A CLINICAL, RADIOLOGICAL AND MICROBIOLOGICAL EVALUATION OF ROOT-FILLED TEETH WITH POST-TREATMENT DISEASE IN A KENYAN POPULATION

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DECLARATION

I declare that this thesis is my original work and has not been presented for the award of a degree in any other university.

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DEDICATION

I dedicate this thesis to Maria, Levania, Lacey, Rehema and Franklin Jnr.

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I thank God for granting me strength and good health throughout this period.

To my dearest parents, Peter Nyaata and Eunice Kemunto, I cannot thank you enough for all the support and encouragement you have given me throughout this journey.

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ACRONYMS

Actino. radici.	*Actinomyces radicidentis*
bp	*base pairs*
Cl	*Confidence level*
C.albicans	*Candida albicans*
E. feacalis	*Enterococcus feacalis*
F. nucleatum	*Fusobacterium nucleatum*
DNA	*Deoxyribonucleic acid*
Fig.	*Figure*
GP	*Gutta percha*
KNH	*Kenyatta National Hospital*
NaOCl	*Sodium hypochlorite*
PCR	*Polymerase Chain Reaction*
PTD	*Post-treatment disease*
SPSS	*Statistical Package for Social Sciences*
Strep. spp.	*Streptococcus species*
TE	*Tris-HCL, Ethylenediamine Tetraacetic acid*
UNSDS	*University of Nairobi, School of Dental Sciences*
UV	*Ultra violet*
Porph. endo.	*Porphyromonas endodontalis*
P. gingivalis	*Porphyromonas gingivalis*
P. intermedia	*Prevotella intermedia*
P. micros	*Peptostreptococcus micros*
P. nigrescens	*Prevotella nigrescens*

DEFINITION OF TERMS

TERMS	DEFINITION
Endodontic treatment:	Cleaning, shaping, disinfection and filling of the pulpal space
Post-treatment disease:	Pain, discomfort, swelling, sinus tract and/or persistent radiolucent lesion associated with a root-filled tooth
Microorganisms:	Bacteria or fungi

ABSTRACT

Background: A number of studies on root-treated teeth in various populations have shown a prevalence rate of post-treatment disease of between 15 to 56.1%, with microbial infection being the main cause.

Objective: The aim of this study was to identify microorganisms isolated from root-treated teeth with post-treatment disease, as well as clinical and radiological features associated with such teeth, in two institutions providing dental health care in Nairobi.

Study design: This was a descriptive cross-sectional study.

Subjects and Methodology: Forty five patients presenting with post-treatment disease at the UNSDS and KNH participated in the study among whom thirty eight had microbial sampling carried out. The dental institutions were selected through convenient sampling. All the patients who presented to these institutions and satisfied the inclusion criteria during the period of study were included in the sample. An interviewer-administered semi-structured questionnaire was used to collect demographic data, the presenting complaint and the date when the endodontic treatment was carried out. Intra-oral clinical examination was done to identify the affected tooth, assess signs and symptoms of post-treatment disease and the state of the coronal restoration. Intra-oral periapical views were taken using the paralleling technique. The radiographs were used to assess the integrity of the coronal restoration, presence of periradicular radiolucency, missed canals and the quality and extent of the root filling. Microbial sample collection was carried out using strict asepsis. After removal of the root canal filling, intra-canal swabs were taken and microorganisms were identified using PCR. The data obtained were analysed using the Statistical Package for Social Sciences (SPSS) programme.

Results: Thirty one patients (68.9%) presented with pain as the only complaint, six(13.3%) had pain and swelling, one(2.6%) had pain, swelling and a sinus tract, one(2.6%) had a sinus tract while six(13.3%) had no symptoms. Posterior teeth with post-treatment disease were found to have been more thirty two(71.1%) than anterior teeth. Forty(88.9%) of the filled canals had associated periradicular radiolucency, twenty four(53.3%) had root filling short of the apex by more than 2mm, while fifteen(33.3%) had voids within the root filling. Microorganisms were present in all the samples collected. The most frequently detected microbes were *Porphyromonas gingivalis, Prevotella nigrescens* and *Candida albicans.* There was no association between post-treatment disease and the quality of root filling or any of the microorganisms.

Conclusion: Pain is the most common symptom in patients with post treatment disease. Most of the teeth evaluated had periradicular radiolucency and inadequate root-fillings. Although microorganisms were identified in all the teeth investigated, only *P. Intermedia* was found to have a statistically significant association with pain and swelling.

Recommendations: There is need to improve on the technical aspects of endodontic treatment in the various dental institutions. More studies are needed to determine whether there is indeed an association between post-treatment disease and the quality of root filling as well as various intra-canal microorganisms.

CHAPTER ONE

1.1: Introduction

Post-treatment disease refers to the presence of signs and symptoms of infection and/or the appearance of a periradicular radiolucency following root canal treatment[1]. The term also refers to cases where the lesion was present pre-operatively and has remained the same in size or reduced slightly over a four-year assessment period. The disease may, therefore, present clinically as pain, swelling, discomfort and presence of a sinus tract. Radiographically, root resorption or enlargement of a previous periradicular radiolucency associated with the root filled tooth may be noted.[1] Diagnosis of this condition, therefore, requires both clinical and radiographic examination.[2]

Endodontic treatment aims at removing all organic debris and microorganisms from the root canal system followed by obturation of the main and any auxillary canal(s). Ideally, following endodontic treatment, the tooth should be asymptomatic. However, several studies have reported unfavourable outcome after endodontic treatment. A study conducted by Gulabivala and others[3] reported unfavourable outcome of between 15% to 32%. A retrospective study in Hong Kong by Chan and others[4] reported a rate of 52% unfavourable outcome within 18 months post treatment. Lumley and others[5] reported 74% survival of root filled teeth with no re-intervention, ten years after treatment. In that study, unfavourable endodontic treatment outcome was defined as re-treatment, apical surgery or extraction of a root filled tooth. A study conducted by Toure and

others[6] reported unfavourable endodontic treatment outcome in 56.1% of previously treated roots.

Several causes of unfavourable endodontic treatment outcome have been reported in the literature. According to Roda and Gettleman[7] iatrogenic procedural errors, such as, poor access cavity design, canals that were inadequately cleaned and obturated, complications following instrumentation and apical transportation were possible causes of unfavourable endodontic treatment outcome. In addition, coronal leakage, missed canals, foreign body reaction and cysts have also been implicated. Iatrogenic errors may result in the re-introduction of intraradicular microorganisms or persistence of the remaining ones.[7,8]

Microbial infection remains the main cause of unfavourable outcome following endodontic treatment.[9,10] *Enterococcus feacalis* has been implicated as the most common microorganism associated with post-treatment disease in endodontically treated teeth.[11,12] Other studies have reported Streptococcus, Lactobacillus, Eubacterium, Dialister and Prevotella species as the predominant microorganisms in root-filled teeth with unfavourable outcome.[13] Studies by Gomes and others,[12,14] associated certain microbes, including Prevotella, Porphyromonas, Streptococcus and Peptostreptococcus species with specific endodontic signs and symptoms. The clinical features assessed in those studies were pain, tenderness to percussion, the presence of a sinus tract and swelling.

1.2: Literature Review

1.2.1: Tooth Structure

The structural anatomy of a tooth comprises enamel, dentine, pulp and cementum.[15,16] Enamel covers the anatomic crown while cementum covers the anatomic root. Dentine forms the largest part of the tooth structure, extending almost the full length of the tooth. Externally, dentine is covered by enamel on the anatomic crown, and cementum on the anatomic root.[15,16] Internally, dentine forms the walls of the pulp cavity in which lies the dental pulp. Anatomically, the dental pulp is divided into coronal pulp and radicular pulp. The radicular pulp is continuous with the periapical tissues through the apical foramen.[16] Accessory canals may extend from the pulp canals laterally through the root dentine to the periodontal tissues.[16] Studies have reported multiple foramina, additional canals, fins, deltas, intercanal connections, loops, C-shaped canals and accessory canals.[17,18,19,20]

1.2.2: Diseased Tooth

In an intact tooth, enamel and dentine enclose the pulpal space and protect it against invasion by bacteria.[20] Dentine usually consists of dentinal tubules which extend across its entire width.[16] Exposed tubules may serve as pathways for microbial invasion of the pulp space.[21] Microorganisms reach the dental pulp through openings in the dental hard tissue wall as a result of caries, clinical procedures or trauma-induced fractures and cracks.[22] When disease involves only the enamel and dentine, placement of a simple restoration may serve to restore the function of the affected tooth.[20,22] However, where the disease

pathology extends to involve the pulp, treatment may either be in the form of extraction or endodontic therapy.[20,23]

1.2.3: Endodontic Treatment

Endodontic treatment is usually indicated in all teeth with pulpal or periradicular pathology. Elective endodontic therapy may also be done in some teeth[1]. The treatment aims at removing all organic debris and the bacterial biofilm colonizing the complex internal root canal surface, followed by obturation of the main canals.[1,23]

