The Effect of Triple Antibiotic Paste as an Intracanal Medication with an Anti-Inflammatory Drug on Post-Operative Pain of Asymptomatic Uniradicular Necrotic Teeth (A Double Blind Randomized Clinical Trial).

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Protocol

Submitted to the Faculty of Oral and Dental Medicine-Cairo University in partial fulfillment of the requirements for Doctorate Degree in Endodontics

By

Mohamed Omaia Ahmed Salah

B.D.S, October 6 University, 2009

M.S.C, Cairo University, 2015

Department of Endodontics

Faculty of Oral and Dental Medicine

Cairo University

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Section 1: Administrative information

1. Title

The effect of triple antibiotic paste as an intracanal medication with an anti-inflammatory drug on post-operative pain of asymptomatic Uniradicular necrotic teeth: a double blind randomized clinical trial.

2. Trial registration

Trial registration number NCT02907489

Trial registration link https://clinicaltrials.gov/show/NCT02907489

3. Protocol version

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4. Funding

Self-funding.

5. a. Roles and responsibilities – contributors

A) *Prof. Dr. Maged M. Negm* ***(N.M.)***

* Professor of Endodontics, Faculty of Oral and Dental Medicine, Cairo University.

** The senior supervisor.

***Responsible for implementation and allocation concealments.

B) *Dr. Yousra M. Nashaat* ***(N.Y.)***

* Associate Professor of Endodontics, Faculty of Dentistry, October 6 University.

**1st assistant supervisor.

***Primary outcome assessor.

C) *Dr. Nehal Nabil* ***(N.N.)***

*Lecturer in Endodontics, Faculty of Oral and Dental Medicine, Cairo University.

**2nd assistant supervisor.

D) *Dr. Amal Sabry Othman* ***(O.A.)***

* Lecturer in Microbiology, Faculty of Applied Medical Sciences, October 6 University.

** 3rd assistant supervisor.

***Secondary outcome assessor.

E) *Dr. Mohamed Omaia Ahmed Salah* ***(A.M.)***

* Assistant lecturer in Endodontics, Faculty of Dentistry, October 6 University.

** Researcher.

*** Responsible for all clinical procedure in this clinical trial.

5. b. Roles and responsibilities – committees

Department of Endodontics, Faculty of Oral and Dental Medicine, Cairo University.

Section 2: Introduction

6. a. Background and rationale – description of research question and justification for undertaking the trial

Description of research question (PICOTS):

(P) Population:

Patients with asymptomatic uniradicular necrotic teeth treated in multiple visits.

(I) Intervention:

Triple antibiotic paste with an anti-inflammatory drug (diclofenac potassium 50 mg) as intra canal medicaments.

(C) Control / Comparator:

Non setting calcium hydroxide.

(O) Outcome:

	Outcome measure	**Measuring device**	**Measuring unit**
1ry outcome	Pain	Visual Analogue Scale (VAS) ***(Cruz Junior et al. 2016)***	Ordinal
2 ry outcomes	Intracanal bacterial reduction	Bacterial culture	Colony forming units per milliliter of blood agar medium

(T) Time:

Dental treatment will be done at baseline and postoperative evaluation of pain change will be done after 24, 48, and 72 hour from the end of the first visit.

(S) Study design:

A double blind, randomized controlled clinical trial.

Justification for undertaking the trial:

Microorganisms are the main cause for pulp and periapical inflammation and disease. The failure to effectively eliminate them and their by-products might result in persistent irritation and impaired healing.

Earlier researches placed great importance on the bacterial contamination of the root canal as it is a major concern in endodontics. Elimination of microorganisms and necrotic tissue from root canal system is essential for successful outcome of treatment **(Drake et al. 1994).**

Some microorganisms as *E. faecalis* is able to form a biofilm that helps it resist destruction by enabling the bacteria to become a thousand times more resistant to phagocytosis, antibodies, and antimicrobials than nonbiofilm producing organisms **(Distel et al. 2002)**.

It is resistant to inter appointment medicaments including calcium hydroxide and may also reside in canals as single species without the support of other organisms **(Orstavik & Haapasalo 1990) and (Haapasalo & Orstavik 1987).**

It was also reported that this microorganism has the ability under specific conditions to infect the whole length of the tubules within 2 days **(Orstavik & Haapasalo 1990).**

Our challenge as endodontists is to implement methods to eliminate this microorganism during and after root canal treatment.

