, we have observed a significant reduction of periapical change by applying measurement methods and PAI scale. This shows that in the long run calcium hydroxide has a beneficial effect on the periapical change in the root canals in teeth diagnosed with CPP. This has been confirmed in other studies (Gusiyska A. 2013), (Siqueira J. F. et al. 2008).

The analysis of data has proven that the adequate isolation of the operative field, the proper mechanical and chemical treatment including only EDTA, sodium hypochlorite 5.25% and saline (distilled water), have been sufficient to control the infection and reduce microorganisms (*Ent.faecalis*, *S. aureus*, *M. catarrhalis*) in the root canal system. In regards to the reduction of microorganisms and the prevention of reinfection between visits, no differences have been established between the various medications for temporary application in the root canals between individual visits. This has not been confirmed in the Ferrari PH study. et al. 2005, based upon repeat microbiological examinations, which established the presence of microorganisms in all examined teeth treated by the multi-visits method without application of intracanal dressing. The main risk associated with the multi-visits method of treatment is depressurization of the cavity and reinfection of the root canal system.

The results of our study have been confirmed by other authors (Haenni S et al. 2003), whose findings proved the increased antimicrobial effect of calcium hydroxide compared to the use of conventional medications. Two studies, Radeva E. (2005), (2012) have established the powerful effect of calcium hydroxide and chlorhexidine on endodontic pathogens while this has not been confirmed in the study by Zancan et al. (2016). According to their findings calcium hydroxide was ineffective for the elimination of *E.*

faecalis. Paikkatt et al. (2017) have confirmed the benefits of using calcium hydroxide against the other major pathogen - *Candida albicans*. Contrary to these conclusions, Tonea A. et al. (2017) have established that calcium hydroxide shows very low efficacy against *Candida albicans* and *Enterococcus faecalis* and should not be used alone as an antimicrobial agent. Other authors have supported this hypothesis (Zancan R. F. 2016) and proved the need to apply calcium hydroxide combined with chlorhexidine to control microbial infections, which has also been confirmed in another study (Zaneva-Hristova D. 2020).

The teeth treated by the method of multiple visits with the application of calcium hydroxide has shown most promise for a successful healing process using PAI. In our opinion, this is due to the fact that, by releasing calcium ions, calcium hydroxide stimulates the mineralization of tissues in the periapical space (Gusiyska A. 2013), (Zancan et al., 2016). The positive aspects of calcium hydroxide treatment also include bactericidal and bacteriostatic effect, stimulation of the healing processes and fibroblasts, neutralization of the low pH of acids, inhibition of the internal absorption (Mustafa M. et al. 2012). Calcium hydroxide has a hygroscopic effect and, therefore, contributes to the reduction of the exudates from root canals (Leonardo MR. Et al. 1993).

The disadvantage of calcium hydroxide application has also been described by other authors (Borisova-Papancheva Ts. Et al. 2018), who reviewed the various techniques for the removal of intracanal medication and established that none of them is able to provide complete elimination.

Results from Task 4

Results based on clinical research

We examine the effect of the applied multi-visits treatment by leaving the tooth hermetically sealed for a week without intracanal medication, by collecting data from clinical and paraclinical research, as well as from the feedback given to us by patients. 9 of these patients were male (39.1%) and the remaining 14 (60.9%) were female patients.

All patients were divided into four subgroups according to their age. No patients were registered in the first subgroup - 16-18 years old. In the second subgroup, aged 19-35 years, seven men (77.8%) and nine women (64.3%) were included. In the third subgroup - 36-60 years old, there were two male patients (22.2%) and four female patients (28.6%). The fourth subgroup included patients over 60 years of age. Only one female patient was registered into this subgroup (7.1%).

Based on the data collected from the patients, two male patients (22.22%) experienced postoperative sensitivity, which was resolved within 48 hours after filling the root canals. Six patients (26.08%) had pain symptoms after treatment and placement of a sterile cotton swab in the root canals. Two of them were male. They also had pain symptoms after the definitive filling of the root canals. Three of them reported no pain in the first stage of assessment. One of them was a man who belonged to the 19-35 age group. Only one female patient (7.1%) aged 19-35 years reported pain which subsided in intensity but persisted for one week after treatment. All patients were re-examined one week after treatment.