In order to facilitate unobstructed instrumentation and obturation of the root canal(s), it is important to prepare a good access cavity.[24] This ensures complete removal of the coronal pulp as well as location and accessibility of the root canals. Cleaning, shaping and disinfection of the root canal space may be achieved by the use of hand or rotary instruments and an irrigant solution.[25] Hand instruments include barbed broaches, K-type and H-type instruments, while rotary instruments include low-speed and engine-driven instruments. Low-speed instruments are Gates-Glidden burs and Peeso reamers, while engine-driven instruments include Hero 624, ProFile and ProFile GT systems.[26] The irrigant solution used should preferably have disinfectant and organic debris dissolving properties.[1] A number of studies have reported sodium hypochlorite to be the most effective canal irrigant[27,28] and non-setting calcium hydroxide as the most effective intra-canal medicament.[29]

Pitt Ford and others[1] stated that following adequate chemo-mechanical preparation, the root canal space should be filled using a suitable root filling

material. The objective of obturation is to prevent the passage of microorganisms and fluid along the root canal by filling the entire root canal system. Filling of this space may be done via lateral or vertical condensation of gutta percha or resin points. A sealer cement should also be used to provide a lateral seal. The authors stated further that a well filled root canal system should not have any voids within the filling material and also between the filling material and the canal wall. In addition, there should be no canal space visible beyond the end-point of the root canal filling material.[1] Following completion of root canal treatment, the tooth should be adequately restored using materials that bind to the tooth such as resin composites or crowns to prevent bacterial contamination of the canal system.[1]

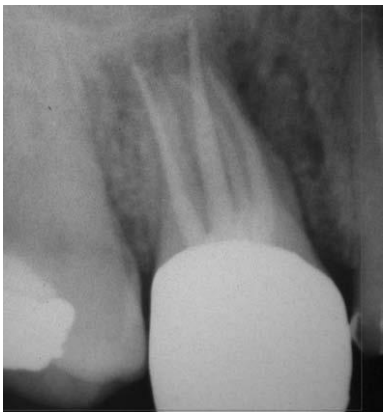

Fig.1.1. A radiograph of a well obturated upper molar[30]

1.2.4: Post-treatment Endodontic Disease

Ideally, following endodontic treatment, the tooth should be asymptomatic with no associated sinus tract, no loss of function and with evidence of a normal periodontal ligament space around the root.[1] However, studies have reported unfavourable outcome after treatment. A retrospective study conducted in Hong Kong by Chan *et al*[4] reported an unfavourable outcome in 52% of the cases

within the first 18 months after treatment. A study by Lumley and others[5] reported 74% survival rate of root filled teeth with no re-intervention ten years after treatment. In that study, unfavourable endodontic treatment outcome was defined as re-treatment, apical surgery or extraction of a root filled tooth. A study conducted in Dakar, Senegal,[6] reported presence of periapical lesions in 56.1% of filled roots. In that study, the technical quality of the root filling was radiologically determined. The criteria used to determine whether or not obturation had been done well, was one described by Pitt Ford *et al.*[1] The authors of the study noted that poorly filled roots were associated with more periapical lesions than those that were adequately filled.

Several factors predispose to unfavourable endodontic treatment outcome.[7,8] These include poor access cavity design, canals that are inadequately cleaned and obturated, complications arising from instrumentation as well as apical transportation. Coronal leakage, missed canals and iatrogenic errors have also been implicated as predisposing factors in post-treatment disease.[7] However, most of these situations are secondary factors which facilitate microbial infection.[31] In addition, foreign body reactions and true cysts may contribute to the persistence of disease after endodontic treatment.[7,8,31]

Studies have reported microbial infection as the main aetiological factor in post-treatment disease.[4,9,10,32] Microorganisms which have been identified from root filled teeth with post-treatment disease include, Streptococcus and Lactobacillus species, *Dialister invisus, Eubacterium infirmum, Prevotella intermedia*, *Selenomonas sputigena*, *Synergistes* oral clone BA121, *Treponema denticola, Enterococcus faecalis,* Actinomyces species and *Bacteroides gracilis.*

Enterococcus faecalis has been reported as the most commonly isolated microorganism.[2,14,33] Gomes and others[2,14] found an association between clinical features and the microorganisms within the canals of root filled teeth. The main culprits identified were, Prevotella species (especially *P.intermedia, P.nigrescens)*, Fusobacterium, Peptostreptococcus, Streptococcus and Actinomyces species.

1.2.5: Methods of Clinical and Radiological Identification of Post-treatment Disease

Root canal treatment should be assessed at least one year after treatment and subsequently as required. Clinically, the presence of pain, swelling, sinus tract and loss of function indicate an unfavourable treatment outcome. Radiographically, the treatment is termed unfavourable if a periradicular lesion has appeared after the treatment or a pre-existing lesion has increased in size or only reduced slightly in size over a four year assessment period, or signs of continuing root resorption are present.[1]

Radiological methods of identification of post-treatment disease

Various radiographic techniques may be used in the diagnosis of post-treatment disease, such as panoramic radiography, intra-oral periapical radiographs, computed tomography and cone beam computed tomography, magnetic resonance imaging and ultrasound.[34,35]

Intra-oral periapical radiographs remain the most frequently used mode of radiography during root canal treatment. Images obtained from such radiographs provide useful information for the presence and location of periradicular lesions,

root canal anatomy and proximity to the root of adjacent anatomical structures. This technique, however, yields limited information since they are two-dimensional and may suffer geometric distortion in areas where the anatomy does not comfortably accommodate the image receptor.[35]

Computed tomography and cone-beam computed tomography have the advantage of providing three-dimensional images. Whereas tuned aperture computed tomography produces images with less anatomical noise than conventional radiographic techniques, it is still only a research tool and has mostly been evaluated *in-vitro*. In addition, cone-beam computed tomography yields superior images at a lower radiation dose than conventional computed tomography. However, cone-beam computed tomographic images may be affected by scatter and beam hardening causes by high density neighbouring structures such as metal posts and restorations.[33,36]

Magnetic resonance imaging has poor resolution and long scanning times compared with simple radiographs in addition to the high hardware costs. Ultrasound is blocked by bone and is therefore useful where there is little or no overlying cortical bone. In addition, positioning the probe when imaging posterior teeth is a challenge.[35]

1.2.6: Methods of Microbial Identification

Over the years, various methods have been used to identify microorganisms isolated from infected root canals. Cultivation of the microorganisms using artificial growth media has been carried out over the past century[37,38]. This method relied on the isolation, growth and laboratory identification by morphology

as well as biochemical tests. A review by Siqueira *et al*[37,38] noted the advantages and limitations of this method. The advantages included the possibility of identifying a great variety of microbial species in a sample, determining antimicrobial susceptibilities of the isolates as well as studying their physiology and pathogenicity. However, the method had limitations such as high cost, prolonged duration in identification of some fastidious anaerobic bacteria and low sensitivity (particularly for fastidious anaerobic bacteria). In addition to the limitations above, the method had low specificity which depended on the experience of the microbiologist, needed a special transport medium, was time consuming and laborious.

Immunological methods have also been used to identify microorganisms associated with endodontic infections.[37,38] Such methods are based on specificity of antigen-antibody reactions. As with any other method, immunological methods have benefits and limitations.[38,37] The advantages as stated by Siqueira and others[37,38] included a shorter time in identifying microorganisms, ability to identify dead microbes, easy standardization and low cost of materials. However, they are limited due to the fact that only target species can be detected, low sensitivity (about 10^4 cells) and specificity that depends on antibodies used.[37,38]

In the recent past, molecular genetic methods have been used to identify microorganisms associated with endodontic infections.[37,38] Identification using this method involves the amplification of the microbial DNA, cloning and sequencing. It is advantageous in that it facilitates the detection of cultivable as well as uncultivable microbial species.[37,38] Siqueira and others[37] state that the number of recognized bacterial phyla has exploded from the original estimate of

11 in 1987 to 36 in 1998 and to 52 by 2005. Identification using molecular genetic methods is highly specific and accurate. The method also allows detection of microbial species directly from the clinical sample without the need for cultivation. The technique is also very sensitive, fast, does not require carefully controlled anaerobic conditions during sampling and transportation of the specimens and allows for storage of samples for analysis at a later date. Limitations of this technique include high cost, laborious procedure, and ability of PCR assays to detect only target species.[37,38]

The aim of this study was to assess the clinical and radiological presentation of root-treated teeth with post-treatment disease in a Kenyan population. In addition, microorganisms isolated from such teeth were also identified using polymerase chain reaction (PCR) technique.