Systemic antibiotics appear to be clinically effective as an adjunct in certain surgical and nonsurgical endodontic procedures. Their administration is not without the potential risk of adverse systemic effects, such as allergic reactions, toxicity and the development of resistant strains of microbes. In addition, the systemic administration of antibiotics relies on patient compliance with the dosing regimens followed by absorption through the gastro-intestinal tract and distribution via the circulatory system to bring the drug to the infected site. Hence, the infected area requires a normal blood supply which is no longer the case for teeth with necrotic pulps and for teeth without pulp tissue. Therefore, local application of antibiotics within the root canal system may be a more effective mode for delivering the drug **(Gilad et al. 1999).**

A successful endodontic treatment is therefore dependent on the initial killing of all the bacteria, i.e. those present in the root canal as well as those already penetrated in depth. The achievement of microbicidal doses becomes critical in the endodontic environment, because in such harsh conditions bacteria may aggregate to form a biofilm or enter a stationary phase, thus acquiring a resistant phenotype. Accordingly, in endodontic therapy the local use of antibiotics allows the use of the necessary very high concentrations. Moreover, some antibiotics like tetracyclines may represent the optimal choice to grant long-lasting antimicrobial effects, since they readily attach to dentine and are gradually released, retaining their antibacterial activity **(Khademi et al. 2006).**

6. b. Background and rationale – choice of comparator

Calcium hydroxide will be used as a comparator because it has been used widely in endodontics because of its properties such as high alkalinity ***(Tronstad et al. 1981)***, anti-bacterial activity ***(Orstavik et al. 1991; Sjögren et al. 1991; Stuart et al. 1991)*** and the ability to create an appropriate environment that favors hard tissue deposition and apical repair ***(Cvek et al. 1976; Ghose et al. 1987).*** Also It has been noted that general indication of intracanal calcium hydroxide is pulpal necrosis. And several studies have found a direct relationship between level of infection and amount of post operative pain. ***(Eleazer & Eleazer 1998; Trope 1991; Mor et al. 1992; Walton & Fouad 1992)***

As well as it has been suggested that calcium hydroxide has pain preventive properties because of its antimicrobial and tissue altering effects. ***(Walton et al. 2003)***

Furtheremore studies have shown that calcium hydroxide has the ability to dissolve necrotic tissues alone or may increase the effect of other solvents. This suggests that calcium hydroxide would be an effective agent in cleaning and disinfecting infected root canals. ***(Safavi et al. 1985)***

7. Objectives

This trial is aiming to answer a clinical question whether in patients with asymptomatic necrotic teeth, the use of triple antibiotic paste as intracanal medication with an anti-inflammatory drug, compared to a calcium hydroxide, reduce postoperative pain and intracanal bacteria or not?

8. Trial design

Randomized, controlled, double blinded, unicenter, parallel, two-arm, superiority trial with 1:1 allocation ratio.

Section 3.a: Methods-participants, interventions, and outcomes

9. Description of study settings

The trial will be conducted in Endodontic clinic, Faculty of Oral and Dental Medicine, Cairo University, Cairo governorate, Egypt.

- Dental unit that will be used: Adec 200 U.S.A.

- X-ray machine that will be used: ViVi, S.r.I, Italy.

10. Eligibility criteria

a- Inclusion criteria :

1. Subject's age between 18-50 years.

   [To study the effects of intracanal medication on permanent teeth; above 18 years to ensure complete root formation to avoid false negative response of electric pulp testing as well as below 50 years to avoid possibility of root canal calcification with false negative response] ***(O'Keefe 1976, Watkins et al. 2002, DiRenzo et al. 2002, Siqueira et al. 2002, Harrison et al. 1981)***

2. Both male and female subjects.

   [To study the effect of different variables on the outcome] ***(Torabinejad et al. 1988; Walton & Fouad 1992; Genet et al. 1987)***

3. Medically free and healthy subjects.

4. Mandibular and maxillary single rooted teeth.

[Because studies have suggested that postoperative pain is more likely to occur in multirooted teeth] ***(Ng et al. 2004; Clem 1970)***