Results based on microbiological research

The analysis of the results obtained from the clinical laboratory, where four patients were tested, showed that in all samples (100%) taken before the root canal treatment *Enterococcus faecalis* was isolated in large quantities $\rightarrow 10^5$ (Fig. 66).

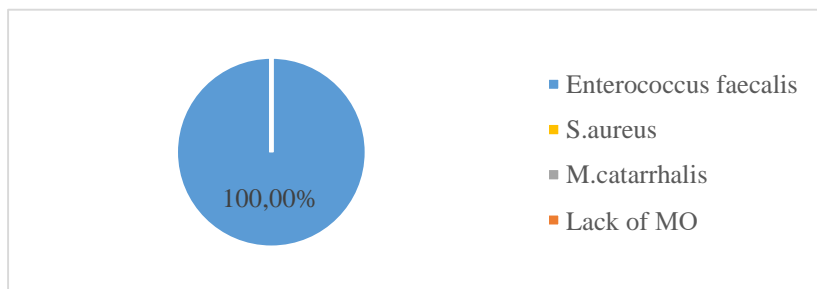


Fig. 66. Isolated microorganisms in teeth treated by the multi-visits method with a dry sterile swab before medication

In all examined patients (100%), complete removal of microorganisms was observed after the mechanical and chemical treatment (Fig. 88). None of the samples taken after one week of dry sterile swab in the cavum showed presence of microorganisms in the root canals.

Results based on paraclinical research

Only three of all 23 studied patients appeared for a follow-up X-ray. (Fig. 67, 68).



Fig.67. Initial X-ray



Fig.68 Follow-up X-ray

Using PAI index (Fig. 69) the following has been established:

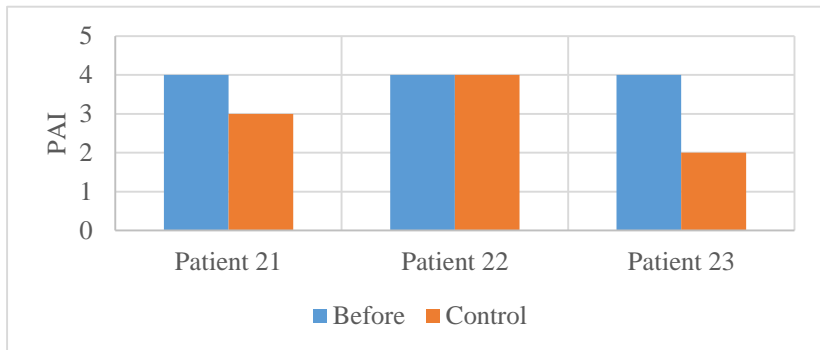


Fig. 69. PAI – before and control without dressing

On the basis of the findings about the average size of the lesion on the PAI scale before and during the sixth postoperative month after the end of treatment, it was established that the size decreased by one on average.

Discussion of the results from task 4

After the conducted study, it has become evident that the multi-visits treatment of CPP by this method gives satisfactory results in terms of postoperative sensitivity. While monitoring the healing process of the teeth during this task, we have established a reduction of the periapical change by applying measurement methods and PAI scale which, however, was not in satisfactory quantities. This demonstrates that in the long run the treatment of teeth with CPP by the multi-visits method and without the use of intracanal medication does not achieve the desired and expected results. Confirmation of our results can be found in the studies of other authors (Shuping GB et al. 2000), (Trope M. et al. 1999) whose findings have not established satisfactory results in terms of reduction of microorganisms from the root canals and improved healing in teeth treated by the multi-visits method and without the use of intracanal medication.

In terms of reducing microorganisms and preventing reinfection between visits, multi-visits treatment with a dry sterile swab showed satisfactory results, which was not confirmed in the Ferrari PH study. et al. 2005, which upon repeated microbiological examination established the presence of microorganisms in all examined teeth, treated by a multi-visits method without application of an intracanal dressing. The main risk with a multi-visits method of treatment is depressurization of the cavity and re-infection of the root canal system.

