FINAL REPORT

VOLUME II

Research report (mini-dissertation) of

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In partial fulfilment of the requirement for the degree of Master of
Dentistry in Prosthodontics, in the School of Dentistry, Faculty of Health
Sciences, University of Pretoria, South Africa.

THE PREVALENCE OF SECOND CANALS IN THE MESIOBUCCAL ROOT OF MAXILLARY MOLARS IN A SOUTH AFRICAN SUBPOPULATION:

A CONE BEAM COMPUTED TOMOGRAPHY STUDY

by

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Submitted in partial fulfilment of the requirements for the degree

MChD (Prosthodontics) in the Department of Prosthodontics,

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JULY 2017

DECLARATION

I, Nelson Alexander Fernandes, hereby declare that this dissertation, submitted in partial fulfilment of the requirements for the degree of MChD (Prosthodontics) at the University of Pretoria, has not previously been submitted for a degree at any other University.

Nelson Alexander Fernandes

DEDICATION

To my amazing wife and life partner, Belinda Bunn, for her love, support and patience. I am eternally grateful for having her in my life. This work is also dedicated to my parents who have always loved me unconditionally and taught me to work hard for the things that I want to achieve.

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to the following people for their contribution in bringing this project to fruition:

Prof. Hemant Dullabh, Head of the Department of Prosthodontics, University of Pretoria, for believing in me and giving me the opportunity to pursue my dreams.

Prof. Leanne Sykes, Head of Clinical Unit of the Department of Prosthodontics, University of Pretoria, for advising me on my protocol submission and always being available to help.

Prof. Tiaan de Jager, Deputy Dean of Research, Dean of Health Sciences, University of Pretoria, for inspiring me to publish and excel in research, after attending his research methodology course.

Dr. Dirk Herbst, Senior Specialist of the Department of Prosthodontics, University of Pretoria, for being the main supervisor and leader of this project, and for being a mentor throughout my post-graduate training.

Prof. Corne Postma, Head of Clinical Unit of the Department of Dental Management Sciences, University of Pretoria, for being my co-supervisor, doing the statistical analysis of this project, and always being available to discuss my queries.

Dr. Belinda Bunn, Head of Clinical Unit of the Department of Oral Pathology and Oral Biology, University of Pretoria, for being my additional co-supervisor, and assisting with the syntax of this document.

Prof. Piet Becker, Biostatistician of the Medical Research Institute, University of Pretoria, for conceiving the sample size and statistical analysis required for protocol submission.

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LIST OF ABBREVIATIONS

MB	Mesiobuccal
MB2	Second mesiobuccal canal
PA	Periapical
2D	Two-dimensional
3D	Three-dimensional
CBCT	Cone beam computed tomography
CT	Computed tomography
MCT	Micro-computed tomography
ROI	Region of interest
FOV	Field of view
AAE	American Association of Endodontists
AAOMR	American Academy of Oral and Maxillofacial Radiology
PPV	Positive predictive value
NPV	Negative predictive value
ALARA	As low as reasonably achievable

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ABSTRACT

THE PREVALENCE OF SECOND CANALS IN THE MESIOBUCCAL ROOT OF MAXILLARY MOLARS IN A SOUTH AFRICAN SUBPOPULATION:

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Introduction: Endodontic therapy is a global routine dental therapeutic procedure. Despite recent and present advances in dentistry and particularly with the popularity of implant dentistry, it remains biologically and aesthetically advantageous for retention of one’s original dentition. Tooth extraction followed by surgical placement of dental implants should remain a last resort in management. Treatment success depends on intricate knowledge and a thorough understanding of the anatomy of the root canal system, in order to adequately debride, disinfect, and obturate teeth affected by irreversible pulpitis or pulp necrosis. High endodontic failure rates are frequent in the maxillary molar tooth owing to the complexity of their root canal anatomy, and variations in the number of mesiobuccal (MB) roots. Conventional radiographic imaging techniques are unreliable for the detection of multiple canals, whereas cone beam computed tomography (CBCT) has proven to be effective for visualization of additional canals of root canal systems. There is little published data on the prevalence of second canals in the MB roots (MB2) of permanent maxillary first and second molars in a South African subpopulation. The aim of this study is to detect the prevalence of MB2 canals by analysing axial views of CBCT scans of adult patients.

Methodology: A total number of 200 patient CBCT scans, from 100 male and 100 female patients respectively, were enrolled in the study. A total of 800 teeth were analysed *in-vivo* on CBCT scans, comprising 200 right maxillary first molar teeth (tooth 16), 200 left maxillary first molar teeth (tooth 26), 200 right maxillary second molar teeth (tooth 17), and 200 left maxillary second molar teeth (tooth 27). Teeth displaying radiological evidence of an additional mesiobuccal canal (MB2) were identified.

Results: First maxillary molar teeth showed a high prevalence of MB2 canals, 92% and 87%, for the 16 and 26 respectively. Second maxillary molar teeth showed a lower, but still significant, prevalence of MB2 canals, 69% and 65%, for the 17 and 27 respectively. An association between patient age, gender and the prevalence of MB2 canals was not noted in the study sample.

Conclusion: Root morphology and anatomy of permanent maxillary first and second molar teeth is highly variable. The prevalence of additional canals in the MB roots is a frequent finding which has previously been underreported. The presence of these variations significantly impact endodontic therapy success, longevity, and retention of the natural dentition. The prevalence of these canals requires documentation for dissemination of this knowledge and greater cognizance thereof with respect to more accurate endodontic outcomes and success.

CHAPTER 1

1.0 INTRODUCTION

Endodontic therapy is routinely practiced daily for the treatment of irreversible pulpal disease in order to retain the natural dentition and the surrounding periodontium. Therapeutic success and increased probability of tooth retention is reliant on the complete disinfection, debridement, and obturation of the endodontic canal system, an intricate knowledge of which is a pre-requisite for accurate treatment.[1-3] In spite of the recent rapid advances in long-term form and function attributed to implant placement in dentistry for the replacement of missing teeth, this treatment option may yet be economically unattainable for many patients, particularly in developing countries. Many patients regard retention of their natural dentition as preferable, notwithstanding the biological advantages of bone maintenance.

Though endodontic therapy remains a viable and successful treatment option, maxillary (upper) permanent molar teeth have amongst the highest endodontic failure rates of all teeth, owing to the complexity of their root canal anatomy, and variations found in the morphology of their mesiobuccal (MB) roots.[2, 4-6] This root has been extensively researched,[7] and while most studies indicate the presence of a second MB canal (MB2), consensus is still lacking regarding its prevalence.[2, 3, 8-11] An inability to locate and adequately treat all canals of a root canal system during endodontic treatment, often leads to proliferation and apical spread of residual bacteria, thereby causing persistent infection, periapical inflammation, and ultimate treatment failure.[12]

The previously reported variation in MB2 prevalence has been attributed in part to the diverse and very disparate methods used to identify these canals. Methods used previously for the recognition of MB2 canals include radiography, tooth sectioning with dye staining, and magnification in the form of dental loupes and operating microscopes.[7, 13, 14] Studies have shown that MB2 detection rates vary considerably, with resultant failure to identify these clinically.[2, 13, 15, 16] In addition, the high incidence of locating these canals during endodontic retreatment cases, followed by uneventful healing, suggests that endodontic failure rates may correlate with the inability to detect additional canals at treatment onset.[12, 13]

Tooth root morphology is usually assessed on pre-operative periapical (PA) radiographs. These images are inadequate for the assessment of additional canals, such as MB2, as they are a two-dimensional (2D) representation of three-dimensional (3D) reality, subject to distortion by surrounding structures. New imaging technology allows for greater and more precise anatomical distinction. Cone beam computed tomography (CBCT) is a non-invasive, non-destructive, economically viable technique to reliably identify MB2 canals clinically at the diagnostic, pre-treatment stage. This is substantiated by previous reports.[6, 17-19] Many studies have reported the prevalence of MB2 canals by CBCT alone.[3, 5, 6, 11, 18, 20-23] The discrepancies in prevalence between reported studies are related to differences in methodology, age, geographical location, and CBCT systems. No such study has been performed within a South African subpopulation, the value of which would be the documented diversity of such morphological variance. These results may be compared with those from previous studies for enhanced anatomical knowledge and greater therapeutic success.