5. Asymptomatic non vital teeth.

   *[**Non vital** because researchers believe that post-operative pain in teeth with non-vital pulp are more common than teeth with vital pulp due to presence of high level of irritants and infectious agents in pulpal and periapical region].* ***(Albashaireh & Alnegrish 1998; Clem 1970; Soltanoff 1978)***

   [Asymptomatic *because studies have shown that the presence of preoperative pain can significantly increase the possibility of postoperative pain]* ***(O'Keefe 1976; Genet et al. 1987; Imura & Zuolo 1995; Torabinejad et al. 1988)***

b- Exclusion Criteria:

1. Teeth with acute dentoalveolar abscess.

   [Because it was felt that emeregency management should include incision and drainage.] ***(Ehrmann et al. 2003)***

2. Subjects having more than one tooth that require root canal treatment.

   [To eliminate the possibility of pain referral and false results]

3. Subjects that have taken analgesic, anti-inflammatory or antibiotic drugs during the 10 days prior to the start of treatment.

   [Because it would alter pain perception by the patients]

4. Pregnant females.

5. Subjects with systemic diseases who have endocrine diseases, Infectious diseases or Psychological disturbance.
6. Teeth with periodontal disease or pulp calcification.
7. Subjects taking chronic pain medications.

   [Because it would alter pain perception by the patients]

11. a. Interventions

1. The subjects will be selected from the regular attendees of Endodontic clinic, Faculty of Oral and Dental Medicine, Cairo University.
2. A written announcement will be hold in Endodontic clinic, Faculty of Oral and Dental Medicine, Cairo University to inform the attendees regarding the trial.
3. Patient examination will be done including medical history, dental history, extra-oral and intra-oral examination to evaluate the patient's health condition and data will be recorded in a diagnostic chart *(Appendix no. 1)*.
4. All subjects fulfilling eligibility criteria will give an informed consent for participation *(Appendix no. 3)*.
5. Preoperative intraoral periapical radiograph will be taken. *(Kodak intraoral periapical films speed D, KODAK)*
6. The teeth will be isolated with cotton rolls, electrical pulp testing will be performed using electric pulp tester that will be positioned at the middle third of the buccal surface of the tooth to check pulp vitality.
7. Tooth will be anaesthetized by local anesthesia with a non-disposable metallic aspirating syringe using 1.8 ml Mepivacaine HCl 2% - Levonordefrin 1:20000

(Carpule Mepecaine-L, Alexandria Company for Pharmaceuticals and Chemical Industries, Egypt).

8. An access cavity preparation will be performed using round bur size 3 and endo-Z bur for deroofing under a continuous irrigation with sterile physiologic saline *(El Nasr Pharmaceutical Chemicals CO., Abu Zaabal, Egypt)*.
9. Rubber dam will be placed.
10. The patency of the canals will be checked using size 15 K-files *(MANI, INC., Tochigi, Japan).*
11. Working length will be determined using an electronic apex locator *(Root ZX, J.Morita USA, Irvine, CA)* at the"0.5" mark then will be confirmed with intraoral periapical radiograph, to be 0.5-1 mm, shorter than radiographic apex. *(Kodak intraoral periapical films speed D, KODAK)*
12. The canals will be instrumented to a size 25 K-type file *(MANI, INC., Tochigi, Japan)* without irrigation.
13. The first pre-chemomechanical bacteriological sample (S1) will be acquired. Each root canal will be filled with sterile distilled water using a sterile plastic syringe. Then a sterile paper point size 25 will be placed in the canal for 1 minute. The paper point will be immediately transferred into test tube with thioglycolate.
14. **Laboratory Procedures:** Samples will be immediately processed in the laboratory inside an anaerobic chamber (85% N_2, 10% H_2 and 5% CO2) *(Coy Laboratory Products, Grass Lake, MI, USA).* The entire 4-mL sample will be vortexed for 1 minute. An aliquot of 1 mL will then be diluted in enriched thioglycolate broth using 10-fold serial dilutions until 10^{-4}. Each diluted sample will be inoculated on a 5% sheep blood CDC anaerobic agar plate with vitamin K and hemin *(BBL Becton Dickinson de Mexico)* and will be

incubated at 37°C for 7 days. Colony-forming units (CFU) for each strain of bacteria will be counted using pour plate method. The remaining 3 mL of each sample will be incubated under anaerobic conditions. After 7 days, the tubes will be assessed for turbidity and digital images of each tube will be captured to detect bacterial growth. ***(Manzur et al. 2007)***

15. Preparation of teeth will be performed using rotary ProTaper Universal instruments *(Dentsply Maillefer, TN, USA)* in an endodontic motor and reducing hand piece *(X-Smart, Dentsply Maillefer, USA)*, according to the manufacturer instructions.