Results from task 5

Results based on clinical research

We take into account the effect of the applied multi-visits treatment by using 2% chlorhexidine in addition to our adopted irrigation protocol, by collecting data from clinical and paraclinical studies, as well as from the information given to us by patients. 13 of these patients were women (56.5%) and the remaining 10 (43.5%) were male patients.

All patients were divided into four subgroups according to their age. In the first subgroup - 16-18 years old no patients were registered. In the second subgroup - 19-35 years there are seven men and ten women. In the third subgroup - 36-60 years there are two male patients and two female patients. The fourth subgroup includes patients over 60 years of age. This group includes one male patient and one female patient.

Based on the data collected from the patients, it was found that four men (40%) reported pain after irrigation with 2% chlorhexidine. Five (50%) reported pain immediately after root canal filling. Only in one patient (10%) we observe prolonged pain, which passes within 48 hours after filling the root canals. No patient reported persistent pain one week after treatment, nor percussion pain. Only one of the studied female patients (7.7%) reported pain that occurred after application of the medication. The same patient reported pain after root canal obturation. 24 hours after treatment, all 13 (100%) women experienced a complete reduction in pain symptoms. All patients returned one week after treatment. Cases are considered successful if the tooth has no clinical symptoms.

Results based on microbiological research

From the analysis of the results obtained from the clinical laboratory, which we applied to four patients, we conclude that in all 6 samples (100%) taken before root canal treatment, *Enterococcus faecalis* was isolated (Fig. 70).

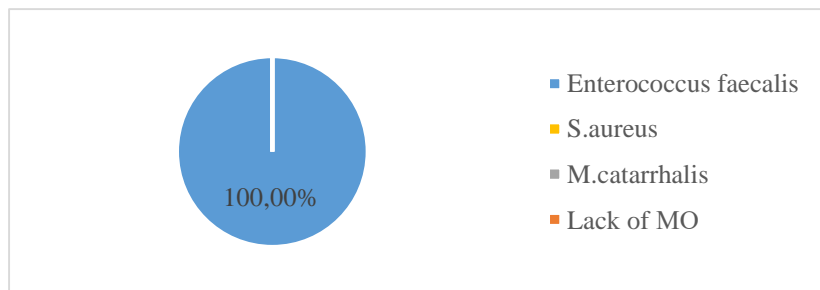


Fig. 70. Isolated microorganisms before medication treatment in teeth treated by a multi-visits method by application of 2% chlorhexidine

In all examined patients (100%) complete removal of microorganisms was observed after mechanical and chemical treatment. None of the samples taken at the second visit reported the presence of microorganisms in the root canals.

Results based on paraclinical research

Only 8 of all patients participating in this task appeared for a follow-up X-ray one year after the treatment (Fig. 71, 72).



Fig. 71. Initial X-ray



Fig.72. Follow-up X-ray

Using PAI-index (Fig. 73), we established the following:

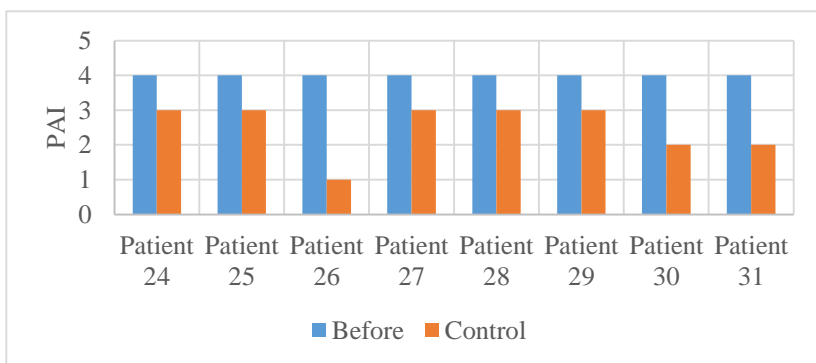


Fig. 73. PAI – before and control after chlorhexidine

From the results obtained for the average size of the lesion on the PAI scale before and on the sixth postoperative month after completion of the treatment, it was found that its size decreased 1.6 times on average.