CHAPTER 2

2.0 LITERATURE REVIEW

Success in endodontics can only be achieved if the entire root canal system is adequately debrided, disinfected, and hermitically sealed. Clinicians need to be acquainted with the structure and form of these systems in various teeth, to achieve long-term endodontic success. Endodontic treatment of maxillary molar teeth is complicated by the diverse and intricate micro-anatomy of their root canal systems.[3, 23, 24] Endodontic failure in these teeth is attributed to incomplete root canal obturation, a complication very frequently associated with the lack of identification of additional canal spaces.[12, 13, 15, 25]

2.1 Maxillary molar root canal morphology and micro-anatomy

Therapeutic success relies on the inherent knowledge of the clinician of the morphological variances which exist at any site.[26] Failure to understand the internal anatomy of the root canal system in any tooth is directly linked to treatment failure.[27] The maxillary first molar has the most complex root canal morphology of all teeth, which can result in treatment failures. It is therefore the most researched tooth in the mouth and subjected to most investigations at present.[23, 26, 28]

One of the initial investigations detailing dental morphology, in particular pertaining to maxillary molar teeth, was performed in 1969 in which 208 extracted maxillary molar teeth were sectioned in order to expose their mesiobuccal root canal spaces. Four characteristic configurations were identified and subsequently classified as indicated in Table 1.[15, 24, 28]

Table 1: Canal configurations of the MB root of maxillary first molars

Type I	Single canal extending from the pulp chamber to apex
Type II	Two canals, larger buccal and smaller palatal, extending from the pulp chamber and merging 1-4mm from the apex into a single canal
Type III	Two distinct canals extending from the pulp chamber to the apex with two distinct apical foramina
Type IV	Single canal extending from the pulp chamber and dividing into two separate canals with separate apical foramina near the apex

Type I represented the simplest morphological form, comprising a single canal from pulp chamber to apex (48.5%). Type II (37.5%) comprised two separate canals converging into a single apical foramen. Types III and IV (14%) were the rarest forms respectively comprising of two morphologically separate and distinct canals, and a solitary canal emanating from the pulp with divergence in the apical aspect respectively.[15] These results were reiterated in another study using haematoxylin dye on 100 extracted maxillary first molar teeth, which had been subjected to alcohol dehydration followed by clearance of the pulpal contents/root canal system with liquid plastic resin, a process known as tooth clearing. This methodology has been favoured for many years, and results of this study showed canals to have Type I (45%), Type II (37%), or Type III (18%) configurations.[25]

The site of root canal origin is located at the cemento-enamel junction (CEJ), an important landmark indicating the site of the pulp chamber and canal orifices.[26] The location of the MB2 canal orifice is highly variable but most frequently mesiopalatally to the main MB canal.[15] Canal identification and negotiation is challenging as its orifice is often undetected due to the presence of a ledge of dentine.[26] This can be bypassed by introducing an endodontic file or explorer from a buccal and distal direction which crosses over the canal opening.[15] Clinically, the use of magnification, in the form of dental loupes or dental operating microscopes, may help locate MB2 canals. Studies have shown significant gains in levels of MB2 canal detection, both in initial treatment and retreatment cases, when magnification is used.[14, 29, 30]

2.2 Conventional radiography in root canal therapy

Radiographs are essential in all phases of endodontic treatment, namely the pre-operative, intra-operative, and post-operative phases.[18] The radiological image quality is clinically significant and the highest image resolution is thus advocated.[31] An accurate image of the dental tissues and surrounding bone is required for correct diagnosis, treatment execution, and appraisal of endodontic treatment outcome.[18, 28, 31]

Conventional (analogue) and digital PA radiographs are pre-operatively used for endodontic assessment of canal anatomy. These radiographs compress 3D structures into 2D images, resulting in a significant loss of diagnostic information and inhibiting the diagnostic ability of the observer.[32, 33] The morphological details provided by such imaging modalities hinders consideration of the sagittal relationships (bucco-lingual), as they are only able to project images in a lateral (mesio-distal) dimension.[21]

Canals that overlap each other are difficult to differentiate on radiographs, as these lie within the same plane as the x-ray beam.[34] This can be overcome by taking radiographs at various angulations of the central x-ray beam to the tooth/area in question, as demonstrated by using the parallax method, whereby the x-ray tube is shifted along the horizontal plane in 10-15^0 increments.[34, 35] This method is not recommended for detecting multiple canals, and more suited for detecting the bucco-lingual positioning of impacted teeth, especially impacted canines.[36]

The most accurate radiological images are obtained by using a paralleling technique, whereby the radiographic film or digital sensor is parallel to the tooth's long axis, and the x-ray beam is positioned at 90^0 to these. The precision of this technique compared with the bisecting angle technique is emphasized by the consistent and reproducible results it achieves.[34, 37] Anatomy affects the accuracy of the paralleling technique, often compromised by difficulty with film/sensor positioning, as occurs in the posterior maxilla due to limited space and increased gag reflex, resulting in poor patient tolerance.[34] Molar tooth roots are typically divergent or convergent, which may result in both image magnification or distortion, precluding the use of the paralleling technique.[34]

Anatomical structures in the posterior maxilla, such as the zygomatic arch or maxillary sinus, may conceal the region of interest. The presence of such structures is termed “anatomical noise” and complicates radiographic image analysis. Complex structures reduce contrast in the region of interest further, often requiring additional radiographs to be taken at different angles to obtain a clearer image.[34, 38 39]

2.3 Advanced imaging techniques in endodontics

The development of 3D imaging modalities has allowed clinicians to view areas of interest as 3D volumes, in which images thereby obtained may be edited and enhanced.[7] These images have high contrast and spatial resolutions which optimises their diagnostic value. Contrast resolution distinguishes between areas on an image with different densities, whilst spatial resolution refers to the ability to display different objects which are in close proximity to each other as being separate.[31] 3D imaging systems which have been utilized in endodontic research include computed tomography, micro-computed tomography, and cone beam computed tomography.

2.3.1 Computed tomography such as medical CT

Computed tomography (CT) has been used in the past for endodontic diagnosis with limited success. CT scanners are composed of a motorised platform which accommodates the patient, and a central rotating gantry with a circular aperture through which the patient is moved as the image is acquired.[19, 34] The first CT scanners consisted of a central gantry containing an x-ray source and a solid-state detector. A fan-shaped x-ray beam is transmitted in a slice-by-slice fashion through the patient in the axial plane, and these slices are then stacked together to form multiple 2D images of the scanned area.[40]

In modern CT scanners, the central gantry contains an x-ray source and multiple x-ray detectors which rotate around a patient as they are moved through the aperture on a motorised platform. This allows up to 64 (multiple) image slices to be taken simultaneously (multi-slice CT), thereby reducing scanning time and radiation exposure to the patient significantly.[19, 40] The obtained image slices are then stacked together and reformatted by specialised software which generates 3D images that can be viewed in multiple planes (coronal, sagittal, and axial). Although multi-slice medical CT can assess root canal anatomy, they require large and costly scanners which are often only located in hospital settings, emit high radiation doses to patients, have low spatial resolution which create streak or scatter artefacts when metallic objects, such as metallic restorations or posts, are present in the scanning area.[34] Nominal slice thickness in these scanners is limited to 200μm, which limits the resolution of fine endodontic structures, but some studies have shown it to be of diagnostic value in identifying MB2 canals.[39, 41]