1.3: Statement of the Problem

Inspite of radiographically adequate root filling and coronal restoration, some patients still present with post-treatment disease. This may present as pain, swelling, sinus tract and/or the presence of a periapical radiolucency associated with the affected tooth. This post-treatment disease is attributed to microorganisms remaining in the root canal space which then multiply to a level that ultimately cause symptoms. In view of the high financial cost of endodontic treatment and the time spent on carrying out the procedure, it is essential to investigate the microorganisms capable of persisting within the root canal space and which may cause post-treatment disease.

1.4: Justification

There is paucity of information on clinical and radiological presentations as well as microorganisms associated with post-treatment disease in root-treated teeth of the Kenyan population. The findings of this study will provide relevant information to clinicians carrying out endodontic treatment. The information will assist clinicians improve on the quality of endodontic treatment.

1.5: Objectives

1.5.1: Main Objective

The aim of this study was to evaluate the clinical and radiological presentation of root-treated teeth with post-treatment disease and identify microorganisms isolated from such teeth, in a Kenyan population.

1.5.2: Specific Objectives

1) To determine clinical presentation of patients with post-treatment disease following root canal treatment.
2) To determine radiographic presentation of root-filled teeth with post-treatment disease.
3) To determine type of microorganisms isolated from root-treated teeth with post-treatment disease using PCR.

1.6: Hypothesis

1.6.1: Null hypothesis

1) Post-treatment disease is not related to the quality of the root filling and microorganisms.

1.7: Variables

Variable	Measurement
Socio-demographic variables	
Age	Number of years
Gender	Male or Female
Independent Variable	
Types of microorganisms	Identification of amplified microbial DNA
Quality of root filling	Presence/absence of voids Apical extent of root filling
Dependent Variables	
Post-treatment endodontic disease	Pain
	Tenderness to percussion
	Swelling
	Sinus tract
	Periradicular radiolucency
	Wet canal
Confounder variable	
Missed canals	Linear radiolucency
Lateral and accessory canals	Linear radiolucency
Quality of the coronal restoration	Marginal integrity

CHAPTER TWO

2.0: Methods and Materials

2.1: Study Design

This was a descriptive cross-sectional study.

2.2: Study Area

The study was conducted at the University of Nairobi, School of Dental Sciences(UNSDS) and the Kenyatta National Hospital(KNH). UNSDS is situated off Argwings Kodhek road. It offers undergraduate and post-graduate training. Patients are attended to by both undergraduate and postgraduate students under supervision. UNSDS serves as a referral centre for patients from all parts of the country. The KNH is the largest referral hospital in Kenya. It is situated off Ngong road in Nairobi. The dental department is located in the old wing of the hospital. It has divisions catering for all specialities in dentistry. It serves as an internship training centre where newly qualified dentists treat patients under the supervision of specialists.

2.3: Study Population

The study population comprised of referred patients with post-treatment endodontic disease.

2.4: Inclusion Criteria

1. Kenyan residents whose treatments were done within the country.
2. Patients with post-treatment disease.
3. Patients who had not taken antibiotics two weeks, prior to collection of the samples.
4. Patients who consented to participate in the study.

2.5: Exclusion Criteria

1. Kenyan residents whose treatments were not done within the country.
2. Patients whose root canal treatment had not been completed.
3. Patients who were taking antibiotics one month prior to collection of the samples.
4. Patients who did not consent to participate in the study.
5. Teeth with periodontal pockets deeper than 4mm.

2.6: Sample size

Using Epi Infor 6, for a descriptive cross-sectional study, with a minimum expected failure rate of 15%, the minimum sample size computed was 48 patients.

2.7: Sampling

Convenient sampling was applied to select the study area. All patients presenting to the dental institutions mentioned in the study area, and who satisfied the inclusion criteria during the period of study, were included in the sample.

2.8: Data Collection Instruments and Technique

Three types of data collection tools were used; a semi-structured questionnaire, a clinical examination form and a laboratory chart.

2.9: Data Collection Instruments

1. Questionnaire (Appendix II)

An interviewer-administered semi-structured questionnaire was used to collect the data. Data collected included; demographic data (age, gender), presenting complaint, use of medication with regard to the presenting complaint, date when the endodontic treatment was carried out and by whom, and the presence of a known systemic illness.

2. Clinical and Radiographic Assessment Form (Appendix III)

The affected tooth was identified. Signs and symptoms of which were noted. The type and state of the coronal restoration, presence or absence of periradicular radiolucency, missed canals (if present) and quality of the root filling (based on the European Society of Endodontology criteria, 2006[1]) were assessed.

3. Laboratory Chart (Appendix IV)

Ten microorganisms were identified and their prevalence noted.

2.10: Clinical Examination

This was carried out in a well lit dental clinic. A sterile dental mirror and probe were used for each patient, and the findings noted as per the clinical assessment form (Appendix III).

2.11: Radiological Examination

Two intra-oral periapical views of the affected tooth were taken using paralleling technique. The radiographs were viewed using a fluorescent light box, and the following noted: the integrity of the coronal restoration, presence of periradicular radiolucency, missed canals and quality of the root filling (extent, presence/absence of voids).

For purposes of this study, the integrity of the coronal restoration was defined as the absence of radiolucency between the tooth structure and the restoration at the margins. Periradicular radiolucency was noted to be present in cases where there was widening of the periodontal ligament or a periapical lesion. Identification of a missed canal was by the presence of a linear radiolucency within the root. Voids in the root filling were detected by the presence of radiolucencies within the root filling. An electronic digital vernier caliper (Shengya Machine & Tools Co., Ltd) was used to measure the extent of the root filling, which was classified as: i) extending to the apex, ii) extending >2mm short of the apex, iii) <2mm short of the apex, and iv) root filling beyond the apex

2.12: Microbial Sample Collection

Samples were collected using strict asepsis[1]. Oral prophylaxis was done using a slurry of pumice prior to the procedure. The patient was then requested to rinse his/her mouth with 15mls. of 0.2% chlorhexidine solution(Corsodyl, GlaxoSmithKline) for 30 seconds before isolation of the affected tooth using a rubber dam.

The isolated tooth and surrounding field were cleaned using 3% hydrogen peroxide (Diarim Enterprises Limited, Kenya) and disinfected with 2.5% sodium

hypochlorite (NaOCl) solution (Jik, Reckitt Benckiser East Africa Limited) for 30 seconds. An endodontic access cavity through the coronal restoration was made using a sterile high-speed carbide bur until the root filling was exposed. Isotonic sterile saline (Normal saline B.P, Infusion Kenya Limited) was used in the reservoir and was randomly sampled for microbial analysis. The saline solution was allowed to run through the system prior to the preparation of the access cavity, so as to minimise microorganisms within the system. The tooth, clamp and adjacent rubber dam were once again disinfected using 2.5% NaOCl. The NaOCl was then inactivated using 5% sodium thiosulphate.

To check the sterility of the access cavity, two sterile, dry cotton pellets were used to swab the wall of the access cavity. One swab was placed in a sterile test tube, and the other in a test tube containing thioglycolate broth (for the anaerobic bacteria). Both samples were transferred to the laboratory for culture of microorganisms. Cases with a contaminated operative area were excluded from the study. In the case of a multi-rooted tooth, only one canal was sampled (the largest symptomatic canal or one with a periapical lesion).

The old root filling was removed using sterile Gates-Glidden burs in the coronal half of the canal, followed by use of sterile endodontic hand instruments (K-files and Hedstrom files) to remove the root filling material in the apical half of the root canal. New burs and files were used for each patient. No intracanal irrigant or solvent was used at this stage. Root filling materials removed from canals were collected in a sterile test tube with TE buffer(10mM Tris-HCL, 1mM ethylenediamine tetraacetic acid, pH 7.6), and used as a sample. Once the root filling material was removed from the canal, a sterile normal saline solution was

introduced into the canal using a sterile syringe to facilitate the collection of an adequate sample. The canal walls were then filed to generate dentine chips, and a sterile size 20 paper point(Produits Dentaire SA) was used to swab the inside of the canal. Two paper point swabs were taken. Each paper point was retained in position for 60 seconds before being transferred to a cryotube containing TE buffer. A sterile paper point was sampled to serve as a negative control. The collected samples were immediately placed in ice-packed containers, and stored at -4^{o}C.

2.13: Microbial Examination

All samples collected were transported in ice-packed containers to the University of Nairobi, Department of Medical Microbiology, within two hours and stored at -20^{o}C.

Microbial identification using molecular analysis

DNA Extraction

The root canal samples in TE buffer were let to thaw at 37^{o}C. 800µl of genomic lysis buffer(Quick-gDNA^{TM} Miniprep, Zymo Research) were added to 200µl of the root canal sample. The mixture was vortexed for 4-6 seconds then let to stand for 5-10 minutes at room temperature. This was then transferred to a Zymo-Spin^{TM} Column in a collection tube and centrifuged at 10,000 *xg* for one minute. The collection tube was discarded with the flow through and the Zymo-Spin^{TM} Column transferred to a new collection tube. 200µl of DNA pre-wash buffer was added to the spin column and was centrifuged at 10,000 *xg* for one minute. 500µl of g-DNA wash buffer was added to the spin column and centrifuged at 10,000 *xg* for one minute. The spin column was transferred to a clean microcentrifuge tube. 50µl

DNA elution buffer was added to the spin column. This was incubated for 2-5 minutes at room temperature then centrifuged for 30 seconds to elute the DNA.