Discussion of the results from task 5

After analysis of the obturation, analysis of radiographs after root canal filling, the clinical studies and data obtained from patients, it was found that the multi-visits treatment we applied had a success rate of 100% in terms of postoperative sensitivity. Following the healing process of the teeth from this task, we report a satisfactory reduction of the periapical lesion by applying measurement methods and PAI scale. This shows that in the long run, the treatment of teeth with CPP by a multi-visits method using chlorhexidine as an intracanal medication leads to the desired and expected results. This is confirmed by the studies of other Bulgarian authors (Radeva E. et al. 2005), (Karayasheva D. et al. 2015), who studied the antimicrobial activity of the most commonly used medications and found that chlorhexidine is slightly more effective compared to sodium hypochlorite, but not as much as calcium hydroxide. The satisfactory results we obtain may be due to the ability of chlorhexidine to maintain its activity in the presence of blood and organic matter, which was confirmed in the study of Denton G.W. from 1991. In addition, CHX can prevent further colonization of microorganisms, even after its removal from the root canal due to the gradual release of CHX. Its antimicrobial activity can be maintained for a period of 48 hours to 7 days (Ercan E. et al. 2004) (Georgieva Sl. Et al. 2017) (Kapoor V. et al. 2017).

Statistical analysis based on the data obtained by monitoring the healing process in a tooth treated by both methods using PAI

The analysis of the experimental data was performed with a specialized statistical analysis package IBM SPSS Statistics 20.

Hypotheses based on PAI values after treatment by single-visit and multi-visits method:

Independent samples were used in this statistical analysis.

Table. 5. T-test for two independent samples based on data, comparing PAI values after treatment by one of the two methods

		Independent Samples Test								
		Levene's Test for Equality of Variances		t-Test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper	
PAI_Index_after	Equal variances assumed	1.436	.236	-1.570	59	.122	-.295	.188	-.670	.081
	Equal variances not assumed			-1.574	58.420	.121	-.295	.187	-.669	.080

The table illustrates the average difference between the two samples as well as the significance of the test (Sig.). The table consists of 2 parts: Levene's Test for Equality of Variances and t-test for Equality of Means. In the Leuven test, the variances are calculated and the significance of their equality is assessed. In the case of Sig. = 0.236 > 0.05, i.e. the statement about the inequality of variances is insignificant. Then we look at the order: Equal variances assumed, in which the T-test has Sig significance. (2-tailed) = 0.122 > 0.05. The table uses a two-tailed test to test the null hypothesis. We modify the two-tailed into one-tailed test and get the following values of p-value - 0.061. We do not have sufficient information to claim that the difference in PAI in single-visit treatment is positive or PAI in single-visit treatment > PAI in multi-visits treatment, at a significance level of $\alpha = 1\%$, $\alpha = 5\%$ or $\alpha = 10\%$.

There is no a statistically significant difference between the results before and after treatment based on the data obtained by monitoring the healing process using PAI.

Discussion of the results, following the healing process by both methods

This task of our study compares the healing process by PAI in teeth treated by both methods. Our results show better healing processes in teeth treated with a single-visit method, although no statistically significant difference was found. Approximately the same results in the healing process are found in a number of other studies (Jamali S. et al. 2018), (Gill GS et al. 2016), (Chhabra A et al. 2017), (De-Deus G. et al. 2017) , (Pegah R. et al. 2019),

These results are explained by the fact that according to the present study, the simplified irrigation protocol, including 5.25% sodium hypochlorite, 17% EDTA and distilled water / saline, is sufficient to eliminate bacterial infection and affect the healing processes in the periapical space, which has been confirmed in other studies (R. Weiger et al. 2000). Therefore, it is clear that single-visit treatment of teeth with chronic asymptomatic apical periodontitis is a good alternative to the already adopted treatment protocol, including multi-visits treatment by application of additional medications such as calcium hydroxide and chlorhexidine. The application of a one-visit method would reduce the treatment time, the number of visits and the discomfort of the patients from the repeated dental procedures, as well as the risk of reinfection in the presence of microleakage from the temporary obturation between the individual visits (Denis D. 2018). This would make it a preferred method of treating teeth with CPP by dentists.