The following sequence will be used for all groups:

- S1 file will be used to prepare two thirds of estimated working length.
- SX file will be used to prepare two thirds of estimated working length.
- S1 file will used to full working length.
- S2 file will used to full working length.
- F1 file will used to full working length.
- F3 file will used to full working length.
- F4 file will used to full working length.
- F5 file will used to full working length.

16. The canals will be thoroughly irrigated using 2ml of sterile physiologic saline *(El Nasr Pharmaceutical Chemicals CO., Abu Zaabal, Egypt)* as an irrigant between each instrument and the other.
17. The second bacteriological sample (S2) (Post-chemomechanical) will be acquired. Each root canal will be filled with sterile distilled water using a

sterile plastic syringe. Then a sterile paper point matching to the apical enlargement size will be placed in the canal for 1 minute. The paper point will be immediately transferred into test tube with thioglycolate.

18. Laboratory procedures will be the same like that done in the first bacteriological sample. ***(Manzur et al. 2007)***
19. At this stage **(N.M.)** will randomly assign subjects with a 1:1 allocation ratio into the following two intracanal medication groups using computer software, (Microsoft Excel).

1. **<u>Group 1: (control)</u>**

   The canal will be filled with calcium hydroxide (Metapex) *(META BIOMED Co, Chungbuk, Korea)* after finishing mechanical preparation which will be applied through disposable tips supplied by the manufacturer.

2. **<u>Group 2:</u>**

   The canal will be filled with triple antibiotic paste after mixing equal amounts of it with anti-inflammatory drug diclofenac potassium 50 mg (Catafast) *(NOVARTIS PHARMA S.A.E., Cairo, Egypt)* after finishing mechanical preparation which will be applied using K-type hand file through counter clockwise motion.

Material	Specification	Composition	Manufacturer
Metapex	Non setting Calcium Hydroxide with Iodoform	Calcium Hydroxide, Iodoform and Silicon oil.	META BIOMED Co, Chungbuk, Korea. [270 Osongsaengmyeong1-ro, Osong-eup, Heungdeok-gu, Cheongju-si, Chungbuk, Korea

			/ Tel :043-218-1981] http://www.meta-biomed.com
Triple antibiotic paste (TAP)	Mixture of ciprofloxacin, metronidazole and minocycline.	Using commercially available tablets of Ciprofloxacin (Ciprofloxacin 500 mg), Metronidazole (Flagyl 500 mg) and Minocycline (Minocin 50 mg). Following the removal of the enteric coating of the tablets, the contents were ground using a mortar and pestle and mixed in an equal amounts by weight (1:1:1) in a mixing pad (100 mg of each) and then will be dissolved in 100 mL of sterile water to prepare 1 mg/mL solution of TAP ***(Takushige et al. 2004)***	Flagyl 500 mg: (Sanofi Aventis, Cairo,Egypt) 3, El Massaneh St. Zietoun, Cairo, Egypt Tel.: +202 22860000 22860060/1/2 www.sanofi.com Ciprofloxacin 500 mg: (Amriya pharm, Alexandria, Egypt) Alexandria-Cairo Desert Rd. Km 25, Amriya, Alexandria, Egypt. Tel.: +20 (3) 470-1001 / 470-1151 / 470-1146 http://amriyapharm.com/ Minocin 50 mg: (Sedico, Giza, Egypt) 1st. industrial zone,6th of October City, Giza, Egypt Tel: +202-38200575/78/90 http://www.sedico.net
Catafast	50 mg NSAIDs granules for oral solution	Every sachet contains 50 mg diclofenac potassium, Potassium hydrogen carbonate, mannitol;	NOVARTIS PHARMA S.A.E., Cairo, Egypt *[El Sawah St. – EL Amiria, Cairo, Egypt]*

	(Diclofenac potassium)	aspartame, saccharin sodium, glyceryl dibehenate, mint flavor, anise flavor.	*Tel: +20 2 24567200* *https://www.novartis.com.eg/*