Reference DNA for each of the target microorganisms was also extracted to serve as positive controls for the primers used.

Taxon-specific oligonucleotide primers were used to detect different species of microorganisms. A pair of ubiquitous bacterial primers that match almost all bacterial 16S rRNA genes at the same position but not eukaryotic cells, were used as a positive control for the polymerase chain reaction (PCR). Table 1.2 lists the primers used.

Table 1.2. Polymerase chain reaction primer pairs used for the detection of microorganisms in cases with post-treatment disease

Target	Primer pairs(5'-3')	Amplicon length(bp)
Bacterial ubiquitos primer[a]	GAT TAG ATA CCC TGG TAG TCC AC	602
	CCC GGG AAC GTA TTC ACC G	
Enterococcus feacalis[a]	GTT TAT GCC GCA TGG CAT AAG AG	310
	CCG TCA GGG GAC GTT CAG	
Streptococcus spp.[b]	AGA GTT TGA TCC TGG CTC AG	500
	GTA CCG TCA CAG TAT GAA CTT TCC	
Peptostreptococcus micros[b]	AGA GTT TGA TCC TGG CTC AG	207
	ATA TCA TGC GAT TCT GTG GTC TC	
Porphyromonas endodontalis[b]	GCT GCA GCT CAA CTG TAG TC	672
	CCG CTT CAT GTC ACC ATG TC	
Porphyromonas gingivalis[b]	AGG CAG CTT GCC ATA CTG CG	404
	ACT GTT AGC AAC TAC CGA TGT	
Prevotella intermedia[b]	CGT GGA CCA AAG ATT CAT CGG TGG A	259
	CCG CTT TAC TCC CCA ACA AA	
Prevotella nigrescens[b]	ATG AAA CAA AGG TTT TCC GGT AAG	804
	CCC ACG TCT CTG TGG GCT GCG A	
Fusobacterium nucleatum[b]	AGA GTT TGA TCC TGG CTC AG	360
	GTC ATC GTG CAC ACA GAA TTG CTG	
Actinomyces radicidentis[a]	AGG CCT TAT TGG CTT GGT TG	550
	CGG TCA CTC ACA TGT CAA GC	
Candida albicans[a]	GCC GGT GAC GAC GCT CCA AGA GCT G	158
	CCG TGT TCA ATT GGG TAT CTC AAG GTC	
Universal 16S rRNA gene-specific primer pair[b]	AGA GTT TGA TCC TGG CTC AG	1500
	ACG GCT ACC TTG TTA CGA CTT	

[a]Primers used in a study done by Siqueira and Rocas[33]

[b]Primers used in a study done by Fouad *et al*[39]

PCR was carried out using taxon-specific primers for all the samples collected. The DreamTaq^{TM}PCR Mastermix was thawed then gently vortexed and briefly centrifuged. 50µl reaction was prepared for each sample by adding each of the following components into a PCR tube; 15µl DNA template, 25µl PCR mastermix,1µl forward primer, 1µl reverse primer and 8µl nuclease-free water. PCR amplification was performed in a thermocycler(2720 Applied Biosystems, Singapore).

Initial denaturation was carried out at 95°C for 3 minutes followed by 25 cycles of denaturation at 95°C for 30 seconds, a primer annealing step at 59°C for 30 seconds, an extension step at 57°C for 30 seconds and a final extension step at 72°C for 10 minutes(for *E. feacalis, A. radicidentis, Prev. intermedia, Prev. nigrescens, Porph. endodontalis, Porph. gingivalis, Fuso. nucleatum* and bacterial ubiquitous primers); 35 cycles for *C. albicans*. The PCR temperature profile for *Streptococcus* spp. *P. micros*. and universal primers was: 30 cycles of a denaturation step at 95°C for 30 seconds, a primer annealing step at 55°C for 1 minute, an extension step at 72°C for 1 minute and a final extension step at 72°C for 15 minutes.

PCR amplicons were analyzed by electrophoresis in a 2% agarose gel at 4V/cm in Tris-borate-EDTA buffer. 200ml of gel solution was stained with 3µl GRGreen Nucleic Acid Gel Stain, 10,000X in water and visualized under ultra-violet(UV) light. Positive reactions were determined by the presence of bands of the expected sizes. A 100-bp DNA ladder (O'GeneRuler^{TM}) served as the molecular size standard.

2.14: Data analysis

The data collected was coded and entered into a computer and analyzed using the Statistical Package for Social Sciences (SPSS) version 11.5 and Microsoft office excel 2007. Data cleaning was done by running frequencies. Data analysis included both descriptive and analytical statistics. The confidence level for this study was 95% and the p-value for statistical significance was set at less than 0.05. The non parametric statistics were the primary choice for exploring the relationship between the independent variables and the dependent variable as the data obtained was mainly categorical. The statistical tests used in this study included the Independent sample t-test, Chi-square test and Fischer's Exact.

2.15: Validity and reliability

A preliminary visit was made to each of the selected study sites, so that appropriate preparation could be made prior to data collection. Pretesting of the data collection instruments was done and adjustments made. The principal investigator was calibrated by the supervisors. Positive controls for the microorganisms identified were obtained and analysed alongside the samples collected. This was in addition to the ubiquitous bacterial primers.

2.16: Control of Biases and Errors

Errors in data collection were minimized by standardization of the examination to control for intra-examiner errors. The principal investigator carried out all the data collection. Only those patients who met the inclusion criteria were included in the study. All data collection tools were pre-tested. All instruments used were

calibrated. According to available records laboratory equipments was serviced annually and quality inspections were also done annually.

2.17: Ethical Consideration

The proposal was submitted to the Kenyatta National Hospital and University of Nairobi Ethics, Research and Standards committee for approval. Permission to carry out the investigation in the various institutions was sought from the relevant authorities. The purpose of the study and procedural details were explained to the participants, and their informed, written consent obtained prior to carrying out any procedure. All the information obtained from the participants was kept confidential. Endodontic retreatment was carried out to completion. Participants were at liberty to terminate participation without victimization.

2.18: Limitations to the Study

1) Radiographs are two dimensional, and this could present some degree of inaccuracies.
2) PCR can only be used to identify microorganisms with known DNA sequences.
3) Only organisms whose primers were used could be identified.

CHAPTER THREE

3.1: Results

3.2: Socio-Demographic Characteristics

Forty five patients were included in the study, among whom 16(35.6%) were male while twenty nine (64.4%) female. The lowest age encountered was 15years, and the highest age was 66years (mean age 36.67 years). Males were older (mean 38.06 ±14.34 years) than females (mean 35.90 ± 11.54 years). However, the difference was not statistically significant (t=0.55, P=0.58). Figure 3.1 shows gender and age distribution of the participants.