2.3.2 Micro-computed tomography

Micro-computed tomography (MCT) has previously been used in endodontic research with excellent results.[42] An x-ray beam is directed through an object (tooth) towards an x-ray detector on the opposite side, which then captures and records non-absorbed x-rays as a single radiograph/projection image. The object is then rotated a fraction of a degree and another image is recorded in this new position. This process repeats itself until the tooth has completed a 360^0 rotation and all images have been acquired. The series of images are then stacked together and reconstructed into a 3D volume by sophisticated software.[43] Fine detail of both internal and external root morphologies can be accurately reproduced simultaneously, with exceptional resolution, in a non-destructive manner. Constant technological advancements have led to the development of high resolution desktop scanners able of detecting structural details at single-digit micron to nanometre resolution levels, which can be of great benefit in endodontic research.[44]

This technology, however, requires hundreds to thousands of scans to generate a 3D volume for an object, resulting in prolonged scanning and image processing at exceedingly high radiation dosages. Hence, MCT cannot be used clinically on live patients, but rather in laboratory (*in vitro*) studies on extracted teeth.[31, 45] Most endodontic research regarding the use of MCT has focused on instrumentation systems and techniques, with only two studies documenting on the canal morphology of the MB root of maxillary first molars.[7, 24]

2.3.3 Cone beam computed tomography

Cone beam computed tomography (CBCT) is currently the most commonly used imaging modality in endodontic research, and ever more prominent in clinical endodontics. CBCT scanners were specifically developed for scanning the maxillo-facial area at lower radiation doses than CT.[19] They were first introduced to the European market in 1996. At present over 40 different CBCT scanners are commercially available to clinicians for this use. CBCT use is predicted to rise in dental practice for its widespread applications and superior image qualities.[46]

Scanners are upright and composed of a rotating gantry containing an x-ray source and a sensor (detector), located on opposite sides.[18] The patient is situated between these, in a seated or standing position, while the source and sensor simultaneously rotate between 180-360 degrees around the patient's head, in much the same fashion as panoramic radiography.[47] A cone-shaped beam of radiation is emitted from the x-ray source and captures the entire 3D volume in a single rotation of the device.[17-19, 34, 40] This 3D volume of the region of interest (ROI) is known as the field of view (FOV), the size of which varies as per manufacturer and user settings.[19] A single rotation/sweep of the scanner around a patient's head produces a series of exposures, known as basis or projection exposures, which are recorded from multiple angles and positions.[34] Manufacturer-specific software is used to reconstruct the set of exposures, whereby axial (transverse), sagittal (cross-sectional), and frontal (coronal) anatomical planes can be simultaneously displayed on a computer screen, thus allowing for a 3D view of the ROI.[17, 40, 48]

As the entire volume is captured in a single rotation of the scanner, scanning times are reduced, thereby reducing effective radiation dosages to patients. These dosages are significantly lower than medical CT, however, they are still much higher than all conventional radiographic procedures. Further limitation of the FOV in order to capture small regions alone, results in an even greater reduction in radiation dosage.[6, 17, 21, 40, 49]

Data volume sets are composed of isotropic voxels (3D pixels), which are equal in height, length, and depth, resulting in geometric accuracy.[17] This allows for imaging of superior accuracy and precision. By comparison, medical CT images comprise anisotropic voxels which may cause image distortion.[18] For endodontic purposes, CBCT allows for more definitive visualization of root canal location and morphology. CBCT image resolution for such purposes should not exceed 200μm, which is the average width of the periodontal ligament space. Studies have, however, shown significantly higher MB2 detection rates with image resolution set at 120μm (0.12mm).[18] Newer scanners are now capable of achieving resolutions of between 75-80μm.[44]

Limitations of CBCT imaging include a lower spatial resolution than conventional radiographs, hence there is poor distinction between different objects of similar density in close proximity to each other.[17, 18] This has not proven to affect the detection of accessory canals, such as the MB2, within a tooth. CBCT images are prone to artefacts, which represent incidental findings on an image that do not occur naturally, but are dependent on the techniques used to acquire the images instead. Such artefacts are the result of deviations between the physical imaging procedure itself and mathematical algorithms utilised by the scanner software to reconstruct the 3D volume.[50] Possible causes of image artefacts in CBCT include the patient, the CBCT scanner system, and streaking artifacts from high density objects.[18]

Patients should remain completely immobile during the scanning process, as movement affects image quality. This is more critical in the acquisition of a scan with a limited FOV, as the x-ray beam is concentrated to a small region. Minute patient movements will negatively affect image quality, with greater obscurity and difficulty in distinguishing specific features or changes.[51] The CBCT system can produce image artefacts by partial volume averaging. This process occurs due to differences in contrast resolution between the scan slice thickness and the object being scanned when a reduced number of basis projection images are chosen for interpretation, and represents undersampling. Divergences of the x-ray beam as the scanner rotates around the patient in the horizontal plane can also occur, which is also known as the cone beam effect.[18, 50, 51] Streaking artefacts are termed “beam hardening”, which reflects differences in the absorption of x-ray photons between high density objects, such as metallic restorations, posts, and implants; and adjacent oral structures. This causes cupping artifacts, or light and dark bands (streaks) between dense objects, both of which reduce the diagnostic value of the image.[18]

The gold standard of MB2 detection is through physical tooth sectioning. Recent studies have found that CBCT is just as effective in detecting the presence of MB2 canals and is comparable to MCT, whilst being significantly more effective than conventional radiography.[6, 18, 21] A joint position statement by the American Association of Endodontists (AAE) and the American Academy of Oral and Maxillofacial Radiology (AAOMR), recommends the use of limited FOV CBCT as the imaging modality of choice in the initial treatment of teeth suspected to have additional complex root canal morphology, as is the case with maxillary molar teeth.[52]

2.4 In-vitro detection of the presence/absence of MB2 canals

The first study to investigate the configuration of the MB root of maxillary first molars was performed in 1914 and involved tooth sectioning. One hundred maxillary first molar teeth were injected with India ink and then sectioned in a bucco-lingual direction. The root canals were then exposed for examination, and revealed a prevalence of 63% for 2 or more canal systems in the MB root.[23, 44]

This was followed by other studies which employed clearing methods in order to reconstruct the root canal morphology. This process results in transparency of the dentine, enamel, and cementum, whilst internal (pulpal) structures are highlighted for analysis and was first achieved in 1925 by injecting 513 extracted first maxillary molar teeth with vulcanized rubber, which revealed an MB2 prevalence of 53.6%.[23, 44]

A prominent tooth clearing method was established in 1974,[53] a process which was later used to develop classifications of root canal structures based on their configurations.[25] This technique became the method of choice for numerous endodontic research reports. In short, freshly extracted teeth are fixed in 10% formalin and then decalcified in 5% hydrochloric acid followed by washing the teeth in tap water for 2 hours. This is followed by injection of a dye, usually haematoxylin, Chinese ink or India ink, into the pulp cavities. The teeth are then stored in a 5% hydrogen peroxide solution for 24 hours and then dehydrated in alcohol, then immersed in a clearing agent (such as methyl salicylate, xylene, or a liquid casting resin). To maintain their translucence, the teeth should remain immersed in the clearing agent, as they will revert to their former state of opaqueness once removed from this agent.[25, 44] This method allows for a three-dimensional view of the pulp cavity and its contents in-situ, as seen relative to the exterior tooth surface. As no instruments are used to navigate the pulp space and canals, the original form and relationship of the canals is largely maintained.[25]

Other *in-vitro* methods for detecting MB2 canals include: pulpal access with instruments (with/out radiographs), pulpal cavity gel infusion with radiography, endodontic treatment on extracted teeth with radiography, evaluating ground sections of teeth, and scanning electron microscopy of the pulpal floor.[23] Though these methods allow for direct visualization of canal spaces, they are technically demanding, time-consuming, and can only be performed on extracted teeth.