VI. CONCLUSIONS

From the clinical research:

1. One-visit treatment of teeth diagnosed with chronic apical periodontitis gives good long-term results. From a microbiological point of view, we find that it creates favorable environmental conditions for periapical recovery.
2. In the long run, the treatment of teeth with CPP by a multi-visits method, without the use of intracanal medication, does not lead to the desired and expected results.
3. In the long run, the treatment of teeth with CPP by a multi-visits method using chlorhexidine as an additional irrigant leads to the desired and expected results.
4. After analyzing the data, we conclude that adequate isolation of the operative field, proper mechanical and chemical treatment, including only EDTA, sodium hypochlorite 5.25% and saline, are sufficient to control infection and reduce microorganisms in the root canal system.
5. Teeth treated by the method of repeated visits with the application of calcium hydroxide showed the greatest healing process using PAI. The weakest healing processes are observed in the group treated by the method of repeated visits without the use of intracanal medication.
6. Following the healing process of the sixth postoperative month, we come to the conclusion that both methods of treatment show satisfactory results.

From the surveys:

1. A large proportion of dentists apply a multi-visits method of treatment for asymptomatic apical periodontitis, applying a calcium hydroxide dressing.
2. Despite the proven benefits and needs for good isolation, achieved through adequate application of a rubber dam and preendodontic build up, many dentists report that they do not apply these methods.

3. A small number of the interviewed dentists order a microbiological examination to identify the pathogen in the root canals. Most of them detect the presence of *Enterococcus faecalis*.
4. The majority of patients report mild pain immediately after root canal filling with a single-visit treatment method, with a minimal proportion reporting discomfort one week after treatment.
5. More frequent pain symptoms in both methods of treatment are observed in men aged 36-60 years.
6. More pronounced symptoms are observed in cases treated by a multi-visits method, after application of a temporary dressing.

VII. CONTRIBUTIONS

1. Confirmation of the high frequency of the presence of *Enterococcus faecalis* in teeth diagnosed with chronic apical periodontitis.
2. Confirmation of the efficacy of sodium hypochlorite and chlorhexidine against *Enterococcus faecalis* in clinical conditions.
3. Confirmation that single-visit treatment is a good alternative to the multi-visits one.

Original contributions

1. An in-depth study of pain was performed after treatment of teeth with chronic apical periodontitis
2. It has been proven that with a correctly performed irrigation protocol according to the methodology described by us, the use of a medication dressing does not improve the healing processes.

VIII. PUBLICATIONS AND PARTICIPATION IN SCIENTIFIC FORUMS

Publications

- Denitsa Zaneva-Hristova, Tsvetelina Borisova-Papancheva. “One-step methods of periodontitis treatment-review of the literature”. *Scripta Scientifica Medicinae Dentalis*. 2017/4/27.
- Denitsa Zaneva-Hristova, Application of chlorhexidine in endodontics, Varna Medical Forum, vol. 9, 2020, issue 2
- Denitsa Zaneva-Hristova, Medications for intracanal dressing used in the multi-visits treatment of apical periodontitis, *Scripta Scientifica Medicinae Dentalis*- Vol 6, No 2 (2020)

Participation in scientific forums

- Denitsa Zaneva-Hristova, Tsvetelina Borisova-Papancheva, Slavena Svetlozarova. Flare-ups after endodontic treatment. Faculty of Dental Medicine Varna 29-th Annual Assembly of IMAB; 9 - 12 May 2019.
- Slavena Georgieva, Tsvetelina Borisova-Papancheva, Denitsa Zaneva-Hristova. Irrigation in Endodontics. Faculty of Dental Medicine Varna 27-th Annual Assembly of IMAB; 11 - 14 May 2017.