20. A cotton pellet will be placed in the pulp chamber and the access cavity will be sealed with a temporary filling Orafil-G *(Prevest Denpro, Digiana, India)*.
21. Postoperative pain will be assessed with a visual analog scale at 24 hours, 48 hours, and 72 hours after the procedures. The VAS consisted of a 100 mm horizontal ruler with no numbers except a 0 at the first part of the scale and a 10 in the last part of the scale. The patients will be asked to mark the point that was equivalent to their pain perception with 0 indicating no pain and 10 indicating extreme pain.
22. The pain levels will be classified as no pain (0), mild pain (1-3), moderate pain (4-7) or severe pain (8-10). Additionally the need for analgesics will also recorded by the patients on their cards *(Appendix no. 2)*.

23. After 72 hour the patient will return for the second visit in which the intracanal medication will be removed by irrigation using sterile physiologic saline *(El Nasr Pharmaceutical Chemicals CO., Abu Zaabal, Egypt)*.
24. The final bacteriological sample (S3) will be acquired. Each root canal will be filled with sterile distilled water using a sterile plastic syringe. Then a sterile paper point matching to the apical enlargement size will be placed in the canal for 1 minute. The paper point will be immediately transferred into test tube with thioglycolate.
25. Laboratory procedures will be the same like that done in the first bacteriological sample. ***(Manzur et al. 2007)***
26. Periapical radiograph *(Kodak intraoral periapical films speed D, KODAK)* will be taken with the master gutta percha ProTaper cone #F5 *(Dentsply Maillefer, TN, USA)* for master cone verification.
27. Obturation will be done in the 2nd visit by single cone technique using ProTaper gutta percha points size F5 *(Dentsply Maillefer, TN, USA)* and ADseal resin sealer *(META BIOMED Co, Chungbuk, Korea)*.
28. A cotton pellet will be placed in the pulp chamber and the access cavity will be sealed with a temporary filling Orafil-G *(Prevest Denpro, Digiana, India)*.
29. Postoperative intraoral periapical radiograph *(Kodak intraoral periapical films speed D, KODAK)* will be taken.

11. b. Interventions-modifications

No protocol for discontinuation of the procedure.

11. c. Interventions-adherence

Face to face adherence session during the first visit will take place to stress on the way and the importance of filling the pain diary chart.

As well as phone calls will be given to the subjects to stress on the importance of pain diary chart and to remind them with it.

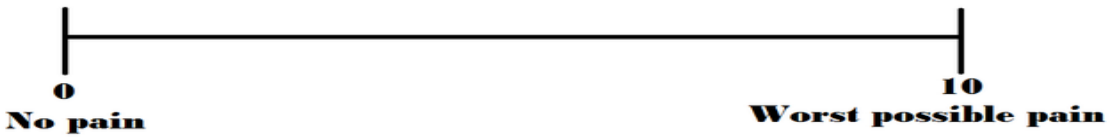
12. Outcomes

1ry outcome: Post-operative pain change.

Measuring device: Visual analogue scale ***(Cruz Junior et al. 2016).***

Measuring unit: Ordinal.

VAS consisted of a 100 mm horizontal ruler with no numbers except a 0 at the first part of the scale and a 10 in the last part of the scale.



0	No pain.
1-3	Mild pain.
4-7	Moderate pain.
8-10	Severe pain.

2ry outcome: Intracanal bacterial reduction of each strain of bacteria.

Measuring device: Bacterial culture. ***(Manzur et al. 2007)***

Measuring unit: Colony forming units per milliliter of blood agar medium.

13. Participant timeline

Activity	-1 Week	Before preparation	After preparation	After (24 hour)	After (48 hour)	After (72 hour)
Allocation	√					
Randomization			√			
Post-operative pain assessment				√	√	√
Intracanal bacterial reduction assessment		√ **S1**	√ **S2**			√ **S3**

14. Sample size

The aim of this study is to assess the effect of triple antibiotic paste as an intracanal medication with an anti-inflammatory drug on post-operative pain of asymptomatic uniradicular necrotic teeth. Prior data by Johns et al., 2014 indicated that the probability of success among conventional is 0.35 (successful treatment based on strict criteria was defined as absence of pain). If the true probability of success among intervention group is 0.65, we will need to study 42 case patients and 42 control patients to be able to reject the null hypothesis that the exposure rates for case and controls are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. We will use an uncorrected

chi-squared statistic to evaluate this null hypothesis. The sample size was calculated by G power program.