2.5 Prevalence of MB2 in retreated maxillary molars

A good indicator of the impact of missed MB2 canals lies in the assessment of differences in their detection following initial endodontic failure and retreated cases. Major causes of endodontic failure are attributed to a failure to recognise and adequately treat all canals of a root canal system.[12] It is an established fact, and accepted by most clinicians, that endodontic failure in maxillary molars is most likely the result of a failure to recognise and adequately treat MB2 canals.[4, 12, 15, 16, 23, 25, 26, 54] Prospective studies comparing endodontic treatment success whereby MB2 canals are located in maxillary molars but left untreated, to those where all canals are located and adequately treated, are ethically inappropriate to perform, resulting in a lack of scientific documentation to validate this assertion.[13]

Indications for non-surgical root canal retreatment as established by the American Association of Endodontists, are stipulated as follows:

- Sustained symptomatic periapical pathology
- Radiographic signs of inadequate root canal obturation with lingering periapical pathology and symptoms following completion of endodontic treatment
- Perseverance of initial clinical symptoms
- Restorative or prosthodontic procedures which may have compromised any previous root canal obturations
- Planned restorative or prosthodontic procedures on a tooth with suboptimal endodontic treatment
- Suspicion of salivary contamination causing bacterial leakage into a root canal system, as seen in cases with inadequate isolation[55]

Two published clinical studies have investigated the differences in incidences of MB2 canals in maxillary molars in initial endodontic treatments to failing maxillary molars requiring retreatments.[13, 30] Results of these studies indicated that these incidences were 67% for retreatments of first maxillary molars, and 59% for initial treatments on maxillary first molars. Thus, there were more MB2 canals located at endodontic retreatments of these teeth. These differences did not apply to second maxillary molars, as far fewer treatments had been performed on these teeth. Failure to locate and treat MB2 canals in maxillary first molar teeth is thus regarded as a significant prognostic factor for successful endodontic therapy.[13, 23, 30]

CHAPTER 3

3.0 AIM AND OBJECTIVES

3.1 Aim

The aim of this study is to determine the prevalence of MB2 canals in permanent maxillary molars in patients attending the Oral and Dental Hospital of the University of Pretoria, South Africa using CBCT.

3.2 Specific objectives

3.2.1 To determine the proportion of patients with an MB2 canal in the first permanent molar.

3.2.2 To document the demographic variables associated with MB2 canals in the first permanent molar (age, gender, position).

3.2.3 To investigate the proportion of MB2 canals in first maxillary molars occurring bilaterally compared with those that were adjacent to MB2 canals of second maxillary molar teeth.

3.2.4 To determine the proportion of patients with an MB2 canal in the second permanent molar.

3.2.5 To document the demographic variables associated with MB2 canals in the second permanent molar (age, gender, position).

3.2.6 To investigate the proportion of MB2 canals in second maxillary molars occurring bilaterally compared with those that were adjacent to MB2 canals of first maxillary molar teeth.

3.2.7 To determine the association between positive outcome and demographic variables (age, gender, position) for the overall data set.

CHAPTER 4

4.0 MATERIALS AND METHODS

4.1 Study design

This is a retrospective cross-sectional study utilizing 200 CBCT scans, taken over a three-year period, to determine the prevalence of MB2 canals in permanent maxillary molar teeth in patients attending the Oral and Dental Hospital of the University of Pretoria, South Africa.

4.2 Study population and sampling

One thousand two hundred and ten CBCT scans, from the CBCT database at the Oral and Dental Hospital, University of Pretoria, from 1 January 2014 to 31 December 2016, were examined. The inclusion and exclusion criteria were as stated below. Two hundred consecutive cases, representing one hundred female and one hundred male patients, which met all the criteria were included in the study. A letter of clearance was obtained from the biostatistician (Annexure A), through which the planned sample size of 200 patients was found to be adequate to estimate prevalence to an accuracy within 10% (0.1).

Inclusion criteria:

1. Patients above the age of 18 years with healthy maxillary permanent first and second molar teeth on both sides.

Exclusion criteria:

1. Teeth with open apices, fractures, periapical lesions, resorption, or

calcifications.

2. Developmental anomalies.

4.3 Measurements

The selected cases were all subjected to scanning with the Planmeca® ProMax 3D Max X-ray unit (Planmeca Oy, Helsinki, Finland), housed in the Oral and Dental Hospital of the University of Pretoria, South Africa. The study was performed using the manufacturer’s software.

Resolution for this unit is established via voxel sizes ranging from 100µm to 600µm with 300-750 basic frames. The focal spot size is 0.6x0.6mm. The anode voltage is 54 – 90 kV and the anode current is 1-14 mA. MB2 canals were identified using sequential axial, coronal, and sagittal slices by careful scrolling through the maxillary permanent molar teeth, from the pulp chamber to the apex. The maximum voxel size for each scan was 200µm, and optimal visualization was achieved by adjusting the brightness and contrast of the chosen image as required, with the systems software tools.

Thirty randomly chosen cases, representing 120 teeth, were assessed and examined by a second observer, a prosthodontist with over 20-years’ clinical experience. This was done to establish inter-observer agreement and reliability of the data.

4.4 Data analysis

Data was initially captured using Excel 2003 (Microsoft Office Excel 2003, Microsoft). Statistical analysis was performed by means of SPSS 23.0 software (IBM SPSS Statistics v23.0; IBM Corp, 2015). Prevalence of MB2 canals in permanent maxillary molars was recorded as a percentage along with a 95% confidence interval. The Pearson's chi-squared test was used to determine the association between MB2 and demographic variables, by which participants were specified as the random component, while age, gender, and tooth position were fixed.

Cohen's kappa coefficient was employed to measure inter-observer reliability. Values < 0.2 are regarded as none to slight agreement, 0.21 - 0.4 as fair agreement, 0.41 - 0.6 as moderate agreement, 0.61 – 0.8 as substantial agreement , and 0.81 – 1.0 as almost perfect agreement.[56] For medical and scientific research validity, inter-observer reliability should be above 0.6.[56]

Among patients with MB2 canals, the probability of contralateral and adjacent occurrence, was calculated for each tooth position. The ability of a prevalent MB2 canal to predict a MB2 canal in the contralateral and/or adjacent molar tooth was measured by determining the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), false-positive and false-negative values for each comparison. Sensitivity refers to the likelihood of having an MB2 canal in a contralateral or adjacent tooth in patients with an MB2 canal, while specificity refers to the likelihood of not having an MB2 canal in a contralateral or adjacent tooth in patients without an MB2 canal.[57, 58] PPV refers to the likelihood of a patient having an MB2 canal in a specific tooth when an MB2 canal is present in a contralateral or adjacent tooth (true positive), while NPV refers to the likelihood of a patient not having an MB2 canal in a specific tooth when an MB2 canal is not present in a contralateral or adjacent tooth (true negative).[57, 58] False-positive results represent patients who are incorrectly identified as having MB2 canals in a specific tooth, while false-negative results refer to patients with MB2 canals in a specific tooth who are incorrectly identified as not having an MB2 canal.[57] For statistical significance, testing was done at the 0.05 level of significance ($p < 0.05$).

4.5 Ethical considerations

Ethics approval was obtained by the Faculty of Health Sciences Research Ethics Committee of the University of Pretoria, South Africa (Annexure B).

Permission was obtained from the Manager of the Oral and Dental Hospital, for the analysis of dental CBCT scans done in the hospital (Annexure C).

Each patient was identified by their hospital file number and assigned a case number from 1-200. Identifying numbers will not be presented in the analyses of the results. Patient anonymity is being respected, and all personal information is being kept strictly confidential.

CHAPTER 5

5.0 RESULTS

Two hundred CBCT scans were included in the study, with an average patient age of 37 years, being similar for both females and males. A total of 800 teeth were analyzed, comprising 200 of each permanent maxillary first and second molar teeth on either side. CBCT scans were taken at voxel sizes not exceeding 200µm. Image contrast and brightness were adjusted by the scanner software for optimal image viewing. Each molar was scrolled axially from the orifice of the canal to the root apex several times until the presence or absence of an MB2 canal could be established with certainty (Figures 1 and 2).