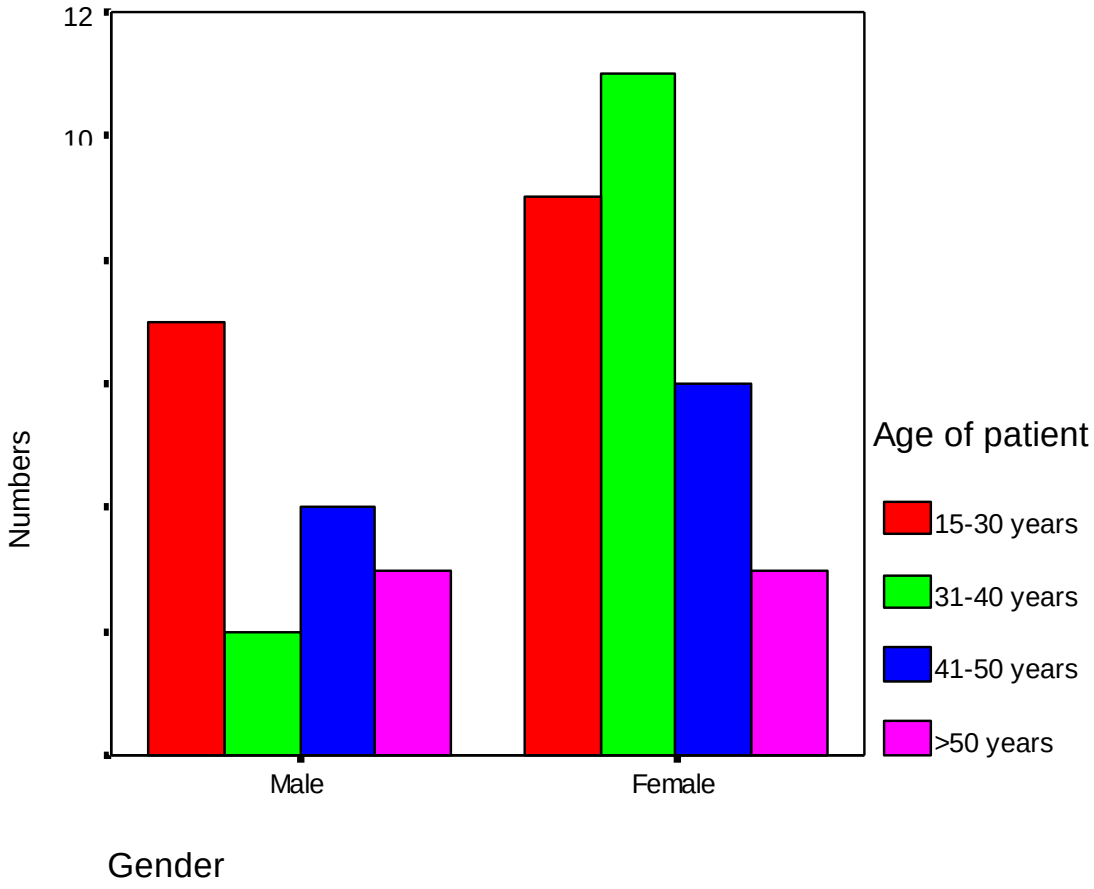

Fig. 3.1 Distribution of participants by age and gender

3.3: Clinical findings

Majority of the patients presented with symptoms 39(86.7%) of which 31(79.5%) had pain, 6(15.4%) had pain and swelling, 1(2.6%) had pain, swelling and a sinus tract, and 1(2.6%) had a sinus tract. Twenty five(55.5%) patients presented with complaints 48 months or more after the root-canal treatment, with 20(44.4%) presenting between 6 to 48 months after treatment. Posterior teeth with post-treatment disease were found to be more (32, 71.1%) than anterior teeth, with maxillary premolars (15, 33.3%) being the most affected followed closely by mandibular molars (14, 31.1%). There was no statistically significant finding between gender and the various symptoms (Table 3.1).

Table 3.1 Distribution of the symptoms of post-treatment disease amongst gender

		GENDER		X^2
SYMPTOMS		Male n (%)	Female n(%)	
Pain only	present	10 (62.5)	21(72.4)	0.36
	absent	6 (37.5)	8 (27.6)	
Pain and swelling	present	3 (18.8)	3 (10.3)	0.36
	absent	13 (81.3)	26 (89.7)	
Sinus tract only	present	1 (6.3)	0 (0.0)	-
	absent	15 (93.8)	29 (100.0)	
Pain, swelling and sinus tract	present	0 (0.0)	1 (3.4)	-
	absent	16 (100.0)	28 (96.6)	
***Neither pain/swelling**	present	2 (12.5)	4 (13.8)	0.64
	absent	14 (87.5)	25 (86.2)	
Total		**16 (35.6%)**	**29 (64.4%)**	

***Post-treatment disease was radiologically determined by the persistence of periapical radiolucency more than four years following root-canal treatment or the appearance of, or increase in size of a periapical radiolucency

Sixteen(35.6%) of the patients presenting with post-treatment disease were between 15 and 30 years of age, 13(28.9%) were between the ages 31 and 40 years, 10(22.2%) were aged between 41 and 50 years while the remaining

6(13.3%) were aged above 50 years. No statistical difference was found between age and the various symptoms.

Table 3.2 Distribution of the symptoms of post-treatment disease amongst the different age groups

		AGE				X^2
SYMPTOMS		15-30yrs n (%)	31-40yrs n (%)	41-50yrs n (%)	>50yrs n (%)	
Pain only	present	11 (68.8)	8 (61.5)	7 (70.0)	5 (83.3)	0.82
	absent	5 (31.3)	5 (38.5)	3 (30.0)	1 (16.7)	
Pain and swelling	present	3 (18.8)	3 (23.1)	0 (0.0)	0 (0.0)	-
	absent	13 (81.3)	10 (76.9)	10 (100.0)	6 (100.0)	
Sinus tract only	present	0 (0.0)	0 (0.0)	1 (11.1)	0 (0.0)	-
	absent	16 (100.0)	13 (100.0)	8 (88.9)	6 (100.0)	
Pain, swelling and sinus tract	present	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	-
	absent	16 (100.0)	13 (100.0)	9 (90.0)	6 (100.0)	
Neither pain/swelling	present	2 (12.5)	2 (15.4)	1 (10.0)	1 (16.7)	0.98
	absent	14 (87.5)	11 (84.6)	9 (90.0)	5 (83.3)	
Total		**16 (35.6%)**	**13 (28.9%)**	**10 (22.2%)**	**6 (13.3%)**	

3.4: Radiological findings

Table 3.3 below shows the distribution of the various radiological findings among participants. Periradicular radiolucency was seen in 40(88.9%) of the patients, 40(88.9%) had intact coronal restorations, while 11(24.4%) were found with missed canals. Two(4.4%) patients had obturation done upto the apex, 2(4.4%) had root filling material extending beyond the apex, 11(24.4%) had root filling extending less than 2mm short of the apex, 30(66.7%) had root filling extending more than 2mm short of the apex, while 41(91.1%) had voids within the root

filling. No statistically significant difference was found between males and females with regards to radiological findings.

Table 3.3 Distribution of the various radiological findings amongst gender

		GENDER		Fisher's Exact
RADIOGRAPHIC FINDINGS		Male n (%)	Female n (%)	
Periradicular radiolucency	present	16 (100.0)	24 (82.8)	-
	absent	0 (0.0)	5 (17.2)	
Coronal restoration	intact	13 (81.3)	27 (93.1)	0.23
	marginal leakage	3 (18.8)	2 (6.9)	
Missed canals	present	5 (31.3)	6 (20.7)	0.33
	absent	11 (68.8)	23 (79.3)	
Extent of root filling	up to apex	0(0.0)	2(100.0)	-
	beyond apex	0(0.0)	2(100.0)	
	<2mm short of apex	4(25.0)	7(28.0)	0.56
	>2mm short of apex	12(75.0)	18(72.0)	
Voids	present	14(87.5)	27(93.1)	0.45
	absent	2(12.5)	2(6.9)	

Sixteen(35.6%) of the participants found to have periradicular radiolucency associated with the affected tooth were aged between 15 and 30 years of age, 10(22.2%) were aged between 31-40 years, while 19(42.2%) were aged above 40 years (Table 3.4).

Table 3.4 Distribution of the various radiological findings among the different age groups

		AGE				X^2
RADIOGRAPHIC FINDINGS		15-30yrs n (%)	31-40yrs n (%)	41-50yrs n(%)	>50yrs n(%)	
Periradicular radiolucency	present	16 (100.0)	10 (76.9)	9 (90.0)	5 (83.3)	-
	absent	0 (0.0)	3 (23.1)	1 (10.0)	1 (16.7)	
Coronal restoration	intact	15 (93.8)	11 (84.6)	8 (80.0)	6 (100.0)	-
	marginal leakage	1 (6.3)	2 (15.4)	2 (20.0)	0 (0.0)	
Missed canals	present	3 (18.8)	2 (15.4)	4 (40.0)	2 (33.3)	0.49
	absent	13 (81.3)	11 (84.6)	6 (60.0)	4 (66.7)	
Extent of root filling	at apex	1(50.0)	1(100.0)	0(0.0)	2(50.0)	-
	beyond apex	1(50.0)	0(0.0)	1(100.0)	2(50.0)	
	<2mm short of apex	3(18.8)	6(54.5)	1(11.1)	1(20.0)	0.12
	>2mm short of apex	13(81.3)	5(45.5)	8(88.9)	4(80.0)	
Voids	present	15(93.8)	11(84.6)	10(100.0	5(83.3)	0.52
	absent	1(6.3)	2(15.4)	0(0.0)	1(16.7)	

Out of the 6(13.3%) patients who did not present with any symptoms, 4(66.7%) had missed canals associated with the teeth with post-treatment disease, while 2(33.3%) did not. The difference between the two groups was found to be statistically significant(P=0.03) (Table 3.5).