15. Recruitment

The subjects will be recruited from the regular attendees of Endodontic clinic, Faculty of Oral and Dental Medicine, Cairo University to meet the target sample size.

Section 3.b: Methods-assignment of interventions

16. a. Allocation-sequence generation

(N.M.) will randomly divide participants into two groups with a 1:1 allocation ratio using computer software, (Microsoft Excel).

16. b. Allocation-concealment mechanism

*All subjects who give consent for participation and fulfil the eligibility criteria will be randomized.

**Number for each member in each group will be written by indispensable pen on large white paper sheet. The sheet will be folded eight times and saved inside opaque well sealed envelope.

16. c. Allocation-implementation

(N.M.) will be responsible for allocation sequence generation and dividing patients into three groups.

17. Blinding

The study will be double-blind in which the patient will not know which intracanal medication he /she will receive, and the assessor will be blinded to the intervention and the comparator applied on the patients, Only the operator knows the type of the intracanal medication used.

N.Y. will be responsible for primary outcome assessing (post-operative pain outcome), while **O.A.** will be responsible for secondary outcome assessing (intracanal bacterial reduction outcome).

Both the pain diary and microbiology sheet will not contain name of the patients or the type of intervention used to ensure blinding and to avoid bias.

Section 3.c: Methods-data collection, management, and analysis

18. a. Data collection method

The operator will obtain baseline data through a paper based case report form for diagnostic data, medical and dental history. *(Appendix no. 1)*

The obtained data for postoperative pain assessment will be recorded in pain diary by the patient. *(Appendix no. 2)*

18. b. Data collection method-retention

*Primary outcome will be assessed by the 1st assistant supervisor **(N.Y.)** through pain diary of visual analogue scale *(Appendix no. 2)* which will be filled by the subjects at 24, 48 and 72 hour after 1st visit to be delivered to the operator at the 2nd visit.

**All subjects will be given a phone call before the 2nd visit not to forget the pain diary.

***Secondary outcome will be assessed by the 2nd assistant supervisor (**O.A.)** through microbiological analysis of the paper point taken before (S1) and after mechanical preparation of the tooth (before placement of the intracanal medication) (S2) as well as after removal of the intracanal medication (after 72 hours) (S3) and will be done in Microbiology laboratory of Faculty of Dentistry of October Six University and then data will be recorded in appendix no. 4.

19. Data management

*All data will be entered electronically.

**Subjects files will be stored in numerical order and stored in secure and accessible place and will be maintained in storage till completion of the study.

20. Statistical methods

Data will be analyzed using IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 21 (SPSS Inc., Chicago, IL). Numerical data will be described as mean and standard deviation or median and range. Categorical data will be described as numbers and percentages. Data will be explored for normality using Kolmogrov-Smirnov test and Shapiro-Wilk test. Comparisons between two groups for normally distributed numeric variables will be done using the Student's t-test while for non-normally distributed numeric variables will be done by Mann-Whitney test. Comparisons between categorical variables will be performed using the chi square test. A p-value less than or equal to 0.05 will be considered statistically significant. All tests will be two tailed.

Section 3.d: Methods-monitoring

21. Data monitoring-formal committee

There is no data monitoring committee for this trial due to minimal risks and short duration of the trial.

22. Harms

Adverse effects are minimal or rare. However, if any harm is seen in the subjects both in intervention or control groups it will be recorded, reported and documented at the end of the trial.

23. Auditing

Auditing of the study design will be done by the Evidence Based Committee – Faculty of Oral and Dental Medicine – Cairo University. The audits will review the processes of participant enrolment, consent, eligibility, allocation to study groups and adherence to trial interventions and policies to protect participants.

Section 4. Ethics and dissemination

24. Research ethics approval

This protocol and the template informed consent form *(Appendix no. 3)* will be reviewed and approved by the Ethics Committee of Scientific Research - Faculty of Oral and Dental Medicine – Cairo University.

25. Protocol amendment

Any modifications to the protocol which may impact the conduct of the study, potential benefit of the patient or may affect patient safety, including changes of study objectives, study design, sample sizes or study procedures will require a formal amendment to the protocol. Such amendment will be agreed upon by the Ethics Committee of Scientific Research and the council of Endodontic department.