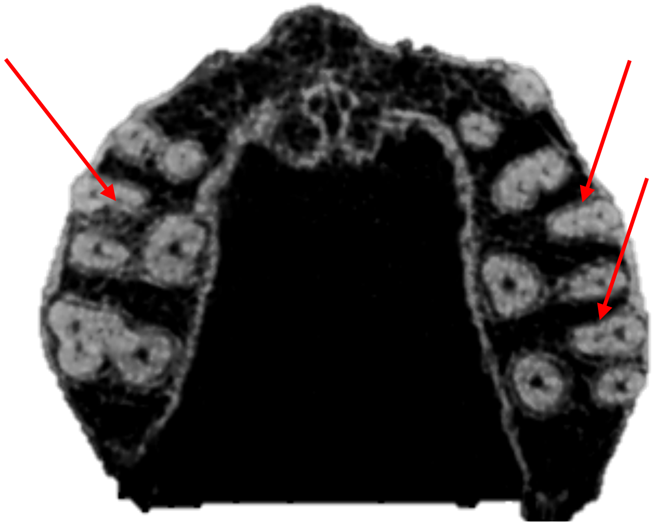


Figure 1: Axial section of the maxillary arch at middle-third root level of the maxillary teeth (note the presence of MB2 canals in teeth 16, 26, and 27 as indicated by the red arrows).

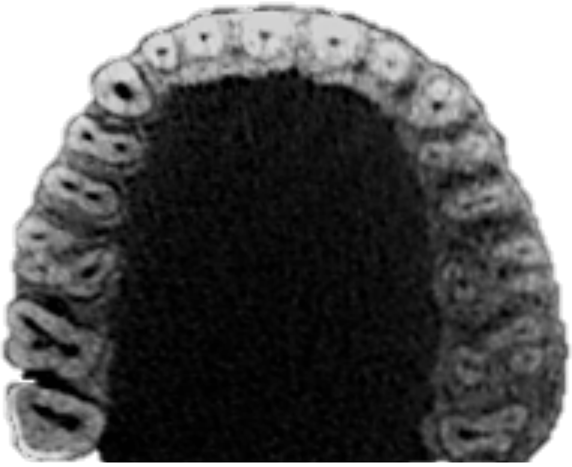


Figure 2: Axial section of the maxillary arch in which MB2 canals are absent in both the first and second maxillary molar teeth.

The prevalence of detecting an MB2 canal was as follows: 92% for permanent first maxillary molars in the first quadrant (16), 87% for permanent first maxillary molars in the second quadrant (26), 69% for permanent second maxillary molars in the first quadrant (17), and 65% for permanent second maxillary molars in the second quadrant (27). Testing was done at 95% confidence for statistical significance.

Inter-observer agreement for the measurements were 0.78, 1.00, 0.82, and 0.57 for teeth 16, 26, 17, and 27 respectively, as measured by Cohen’s kappa.

The prevalence of MB2 canals was also calculated according to patient gender (Table 2), and age for each tooth (Table 3). The Pearson’s chi-square test did not show a significant association between patient gender and the presence of MB2 canals, as well as between patient age and the presence of MB2 canals, for both first and second maxillary permanent molars.

Table 2: Prevalence of MB2 canals in the first and second permanent maxillary molars according to gender

Gender	n	Tooth 16 %	Tooth 26 %	Tooth 17 %	Tooth 27 %
Female	100	91	84	66	64
Male	100	93	90	72	66

Table 3: Prevalence of MB2 canals in the first and second permanent maxillary molars according to age

Age (y)	n	Tooth 16 %	Tooth 26 %	Tooth 17 %	Tooth 27 %
18-30	79	92.4	86.1	69.6	65.8
31-50	85	91.8	88.2	69.4	64.7
>50	36	91.7	86.1	66.7	63.9

Among patients with MB2 canals, the probability of contralateral and adjacent occurrence, was calculated for each tooth position. Contralateral occurrence of MB2 canals was found to be 97.13% for the permanent first maxillary molars (16,26) (Table 4), and 88.46% for the permanent second maxillary molars (17,27) (Table 5).

Adjacent occurrence was similar for both sides, 94.52% in the first quadrant (16,17) (Table 6), and 94.62% in the second quadrant (26,27) (Table 7).

Table 4: Contralateral occurrence of MB2 canals in permanent maxillary first molars (16, 26)

	16 with MB2 n (%)	**16 without MB2 n (%)**
26 with MB2	169 (97.13)	5 (2.87)
26 without MB2	15 (57.69)	11 (42.31)

Table 5: Contralateral occurrence of MB2 canals in permanent maxillary second molars (17, 27)

	17 with MB2 n (%)	**17 without MB2 n (%)**
27 with MB2	115 (88.46)	15 (11.54)
27 without MB2	23 (32.86)	47 (67.14)

Table 6: Adjacent occurrence of MB2 canals in permanent maxillary molars in the first quadrant (16,17)

	16 with MB2 n (%)	**16 without MB2 n (%)**
17 with MB2	138 (94.52)	8 (5.48)
17 without MB2	54 (87.10)	8 (12.90)

Table 7: Adjacent occurrence of MB2 canals in permanent maxillary molars in the second quadrant (26,27)

	26 with MB2 n (%)	**26 without MB2 n (%)**
27 with MB2	123 (94.62)	7 (5.38)
27 without MB2	51 (72.86)	19 (27.14)

The validity of a present MB2 canal to predict a MB2 canal on the contralateral side is presented in Table 8. With a sensitivity of 92%, a PPV of 97%, and the least false-positive and false-negative results, the likelihood of contralateral occurrence of MB2 canals in permanent first maxillary molars (16,26), was considerably better than for other tooth positions.

Table 8: Probability accuracy assessment of MB2 canals in different tooth positions

	Probability of an MB2 canal in the contralateral tooth		**Probability of an MB2 canal in the adjacent tooth**	
Test measurement	**16*/26**	**17*/27**	**16*/17**	**26*/27**
Sensitivity (%)	92	83	72	71
Specificity (%)	69	76	50	73
PPV (%)	97	88	95	95
NPV (%)	42	67	13	27
False-positive result (%)	3	8	4	4
False-negative result (%)	8	12	26	26

* Demarcates the reference tooth used in the calculation

CHAPTER 6

6.0 DISCUSSION

The present study examined the prevalence of MB2 canals, as detected on CBCT scanning images, in maxillary molars of patients attending the Oral and Dental Hospital of the University of Pretoria. Understanding root canal anatomy is essential for endodontic treatment success.[11, 16, 23, 59] Maxillary permanent molars, particularly the first molars, are one of the most commonly endodontically treated teeth, with clinical failure rates which are amongst the highest. This is due to the complexity of their root canal morphology and anatomy particularly in association with their MB roots. An inability to locate and adequately treat MB2 canals poses the greatest challenge to long-term endodontic treatment success in these teeth.[12, 13, 20, 26] The prevalence of MB2 canals in maxillary molar teeth is far greater than is currently known and their existence should be routinely highlighted and taught in endodontic training in order to increase the rate of treatment success.

6.1 Statement of principle findings

This study utilized CBCT to evaluate the prevalence of MB2 canals in permanent first and second maxillary molars in 200 patients attending a university hospital institution. Our results show up to 92% of right maxillary first molars (16) and up to 87% of left maxillary first molars (26) have MB2 canals. MB2 canals were identified less frequently in maxillary second molars in the region of 69% and 65% for the right (17) and left (27) maxillary second molars respectively.

Inter-observer reliability was tested by the assessment of thirty random CBCT scans by a second observer, a prosthodontist with over 20 years' clinical experience, in this study. The inter-observer agreement, as measured by Cohen's kappa, for teeth 16, 26, 17, and 27 were 0.78, 1.00, 0.82, and 0.57 respectively. Thus, for the 16 tooth position the level of agreement was moderate (0.60-0.79), almost perfect for the 26 tooth position (>0.90), strong for the 17 tooth position (0.80-0.90), and weak for the 27 tooth position (0.40-0.59).[56]

Our results did not show a significant association between patient gender and the prevalence of MB2 canals in maxillary first and second molar teeth. They also failed to show a significant reduction in prevalence of MB2 canals with age, as these were equally prevalent across all age groups. Research has shown that an increase in age may be associated with a reduction in size of the pulp cavity. This occurs due to continuous secondary dentin deposition, as well as increased peritubular primary dentin formation, leading to sclerotic dentin which obliterates the pulp.[60]

In patients with MB2 canals, the probability of these being found in contralateral teeth was 97% and 88% for the maxillary first and second molars respectively. The probability of occurring in adjacent teeth, that is between the first and second maxillary molars on either side, was 95%. This indicates that when MB2 canals are found on a molar, it is highly likely that these canals will also be present in adjacent and contralateral molars.