Table 3.5 Association between radiographic findings and symptoms of post-treatment disease

		RADIOGRAPHIC FINDINGS								
		Coronal restoration (P<0.05)			**Periradicular radiolucency (P<0.05)**			**Missed canals (P<0.05)**		
SYMPTOMS OF POST-TREATMENT DISEASE		intact n (%)	marginal leakage n (%)	**p**	present n(%)	absent n(%)	**p**	present n(%)	absent n(%)	**p**
Pain only	present	28(70.0)	3(60.0)	0.50	27(67.5)	4(80.0)	0.50	7(63.6)	24(70.6)	0.47
	absent	12(30.0)	2(40.0)		13(32.5)	1(20.0)		4(36.4)	10(29.4)	
Neither pain nor swelling	present	5(12.5)	1(20.0)	0.53	5(12.5)	1(20.0)	0.53	4(36.4)	2(5.9)	0.03
	absent	35(87.5)	4(80.0)		35(87.5)	4(80.0)		7(63.6)	32(94.1)	
Pain and swelling	present	5(12.5)	1(20.0)	0.53	6(15.0)	0(0.0)	0.47	0(0.0)	6(17.6)	-
	absent	35(87.5)	4(80.0)		34(85.0)	5(100.0)		11(100.0)	28(82.4)	
Sinus tract	present	2(5.0)	0(0.0)	-	2(5.0)	0(0.0)	-	0(0.0)	2(5.9)	-
	absent	38(95.0)	5(100.0)		38(95.0)	5(100.0)		11(100.0)	32(94.1)	
Pain, swelling and sinus tract	present	1(2.5)	0(0.0)	-	1(2.5)	0(0.0)	-	0(0.0)	1(2.9)	-
	absent	39(97.5)	5(100.0)		39(97.5)	5(100.0)		11(100.0)	33(97.1)	

Table 3.6 shows that most patients who presented with pain had voids(29, 93.5%) and the root filling extending more than 2mm short of the apex(22, 48.9%). However this was not statistically significant(P<0.05). The null hypothesis which states that post-treatment disease is not associated with the quality of the root filling was therefore accepted.

Table 3.6 Association between quality of root filling and signs and symptoms of post-treatment disease

		QUALITY OF ROOT FILLING								
		Extent of root filling			**Extent of the root filling**			**Presence of voids in the root filling**		
SYMPTOMS OF PTD		Up to apex n(%)	Beyond the apex n(%)	p<0.05	<2mm short of apex n (%)	>2mm short of apex n (%)	**P<0.05**	present n (%)	absent n (%)	**P<0.05**
Pain only	present	1(50.0)	9(29.0)	0.83	7(63.6)	22(73.3)	0.47	29(70.0)	2(50.0)	0.37
	absent	1(50.0)	6(42.9)		4(36.4)	8(26.7)		12(30.0)	2(50.0)	
Neither pain nor swelling	present	1(50.0)	3(50.0)	0.83	1(9.1)	3(10.0)	0.71	5(12.2)	1(25.0)	0.45
	absent	1(50.0)	12(30.8)		10(90.9)	27(90.0)		36(87.8)	3(75.0)	
Pain and swelling	present	0(0.0)	3(50.0)	-	3(27.3)	3(10.0)	0.18	5(12.2)	1(25.0)	0.45
	absent	2(100.0)	12(30.3)		8(72.7)	31(90.0)		36(87.8)	3(75.0)	
Sinus tract	present	0(0.0)	0(0.0)	-	0(0.0)	2(6.7)	-	2(4.9)	0(0.0)	-
	absent	2(100.0)	2(100.0)		11(100.0)	28(93.3)		39(95.1)	4(100.0)	
Pain, swelling, sinus tract	present	0(0.0)	0(0.0)	-	0(0.0)	1(3.3)	-	1(2.4)	0(0.0)	-
	absent	2(100.0)	2(100.0)		11(100.0)	29(96.7)		40(97.6)	4(100.0)	

3.4: Microbial analysis

Samples for microbial analysis were collected from 38 patients. *Porphyromonas gingivalis, Prevotella nigrescens* and *Candida albicans* were isolated from all the samples. Streptococcus species had the least prevalence at 74%(28). Fig. 3.2 shows the frequency of the microorganisms isolated. All examined cases reacted positively with the bacterial ubiquitous primer.

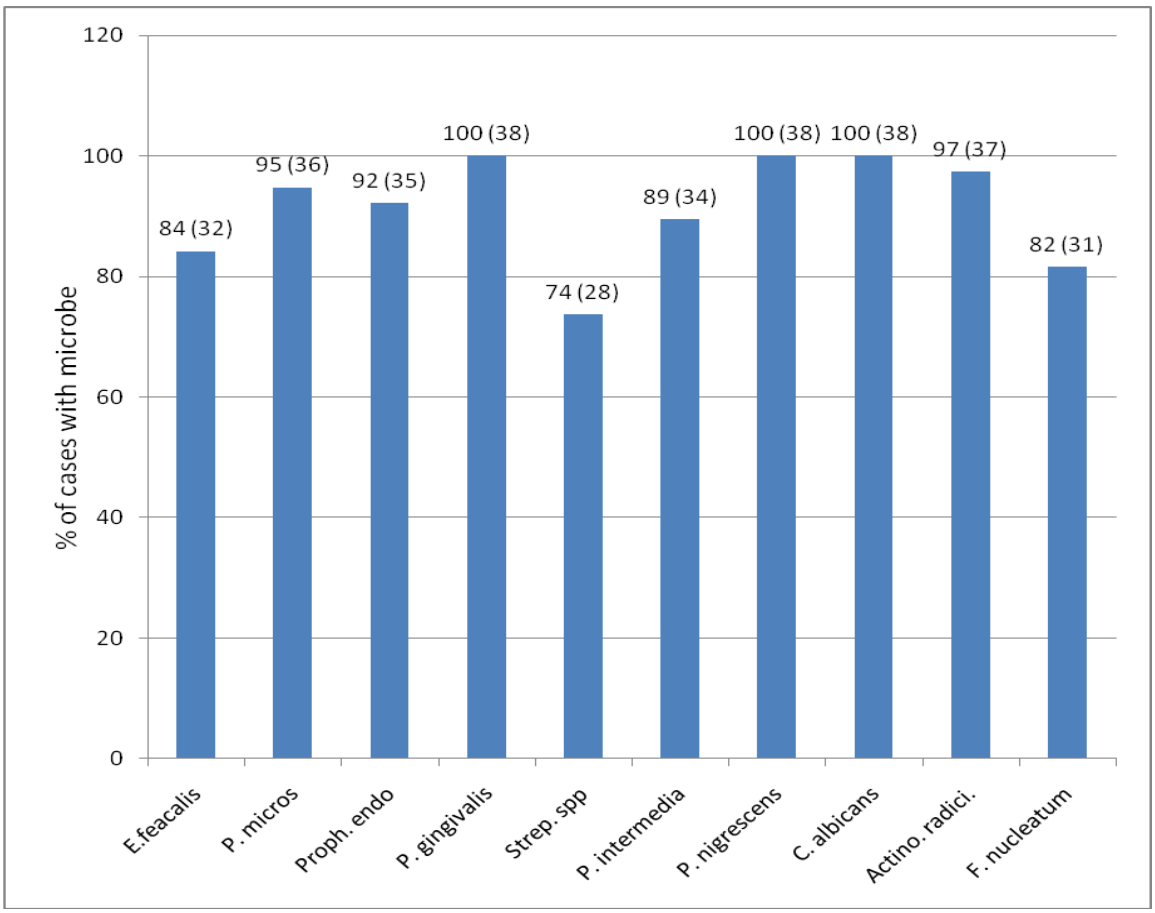

Fig.3.2 Distribution of the various microorganisms

Tables 3.7 and 3.8 show the distribution of the microorganisms amongst the participants. No statistically significant difference was noted between males and females or amongst the different age groups($P<0.05$).

Table 3.7 Distribution of the various microorganisms amongst gender

		GENDER		Fisher's Exact
MICROORGANISMS		Male n (%)	Female n (%)	
E. feacalis	present	9 (69.2)	23 (92.0)	0.09
	absent	4 (30.8)	2 (8.0)	
P. micros	present	13 (100.0)	23 (92.0)	-
	absent	0 (0.0)	2 (8.0)	
Strep. spp	present	9 (69.2)	19 (76.0)	0.47
	absent	4 (30.8)	6 (24.0)	
Actino.radici.	present	12 (92.3)	25 (100.0)	-
	absent	1 (7.7)	0 (0.0)	
Porph. endo	present	13 (100.0)	22 (88.0)	-
	absent	0 (0.0)	3 (12.0)	
P. intermedia	present	11 (84.6)	23 (92.0)	0.42
	absent	2 (15.4)	2 (8.0)	
F. nucleatum	present	12 (92.3)	19 (76.0)	0.22
	absent	1 (7.7)	6 (24.0)	

Table 3.8 Distribution of the various microorganisms amongst the different age groups

		AGE				X^2
MICROORGANISMS		15-30yrs n (%)	31-40yrs n (%)	41-50yrs n (%)	>50yrs n (%)	
E. feacalis	present	11 (73.3)	10 (90.9)	6 (100.0)	5 (83.3)	-
	absent	4 (26.7)	1 (9.1)	0 (0.0)	1 (16.7)	
P. micros	present	14 (93.3)	10 (90.9)	6 (100.0)	6 (100.0)	-
	absent	1 (6.7)	1 (9.1)	0 (0.0)	0 (0.0)	
Strep. spp	present	9 (60.0)	11 (100.0)	3 (50.0)	5 (83.3)	-
	absent	6 (40.0)	0(0.0)	3 (50.0)	1 (16.7)	
Actino.radici.	present	15 (100.0)	11(100.0)	6 (100.0)	5 (83.3)	-
	absent	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.7)	
Proph. endo	present	15 (100.0)	10 (90.9)	5 (83.3)	5 (83.3)	-
	absent	0(0.0)	1 (9.1)	1 (16.7)	1 (16.7)	
P. intermedia	present	12 (80.0)	10 (90.9)	6 (100.0)	6 (100.0)	-
	absent	3 (20.0)	1 (9.1)	0 (0.0)	0 (0.0)	
F. nucleatum	present	12 (80.0)	9 (81.8)	4 (66.7)	6 (100.0)	-
	absent	3 (20.0)	2 (18.2)	2 (33.3)	0 (0.0)	

Despite the high incidence of microorganisms identified in all the samples, only *P. intermedia* was found to be associated with pain(P=0.01) and pain and swelling(P=0.01)(Table 3.9).