26. Consent

Researcher will discuss the trial with all subjects. Subjects will then be able to have an informed discussion with the researcher. The Researcher will obtain written consent from all subjects willing to participate in the trial. All information sheets and consent forms have been translated into Arabic. *(Appendix no. 3)*.

27. Confidentially

All study-related information will be stored securely. All participant information will be stored in locked file cabinets in areas with limited access. All laboratory specimens, reports, data collection, process, and administrative forms will be identified by a coded file and each file containing patient's database including name, age, sex, identification number, address, phone number, work address and any other personal identifiers and won't be released outside the study without written permission of the participant.

28. Declaration of interests

There is no conflict of interests.

29. Access to data

All principal contributors will be given access to the data sets. All data sets will be password protected. To ensure confidentiality, data dispersed to project team members will be blinded of any identifying participant information.

30. Ancillary and post-trial care

All patients will receive postoperative instructions.

Post-trial care will include referral of the subjects to Operative or Fixed Prosthodontics clinic in Faculty of Oral and Dental Medicine Cairo University for fixed restoration placement after completion of the root canal treatment.

31. Dissemination policy

Study results will be published internationally as partial fulfillment of the requirements for PhD degree in Endodontics.

32. Appendices

Appendix no. 1: Diagnostic chart.

Appendix no. 2: Pain Diary.

Appendix no. 3: Patient consent "in Arabic"

Appendix no. 4: Microbiology Sheet.

Diagnostic Chart

Chart No:

Patient No:

Date:

Dr Name:

Personal information

Patient name:

Gender:

Patient age:

Marital status:

Phone no.:

Address:

Occupation:

Medical history

	YES	NO
Hypertension		
Hypotension		
Diabetes		
Anemia		
Cardiac disease		
Bone disease		
Liver disease		
Kidney disease		
Sexual disease		
Hereditary disease		
Past surgeries		
Epileptic		

Others.----------

Past dental history

Extraction _____

Endodontic treatment_____

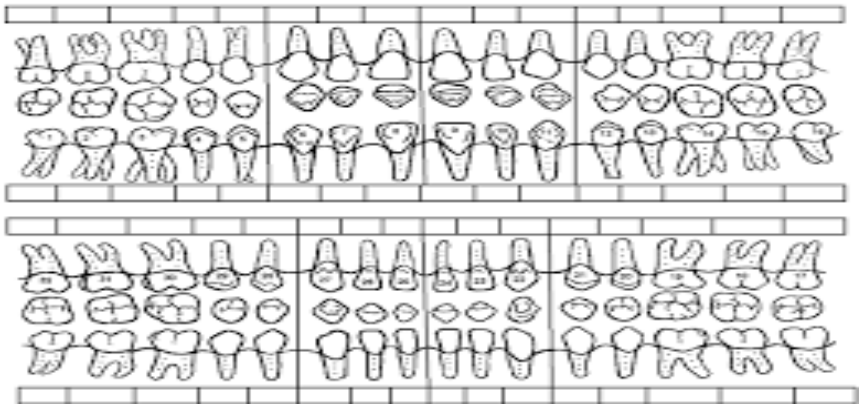
Filling (f): _____

Orthodontic treatment_____

Prosthetic treatments _____

Periodontal treatment _____

Surgical treatment _____



Extra oral examination

Intra oral examination

Patient chief complain----------

Pain Scale Chart

Operator name: …… Operator telephone number: …….

Patient number: ……. Age: …….. Sex: ……

Please put mark in the following scale to indicate severity of the pain with zero indicating no pain and 10 indicating severe pain.

(1) Patient training:

(2) **After 24 hours**

(Tommorow)

(3) **After 48 hours (after 2 days)**

(4) **After 72 hours (after 3 days)**

- **Did you need to take analgesic? .....**
- **When you took the first tablet of the analgesic? .....**
- **Date: / /**
- **Patient Signature: ……..**

Microbiology Sheet

Operator name: …… Operator telephone number: …….

Patient number: ……. Age: …….. Sex: ……

Type of bacteria	Bacterial count in S1	Bacterial count in S2	Bacterial count in S3
1.			
2.			
3.			
4.			
5.			
6.			
7.			

- **Date: / /**
- **Supervisor Signature: ……..**

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