6.2 Critical analysis of research methodology and study limitations

CBCT has been established as the imaging method of choice in the preoperative assessment of teeth suspected of having complex anatomies, and represents the only reliable technique which may be used intra-operatively to detect the presence of MB2 canals.[21, 52] The analysis of CBCT images in our study produced a comprehensive, accurate, and reproducible set of quantitative data. Statistical analysis confirmed the reproducibility of the data, whilst correlation statistics confirmed little inter-observer variability for tooth positions 16, 26, and 17. A limitation of our study was the low inter-observer agreement achieved for tooth 27, which could have resulted from more complex root canal anatomy and morphology often associated with second molar teeth.[3]

The sample size of 200 patients consisted of 100 consecutive female and 100 consecutive male patient scans which met the inclusion criteria. This provided a sample number of statistical significance, whilst the analysis of consecutive cases provided consistency and eliminated bias. Whilst gender and tooth position were fixed, age was variable. A limitation of our study was that only 18% of patients representing our sample size were over the age of 50-years, and thus no reduction in MB2 prevalence, as expected with aging, was noted. However, our inclusion criteria required that both maxillary molar teeth be present bilaterally, which is expected to occur less frequently with increased age, as tooth loss is expected to be higher in this age group.

Our inclusion criteria also allowed us to examine the probability of MB2 canals occurring in contralateral and/or adjacent teeth. With very high levels of sensitivity, specificity, and precision, particularly in the 16 / 26 tooth positions, our results were shown to be accurate, which confirmed the suitability of our study design.

Detecting MB2 canals on maxillary first and second molars involved scrolling through the teeth several times axially from root canal orifice to root tip apically several times. This was done solely to establish whether MB2 canals were absent or present, and did not involve following the course of these canals. Thus, it was not established whether these canals remained separate from orifice to apex, or if they joined the main MB canal at any stage. This could have implications for treatment, as MB2 canals which join the main MB canal some distance away from the apex, may be inadvertently obturated, when only obturating the main MB canal on completion of endodontic therapy, resulting in a positive treatment outcome.

Scans that were included in our study required a minimum resolution of 200µm, which is the width of the periodontal ligament space. These scans were performed for a variety of investigations, which mainly included pre-operative implant assessment and facial trauma. Higher levels of isotropic voxel resolution may have provided clearer images of the MB2 canals, which could have enabled their classification into the different configurations which have been previously described.[25]

The retrospective analysis of CBCT scans in our study proved to be a non-invasive, reliable and accurate method for determining the prevalence of MB2 canals in maxillary molars. Our results provide a greater understanding of the levels of MB2 canal prevalence that can be expected in maxillary molar teeth.

6.3 Comparison of results with those observed in similar studies

A multitude of methods have been employed and documented for the determination of MB2 presence, both *in-vitro* and *in-vivo*. *In-vitro* studies require extraction of teeth often followed by destruction through sectioning, tooth clearing and root canal staining, and MCT analysis. These methods have formed the main reference standards in the determination of the presence or absence of MB2 canals.[3, 5, 11, 20, 22, 23, 25, 61-66] Results from these *in-vitro* studies have revealed an MB2 prevalence of between 90% (MCT)[7] and 93.5% (tooth clearing and root canal staining)[67], which compares favourably with our results (87-92%). The similarity of these results validates the findings in the present study and confirms that CBCT examination of teeth may be used for the detection of additional canals.

Methods which may be used *in-vivo* include analogue and digital radiography, the use of contrast media followed by radiological examination, clinical use of magnifying loupes, intra-operative microscopes and ultrasonics.[6, 23, 54, 67, 68] These methods have not been shown to be consistently successful in the determination of MB2 canals, as they are too reliant on subjective interpretation by clinicians. This has created a lack of consensus regarding the prevalence of MB2 canals, and conclusions obtained from these studies should be interpreted with caution.

Other methods of determining the prevalence of MB2 canals include the use of advanced imaging techniques, such as medical CT and CBCT. Studies have shown results from these studies to be comparable with those of tooth sectioning and MCT, the gold-standards for detecting MB2 canals, in terms of accuracy.[6, 21] As CBCT has become more widely available, is more economical, and produces images at significantly lower levels of ionising radiation (compared to medical CT), it has become the method of choice for locating MB2 canals.

There have been numerous CBCT studies investigating root canal morphology.[1, 5, 6, 11, 20-22, 69] Regarding the prevalence of MB2 canals in maxillary molars, our results (87-92%) are similar to another study (86-91%).[22] In that study, 100 male and female patients with healthy, untreated, and fully developed maxillary molars were assessed for the presence of MB2 canals. Criteria (inclusion and exclusion) were similar for both studies, as was the voxel size (200μm) for obtaining the CBCT images. This may explain the similarities in results.

Other CBCT studies, however, have shown significantly lower levels of MB2 canal prevalence. One study[3] which evaluated root canal configuration of maxillary molars by CBCT, reported a low incidence of MB2 detection (34-42%). Though these scans were obtained at similar voxel sizes of 200μm to our study, and the sample size was similar, the difference in results could be explained in terms of manufacturer software. Images from this study were obtained between 2010-2012, whereas our study's images were obtained from 2014-2016. Constant software developments and improvements of CBCT systems have greatly enhanced the resolution of images, especially when viewed (assessed) on a computer screen. Another possible explanation for the different results could be ethnic diversities, however, the comparative study mentioned in the previous paragraph, and the one mentioned here, were both carried out in the same country. Thus, ethnicity cannot be regarded as a possible reason for these differences.

CBCT parameter settings and software differences could also be the reason for reduced MB2 prevalence rates seen in other studies, such as 52% seen in a Chinese study carried out in 2009,[20] 64% in a Korean study carried out in 2011,[5] and 66% in a North American study carried out between 2007-2012.[11]

Our results did not show a significant association between patient gender and MB2 prevalence. This is similar to two other studies in the literature.[11, 22] A significant association between patient gender and MB2 prevalence have, however, been documented previously.[5, 67]

MB2 prevalence is reduced with increasing age, showing a statistically significant association.[5, 11, 20, 22, 23, 67, 69] This is to be expected, as dental structural changes occur with aging. The most significant of these is the continued deposition of secondary dentine, leading to dentinal sclerosis and pulpal recession.[60, 70] Thus, canals become obliterated as there is a reduction in pulpal volume, making it difficult to locate the MB2 canal, if it is at all present. Our study, however, had fewer patients over the age of 50, with oldest patient in our sample being 72-years old. Though, our results showed MB2 canals to be equally prevalent across all age groups, which is unexpected, this has also been documented in the study performed by Kim *et al*[5], whose oldest patient was 69-years old. Clinicians should therefore be made aware that MB2 canals can present at any age.

Adjacent and contralateral occurrence of MB2 canals has only been reported in one other study.[5] Our results of bilateral symmetrical occurrence of 97% and 88% for the maxillary first and second molars respectively, are significantly higher than that obtained in the study by Kim *et al,*[5] which showed an occurrence of 88% and 82% for the same tooth positions respectively. Probability of adjacent occurrence, that is between molars on the same side, was also significantly higher in our study (95%), when compared to the study by Kim *et al*[5](64%). Though the sample size of the study by Kim *et al*[5](n=351) was significantly higher than ours (n=200), their study occurred five years before ours. Continuous software improvements by CBCT manufacturers have allowed for improvements in image resolution, leading to greater diagnostic accuracy and precision.