Table 3.9 Association between the various microorganisms and symptoms of post-treatment disease

		Pain only			**Pain and swelling**			**Neither pain nor swelling**			**Sinus tract**			**Pain, swelling and sinus tract**		
MICROORGANISMS		present n(%))	absent n(%)	P<0.05	present n(%)	absent n(%)	P<0.05	present n(%)	absent n(%)	P<0.05	present n(%)	absent n(%)	P<0.05	present n (%)	absent n(%)	P<0.05
E. feacalis	present	21(80.8)	11(91.7)	0.37	4(80.0)	28(84.8)	0.60	5(100.0)	27(81.8)	-	2(100.0)	30(83.3)	-	1(100.0)	31(83.8)	-
	absent	5(19.2)	1(8.3)		1(20.0)	5(15.2)		0(0.0)	6(18.2)		0(0.0)	6(16.7)		0(0.0)	6(16.2)	
P.micros	present	25(96.2)	11(91.7)	0.54	5(100.0)	31(93.9)	-	4(80.0)	32(97.0)	0.25	2(100.0)	34(94.4)	-	1(100.0)	35(94.6)	-
	absent	1(3.8)	1(8.3)		0(0.0)	2(6.1)		1(20.0)	1(3.0)		0(0.0)	2(5.6)		0(0.0)	2(5.4)	
Strep.spp	present	19(73.1)	9(75.0)	0.62	3(60.0)	25(75.8)	0.40	5(100.0)	23(69.7)	0.20	1(50.0)	27(75.0)	0.46	0(0.0)	28(75.7)	-
	absent	7(26.9)	3(25.0)		2(40.0)	8(24.2)		0(0.0)	10(30.3)		1(50.0)	9(25.0)		1(100.0)	9(24.3)	
Actino.radici.	present	26(100.0)	11(91.7)	-	5(100.0)	32(97.0)	-	4(80.0)	33(100.0)	-	2(100.0)	1(100.0)	-	1(100.0)	36(97.3)	-
	absent	0(0.0)	1(8.3)		0(0.0)	1(3.0)		1(20.0)	0(0.0)		0(0.0)	0(0.0)		0(0.0)	1(2.7)	
Porph. endo	present	23(88.5)	12(100.0)	-	5(100.0)	30(90.9)	-	5(100.0)	30(90.9)	-	2(100.0)	33(91.7)	-	1(100.0)	34(91.9)	-
	absent	3(11.5)	0(0.0)		0(0.0)	3(9.1)		0(0.0)	3(9.1)		0(0.0)	3(8.3)		0(0.0)	3(8.1)	
P. intermedia	present	26(100.0)	8(66.7)	0.01	2(40.0)	32(97.0)	0.01	4(80.0)	30(90.9)	0.45	2(100.0)	32(88.9)	-	1(100.0)	33(89.2)	-
	absent	0(0.0)	2(33.3)		3(60.0)	1(3.0)		1(20.0)	3(9.1)		0(0.0)	4(11.1)		0(0.0)	4(10.8)	
F. nucleatum	present	20(76.9)	11(91.7)	0.27	4(80.0)	27(81.8)	0.66	5(100.0)	26(78.8)	-	2(100.0)	29(80.6)	-	1(100.0)	30(81.1)	-
	absent	6(23.1)	1(8.3)		1(20.0)	6(18.2)		0(0.0)	7(21.2)		0(0.0)	7(19.4)		0(0.0)	7(18.9)	

Although most of the teeth with post-treatment disease presented with periradicular radiolucency(40, 88.9%), there was no statistically significant finding between this and the various microorganisms identfied(Table 3.10).

Table 3.10. Association between microorganisms and radiological findings

		RADIOLOGICAL FINDINGS					
		Periapical radiolucency			Missed canals		
MICROORGANISMS		present n (%))	absent n (%)	P <0.05	present n (%)	absent n (%)	P<0.05
E. feacalis	present	29(85.3)	3(75.0)	0.51	9(100.0)	23(79.3)	-
	absent	5(14.7)	1(25.0)		0(0.0)	6(20.7)	
P.micros	present	32(94.1)	4(100.0)	-	8(88.9)	28(96.6)	0.42
	absent	2(5.9)	0(0.0)		1(11.1)	1(3.4)	
Strep.spp	present	24(70.6)	4(100.0)	-	7(77.8)	21(75.4)	0.56
	absent	10(29.4)	0(0.0)		2(22.2)	8(27.6)	
Actino.radici.	present	33(97.1)	4(100.0)	-	8(88.9)	29(100.0)	-
	absent	1(2.9)	0(0.0)		1(11.1)	0(0.0)	
Porph. endo	present	32(94.1)	3(75.0)	0.291	8(88.9)	27(93.1)	0.57
	absent	2(5.9)	1(25.0)		1(11.1)	2(6.9)	
P. intermedia	present	30(88.2)	4(100.0)	-	8(88.9)	26(89.7)	0.68
	absent	4(11.8)	0(0.0)		1(11.1)	3(10.3)	
F. nucleatum	present	27(79.4)	4(100.0)	-	8(88.9)	23(79.3)	0.46
	absent	7(20.6)	0(0.0		1(11.1)	6(20.7)	

There was no statistically significant difference between the quality of root filling and the various microorganisms, as depicted in Table 3.11.

Table 3.11. Association between microorganisms and the quality of root filling

		QUALITY OF ROOT FILLING								
		Extent of the root filling			Extent of the root filling			Presence of voids in the root filling		
MICROORGANISMS		up to apex n (%))	beyond apex n (%)	P<0.05	<2mm short of apex n(%)	>2mm short of apex n(%)	P <0.05	present n(%)	absent n(%)	P<0,05
E. feacalis	present	2(100.0)	2(100.0)	-	6(66.7)	22(88.0)	0.17	30(88.2)	2(50.0)	0.11
	absent	0(0.0)	0(0.0)		3(33.3)	3(12.0)		4(11.8)	2(50.0)	
P.micros	present	1(50.0)	2(100.0)	-	9(100.0)	24(96.0)	-	32(94.1)	4(100.0)	-
	absent	1(50.0)	0(0.0)		0(0.0)	1(4.0)		2(5.9)	0(0.0)	
Strep.spp	present	1(50.0)	2(100.0)	-	8(88.9)	17(68.0)	0.23	25(73.5)	3(75.0)	0.72
	absent	1(50.0)	0(0.0)		1(11.1)	8(32.0)		9(26.5)	1(25.0)	
Actino.radici.	present	2(100.0)	2(100.0)	-	8(88.9)	25(100.0)	-	34(100.0)	3(75.0)	-
	absent	0(0.0)	0(0.0)		1(11.1)	0(0.0)		0(0.0)	1(25.0)	
Porph. endo	present	1(50.0)	1(50.0)	0.83	8(88.9)	25(100.0)	-	31(91.2)	4(100.0)	-
	absent	1(50.0)	1(50.0)		1(11.1)	0(0.0)		3(8.8)	0(0.0_	
P. intermedia	present	1(50.0)	2(100.0)	-	8(88.9)	23(92.0)	0.62	30(88.2)	4(100.0)	-
	absent	1(50.0)	0(0.0)		1(11.1)	2(8.0)		4(11.8)	0(0.0)	
F. nucleatum	present	1(50.0)	2(100.0)	-	7(77.8)	21(84.0)	0.51	28(82.4)	3(75.0)	0.57
	absent	1(50.0)	0(0.0)		2(22.2)	4(16.0)		6(17.6)	1(25.0)	

CHAPTER 4

DISCUSSION

The present study was designed to evaluate the clinical and radiological presentation as well as determine the microorganisms associated with root-filled teeth with post-treatment disease in a Kenyan population. The topic was chosen mainly because no studies of this nature have been conducted within the country or region. The present study would therefore provide baselin data on the same. In addition, the data obtained would be useful to dentists carrying out endodontic re-treatment on patients of Kenyan origin.