6.4 Clinical and therapeutic implications

Documentation reaffirms that a greater prevalence of MB2 canals is present in patients than is known or conventionally taught. Of primary significance, should be the emphasis on the importance of being clinically aware of the presence of such canals, which may allow for better (improved) endodontic therapy, as well as allocation of sufficient treatment time. The overall outcome would thus be an improvement of endodontic success in the treatment of these teeth.

Additional benefits of this success would be retention of the natural dentition, which allows for retention of bone in the area with less incidences of periapical pathology, and maintain periodontal health in the region. Quality of life is improved, as optimal chewing and function is maintained. Preservation of oral health is maintained, especially in systemically compromised and elderly patients. This would negate the need for advanced surgery, such as dental implant placement, in these patients.

By reducing the need for dental implants, and apical surgery in the likelihood of treatment failure, there are further cost savings. Awareness of MB2 prevalence is also essential knowledge for any failed cases where surgical endodontics is being contemplated. All root canals, including accessory canals such as the MB2, must be adequately treated before performing any apical surgery. This should be taught at undergraduate level, and these canals should be actively sought pre-operatively when CBCT is used prior to treatment.

As CBCT utilises ionising radiation, there are radiation dosage implications. The ALARA principle[71] (as low as reasonably achievable) should always be applied to limit patient radiation dosage risk, and the smallest field of view should be chosen. The benefits of identifying accessory canals, however, still outweigh the risks of patient radiation exposure.

CHAPTER 7

7.0 CONCLUSION

In the present *in-vivo* CBCT study, it was shown that the prevalence of MB2 canals in permanent maxillary molars is very high, up to 92% in maxillary first molar teeth. The likelihood of these canals being present in contralateral and adjacent molar teeth was also found to be high. Though endodontic therapy remains a viable treatment option for most teeth with high levels of success, the reasons for treatment failure in maxillary molars may in part be attributed to a lack of knowledge regarding the prevalence of these canals, and difficulty in locating them intra-operatively. Untreated MB2 canals remain a source of persistent microbial infection and contamination contributing to endodontic treatment failure.

This study highlights the prevalence of MB2 canals in both first and second maxillary molar teeth, as well as the consistent coexistence of contralateral and adjacent MB2 canals. Furthermore, it also confirms the suitability of CBCT as an appropriate investigative imaging modality for pre-operative endodontic treatment planning. The awareness of this prevalence should be taken into account in endodontic treatment planning in order to improve the clinical success of endodontic therapy of these teeth.

CHAPTER 8

8.0 REFERENCES

1. Peters OA, Laib A, Ruegsegger P, Barbakow F. Three-dimensional analysis of root canal geometry by high-resolution computed tomography. J Dent Res. 2000; 79(6): 1405-9.
2. Zhang Q, Chen H, Fan B, Fan W, Gutmann JL. Root and root canal morphology in maxillary second molar with fused root from a native Chinese population. J Endod. 2014; 40(2): 871-5.
3. Silva EJ, Nejaim Y, Silva AJ, Haiter-Neto F, Zaia AA, Cohenca N. Evaluation of root canal configuration of maxillary molars in a Brazilian population using cone-beam computed tomographic imaging: an in vivo study. J Endod. 2014; 40(2): 173-6.
4. Degerness RA, Bowles WR. Dimension, anatomy and morphology of the mesiobuccal root canal system in maxillary molars. J Endod. 2010; 36(6): 985-9.
5. Kim Y, Lee SJ, Woo J. Morphology of maxillary first and second molars analyzed by cone-beam computed tomography in a Korean population: variations in the number of roots and canals and the incidence of fusion. J Endod. 2012; 38(8): 1063-8.
6. Blattner TC, George N, Lee CC, Kumar V, Yelton CD. Efficacy of cone-beam computed tomography as a modality to accurately identify the presence of second mesiobuccal canals in maxillary first and second molars: a pilot study. J Endod. 2010; 36(5): 867-70.
7. Verma P, Love RM. A Micro CT study of the mesiobuccal root canal morphology of the maxillary first molar tooth. Int Endod J. 2011; 44(3): 210-7.

8. Smadi L, Khraisat A. Root canal morphology of the mesiobuccal root in maxillary first molars of a Jordanian population. General Dentistry. 2006; 54(6): 413-6.

9. Shahi S, Yavari HR, Rahimi S, Ahmadi A. Root canal configuration of maxillary first permanent molars in an Iranian population. J Dent Res Dent Clin Dent Prospects. 2007; 1(1): 1-5.

10. Singh S, Pawar M. Root canal morphology of South Asian Indian maxillary molar teeth. Eur J Dent. 2015; 9(1): 133-44.

11. Guo J, Vahidnia A, Sedghizadeh P, Enciso R. Evaluation of root and canal morphology of maxillary permanent first molars in a North American population by cone-beam computed tomography. J Endod. 2014; 40(5): 635-9.

12. Cantatore G, Berutti E, Castellucci A. Missed anatomy: frequency and clinical impact. Endod Topics. 2006; 15(1): 3-31.

13. Wolcott J, Ishley D, Kennedy W, Johnson S, Minnich S. Clinical investigation of second mesiobuccal canals in endodontically treated and retreated maxillary molars. J Endod. 2002; 28(6): 477-9.

14. Stropko JJ. Canal morphology of maxillary molars: clinical observations of canal configurations. J Endod. 1999; 25(6): 446-50.

15. Weine FS, Healey HJ, Gerstein H, Evanson L. Canal configuration in the mesiobuccal root of the maxillary first molar and its endodontic significance. Oral Surg Oral Med Oral Pathol. 1969; 28(3): 419-25.

16. Kulild JC, Peters DD. Incidence and configuration of canal systems in the mesiobuccal root of maxillary first and second molars. J Endod. 1990; 16(7): 311-7.

17. Patel S. New dimensions in endodontic imaging: Part 2. Cone beam computed tomography. Int Endod J. 2009; 42(6): 463-75.

18. Scarfe WC, Levin MD, Gane D, Farman AG. Use of cone beam computed tomography in endodontics. Int J Dent. 2009; 2009(1): 1-20.

19. Patel S, Dawood A, Pitt Ford T, Whaites E. The pottential applications of cone beam computed tomography in the management of endodontic problems. Int Endod J. 2007; 40(10): 818-30.

20. Zhang R, Yang H, Yu X, Wang H, Hu T, Dummer PM. Use of CBCT to identify the morphology of maxillary permanent molar teeth in a Chinese subpopulation. Int Endod J. 2011; 44(2): 162-9.

21. Domark JD, Hatton JF, Benison RP, Hildebolt CF. An ex vivo comparison of digital radiography and cone-beam and micro computed tomography in the detection of the number of canals in the mesiobuccal roots of maxillary molars. J Endod. 2013; 39(7): 901-5.

22. Reis AG, Grazziotin-Soares R, Barletta FB, Fontanella VR, Mahl CR. Second canal in mesiobuccal root of maxillary molars is correlated with root third and patient age: a cone-beam computed tomographic study. J Endod. 2013; 39(5): 588-92.

23. Cleghorn BM, Christie WH, Dong CCS. Root and root canal morphology of the human permanent maxillary first molar: A literature review. J Endod. 2006; 32(9): 813-21.

24. Somma F, Leoni D, Plotino G, Grande NM, Plasschaert A. Root canal morphology of the mesiobuccal root of maxillary first molars: a micro-computed tomographic analysis. Int Endod J. 2009; 42(2): 165-74.

25. Vertucci FJ. Root canal anatomy of the human permanent teeth. Oral Surg. 1984; 58(5): 589-99.

26. Vertucci F. Root canal morphology and its relationship to endodontic procedures. Endod Topics. 2005; 10(1): 3-29.

27. Cohenca N, Shemesh H. Clinical applications of cone beam computed tomography in endodontics: a comprehensive review. Quintessence Int. 2015; 46(6): 465-80.