A total of forty five patients were included in the study. This is much less than that of similar studies carried out elsewhere. The low sample size may be attributed to the high cost of endodontic treatment and hence many patients poting for extravtion of the affected teeth.

In the present study more than half the patients had complaints of pain, 6(13.3%) had both pain and swelling, one had both pain and swelling while one had a sinus tract as the clinical presentation. This was consistent with the findings of two studies done in Manchester.[40,41] However in these studies which looked at teeth with primary infection as well as root-filled teeth, the authors did not specify the number of root-filled teeth with swelling and sinus tracts.

The present study identified periradicular radiolucency in 40(88.9%) of the patients and inadequate root filling in most of the cases. This suggests an association between periradicular radiolucency as well as quality of root-filling and post-treatment disease. However, there was no statistically significant finding between the presence of periapical radiolucency or quality of the root filling and

the presence of post-treatment disease. This could have been as a result of the small sample size realised in the present study. Several studies have assessed the quality of root fillings in teeth with post-treatment disease.[6,42-44] In Senegal, Toure *et al* examined 344 cases of filled roots in which 56.1% had periapical pathosis with 62.5% of this having unacceptable fillings.[6] Boltacz-Rzepkowska and Pawlicka sampled 282 root-filled teeth among which 49% were adequately filled(within 0-2mm of the radiographic apex).They observed periapical radiolucencies in 25%(69) of the root-filled teeth. Of these, 78.2%(54) had the root filling extending >2mm short of the apex, 4.4%(3) were filled beyond the apex, and 17.4%(12) were adequately filled.[42] In the preceeding studies, the difference between acceptable and unacceptable root fillings and the presence of periapical pathosis was found to have been significant. In the present study, majority (41, 91.1%) of the patients presenting with post-treatment disease had inadequate root fillings, which was consistent with other studies.

Amongst the studies investigating microorganisms associated with post-treatment disease,[2,10,14,33,43,44] the investigators found *E. feacalis* to have been the most prevalent microorganism isolated. However, the present study identified *P. gingivalis, P.nigrescence* and *C.albicans* in all the samples, whereas *E. feacalis* was detected in 84%(32) of the cases. The difference in the type and number of microorganisms identified could be attributed to the microbial method of identification and mechanical and technical factors. Molecular methods of microbial identification are more sensitive than culture methods.[37,38] Studies in which culture methods were used to identify the microorganisms, found one to two microbes in canals with post-treatment disease, while those that used PCR

found more than three microorganisms.[2,10,33] It has been suggested that the bacterial profile may vary among populations from different regions.[33] This could contribute to the difference in the microorganisms identified in this study.

Patients seen in the current study with post-treatment disease, had their primary treatment done in different clinics within the region. The level of qualification of all the practitioners(whether specialised endodontists or general practitioners) who carried out this treatment was difficult to establish as the patients did not have this information. Given that there is only one registered endodontist in the country, it can safely be assumed that the primary endodontic treatment for these patients was carried out by general practitioners. In addition, the conditions(use of rubber dam, irrigants, sealants, hand or rotary instruments) under which the treatment was carried out could not be established. However, the quality of the root filling seen in most of the teeth is suggestive of inadequate cleaning and shaping of the canals. Various authors[31,45] have suggested that inadequately filled canals harbour more microorganisms than those with adequate filling material. It is likely that the flora in such teeth is similar to that found in untreated canals. This could most probably be the case in the current study, as microbes such as *P. endodontalis, P. gingivalis* and *P. intermedia*, which have been isolated from primary endodontic infections in other studies,[45-47] were identified in majority of the cases.

The current study did find a statistically significant association between *P. intermedia* and pain, as well as pain and swelling. Other studies[45-47] have linked signs and symptoms of PTD with particular combinations of specific bacteria. It is

recommended that further research be carried out in the region, under controlled conditions, to ascertain whether there is indeed a geographical difference in the microorganisms identified, and their association with the various signs and symptoms.

CONCLUSION

Based on the findings of this study, it can be concluded that;

1) Statistically, there was no significance between gender and prevalence of post-treatment disease.
2) Pain is the most common symptom in patients with post-treatment disease (79.5%), followed by pain and swelling(15.4%), pain, swelling and sinus tract(2.6%) and sinus tract only(2.6%).
3) Periradicular radiolucency is a common finding in teeth with post-treatment disease, 88.9%.
4) The commonest microorganisms found in teeth with post-treatment disease in a Kenyan population are *P. gingivalis, P. nigrescens* and *C. albicans* (100%), *Actino. radicidentis*(97%), *P. micros*(95%), *P. endodontalis*(92%), *P. intermedia*(89%), *E.feacalis*(84%), *F. nucleatum*(82%) and *Strep. spp*(74%)
5) Although microorganisms were identified in all the teeth investigated, only *P. intermedia* was found to have a statistically significant association with pain and swelling

RECOMMENDATIONS

Based on the findings of this study, there is need for the improvement of the technical aspects of endodontic treatment among dental practitioners. This is likely to increase the chances of a favourable outcome. It is also recommended that other studies be done under controlled conditions, with larger sample sizes, so as to determine whether there is indeed an association between the various variables.

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APPENDIX I

Consent Form

Introduction

I am a postgraduate student, pursuing a master's degree in Prosthodontics at the University of Nairobi, Dental School. I wish to conduct a study titled **'Clinical, Radiological and Microbiological Study of Root-filled teeth with Post-treatment Disease in a Kenyan population'**. Root canal treatment is one of the methods used to treat diseased teeth. Occasionally this treatment may fail. The major cause of failure has been found to be bacteria/germs which remain within the treated tooth. There is scanty information on the presentation of failed root canal treatment and the type of bacteria associated with this failure in our set-up. Data obtained from this study will therefore be useful in improving the outcome of root canal treatment in our set-up.

Voluntary participation

I understand that I have entered the study voluntarily and that no guarantee can be made to the ultimate outcome of the programme. I also understand that I can terminate my participation in the study at will without any consequences. I understand that the participation in the study does not entail financial benefit.

Anticipated risk

There are no anticipated risks in participating in this study.

Confidentiality

The information given to the researcher will be kept in strict confidence. No information, by which your identity can be revealed, will be released or published.

I the undersigned _____ having been informed about the study/having read all the above, had time to ask questions, received answers concerning issues I did not understand do wilfully give consent to participate in the study.

_____ _____

(Participant's signature) (Date)

_____ _____

(Researcher's signature) (Date)

APPENDIX II

Questionnaire

1). Age: _____years

2). Gender: Male _ Female_

3). Occupation; _____

4).Presenting complaint: i) Pain ____

ii) Swelling _____

iii) Both (stated above) _____

iv) None of the above (specify) _____

5).Use of medication for the presenting complaint; Yes _____ (if yes, answer Q. 6 below) No _____

6).(if yes, in Q. 5 above) i).Which medications have you been on? _____

ii). How long ago was the medication taken?_____

7). How long ago was the root canal treatment done? _____

8). Where was the root canal treatment done? (Dental school, private clinic, hospital-specify)_____

9). (for those treated at the dental school)Who did the treatment, student or qualified doctor?

_____

10). Systemic illness i). Yes/No_____ ii). Which illness_____

APPENDIX III

Clinical and Radiographic Assessment Chart

1. Signs and symptoms;

   i).Pain_____

   ii).Tenderness to percussion_____

   iii).Swelling_____

   iv).Sinus tract_____

   iv).Periradicular radioluscency (Assess radiographs) Yes/No_____

   v).Intracanal exudates_____

2. Affected tooth_____
3. State of the coronal restoration(root filling visible or not)_____
4. Depth of periodontal pocket, if present_____
5. Missed canals, if present. Specify._____
6. Quality of root filling; i)radiographic assessment_____

   (extent of root filling, presence/absence of voids)

   ii). Clinical findings_____

APPENDIX IV

Laboratory Chart

Sample: _____

Microorganism (type)	Present(yes)	Absent(no)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.		
12.		

APPENDIX V

Ethical Approval Letter

KENYATTA NATIONAL HOSPITAL
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Ref: KNH-ERC/ A/567

18th August 2010

Dr. Fane Bosibori Nyaata
Department of Conservative and Prosthetic Dentistry
School of Dental Sciences
University of Nairobi

Dear Dr. Nyaata

RESEARCH PROPOSAL: "A CLINICAL, RADIOLOGICAL AND MICROBIOLOGICAL STUDY OF ROOT-FILLED TEETH WITH POST-TREATMENT DISEASE IN A KENYAN POPULATION" (P175/06/2010)

This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and **approved** your above revised research proposal for the period 18th August 2010 to 17th August 2011.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

On behalf of the Committee, I wish you a fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH/UON-ERC

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