28. Barton DJ, Clark SJ, Eleazer PD, Scheetz JP, Farman AG. Tuned-aperture computed tomography versus parallax analog and digital radiographic images in detecting second mesiobuccal canals in maxillary first molars. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003; 96(2): 223-8.

29. Buhrley LJ, Barrows MJ, BeGole EA, Wenckus CS. Effect of magnification on locating the MB2 canal in maxillary molars. J Endod. 2002; 28(4): 324-7.

30. Wolcott J, Ishley D, Kennedy W, Johnson S, Minnich S, Meyers J. A 5yr clinical investigation of second mesiobuccal canals in endodontically treated and retreated maxillary molars. J Endod. 2005; 31(4): 262-4.

31. Nair MK, Nair UP. Digital and advanced imaging in endodontics: a review. J Endod. 2007; 33(1): 1-6.

32. Nance R, Tyndall D, Levin LG, Trope M. Identification of root canals in molars by tuned-aperture computed tomography. Int Endod J. 2000; 33(4): 392-6.

33. Webber RL, Messura JK. An in vivo comparison of digital information obtained from tuned-aperture computed tomography and conventional dental radiographic imaging modalities. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999; 88(2): 239-47.

34. Patel S, Dawood A, Whaites E, Pitt Ford T. New dimensions in endodontic imaging: part 1. Conventional and alternative radiographic systems. Int Endod J. 2009; 42(6): 447-62.

35. Clark CF. A method of ascertaining the relative position of unerupted teeth by means of film radiographs. Proc R Soc Med 1910; 3(Odontol Sectn): 87-90.

36. Gupta S, Jitender S, Tomar D. Evaluation of radiographic techniques for localization of impacted maxillary canines. Int J Stomatol Occlusion Med. 2015; 8(4): 97-104.

37. Forsberg J. A comparison of the paralleling and bisecting-angle radiographic techniques in endodontics. Int Endod J. 1987; 20(4): 282-6.

38. Huumonen S, Ørstavik D. Radiological aspects of apical periodontitis. Endod Topics. 2002; 1(1): 3-25.

39. Huumonen S, Kvist T, Gröndahl K, Molander A. Diagnostic value of computed tomography in re-treatment of root fillings in maxillary molars. Int Endod J. 2006; 39(10): 827-33.

40. Scarfe WC, Farman AG, Sukovic P. Clinical applications of cone-beam computed tomography in dental practice. J Can Dent Assoc. 2006; 72(1): 75-80.

41. Tachibana H, Matsumoto K. Applicability of x-ray computerized tomography in endodontics. Endod Dent Traumatol. 1990; 6(1): 16-20.

42. Nielsen RB, Alyassin AM, Peters DD, Carnes DL, Lancaster J. Microcomputed tomography: an advanced system for detailed endodontic research. J Endod. 1995; 21(11): 561-8.

43. Rhodes JS, Pitt Ford TR, Lynch JA, Liepins PJ, Curtis RV. Micro-computed tomography: a new tool for experimental odontology. Int Endod J. 1999; 32(3): 165-70.

44. Kato A, Ziegler A, Utsumi M, Ohno K, Takeichi T. Three-dimensional imaging of internal tooth structures: Applications in dental education. J Oral Biosci. 2016; 58(3): 100-11.

45. Swain MV, Xue J. State of the art of micro-ct applications in dental research. Int J Oral Sci. 2009; 1(4): 177-88.

46. Nemtoi A, Czink C, Haba D, Gahleitner A. Cone beam CT: a current overview of devices. Dentomaxillofac Radiol. 2013; 42(8): 20120443.

47. Cotton TP, Geisler TM, Holden DT, Schwartz SA, Schindler WG. Endodontic applications of cone-beam volumetric tomography. J Endod. 2007; 33(9): 1121-32.

48. Nesari R, Rossman LE, Kratchman SI. Cone-beam computed tomography in endodontics: are we there yet? Compend Contin Educ Dent. 2009; 30(6): 312-24.

49. Bornstein MM, Scarfe WC, Vaughn VM, Jacobs R. Cone beam computed tomography in implant dentistry: a systematic review focusing on guidelines, indications, and radiation risks. Int J Oral Maxillofac Implants. 2014; 29(Suppl): 55-77.

50. Schultze R, Heil U, Groß D, et al. Artefacts in CBCT: a review. Dentomaxillofac Radiol. 2011; 40(5): 265-73.

51. Soin-Neto R, Mudrak J, Matzen LH, Christensen J, Gotfredsen E, Wenzel A. Cone beam CT image artefacts related to head motion simulated by a robot skull: visual characteristics and impact on image quality. Dentomaxillofac Radiol. 2013; 42(2): 32310645.

52. Fayad MI, Nair M, Levin MD, et al. AAE and AAOMR joint position statement: use of cone beam computed tomography in endodontics 2015 Update. Oral Surg Oral Med Oral Pathol Oral Radiol 2015; 120(4): 508-12.

53. Vertucci F, Seelig A, Gillis R. Root canal morphology of the human maxillary second premolar. Oral Surg Oral Med Oral Pathol. 1974; 38(456-64.

54. Neaverth EJ, Kotler LM, Kaltenbach RF. Clinical investigation (in vivo) of endodontically treated maxillary first molars. J Endod. 1987; 13(10): 506-12.

55. Guide to clinical endodontics. 6th ed. Chicago: American Association of Endodontists, 2013, p. 15-7.

56. McHugh ML. Interrater reliability: the kappa statistic. Biochemia Medica. 2012; 22(3): 276-82.

57. Postma TC. Screening tools to prioritize routine dental care in an institutional environment. Mil Med. 2007; 172(12): 1287-92.

58. van Stralen KJ, Stel VS, Reitsma JB, Dekker FW, Zoccali C, Jager KJ. Diagnostic methods I: sensitivity, specificity, and other measures of accuracy. Kidney Int. 2009; 75(12): 1257-63.

59. Rwenyonyi CM, Kutesa AM, Muwazi LM, Buwembo W. Root and canal morphology of maxillary first and second permanent molar teeth in a Ugandan population. Int Endod J. 2007; 40(9): 679-83.

60. Morse DR. Age-related changes of the dental pulp complex and their relationship to systemic aging. Oral Surg Oral Med Oral Pathol. 1991; 72(6): 721-45.

61. al Shalabi RM, Omer OE, Glennon J, Jennings M, Claffey NM. Root canal anatomy of maxillary first and second permanent molars. Int Endod J. 2000; 33(5): 405-14.

62. Alavi AM, Opasanon A, Ng YL, Gulabivala K. Root and canal morphology of Thai maxillary molars. Int Endod J. 2002; 35(5): 478-85.

63. Gilles J, Reader A. An SEM investigation of the mesiolingual canal in human maxillary first and second molars. Oral Surg Oral Med Oral Pathol. 1990; 70(5): 638-43.

64. Imura N, Hata GI, Toda T, Otani SM, Fagundes MI. Two canals in mesiobuccal roots of maxillary molars. Int Endod J. 1998; 31(6): 410-4.

65. Pecora JD, Woelfel JB, M.D. SN, Issa EP. Morphologic study of the maxillary molars. Part II: internal anatomy. Braz Dent J. 1992; 3(1): 53-7.

66. Seidberg BH, Altman M, Guttuso J, Suson M. Frequency of two mesiobuccal root canals in maxillary permanent first molars. J Am Dent Assoc. 1973; 87(4): 852-6.

67. Sert S, Bayirli GS. Evaluation of the root canal configurations of the mandibular and maxillary permanent teeth by gender in the Turkish population. J Endod. 2004; 30(6): 391-8.

68. Hartwell G, Bellizzi R. Clinical investigation of in vivo endodontically treated mandibular and maxillary molars. J Endod. 1982; 8(12): 555-7.

69. Lee JH, Kim KD, Lee JK, et al. Mesiobuccal root canal anatomy of Korean maxillary first and second molars by cone-beam computed tomography. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011; 111(6): 785-91.

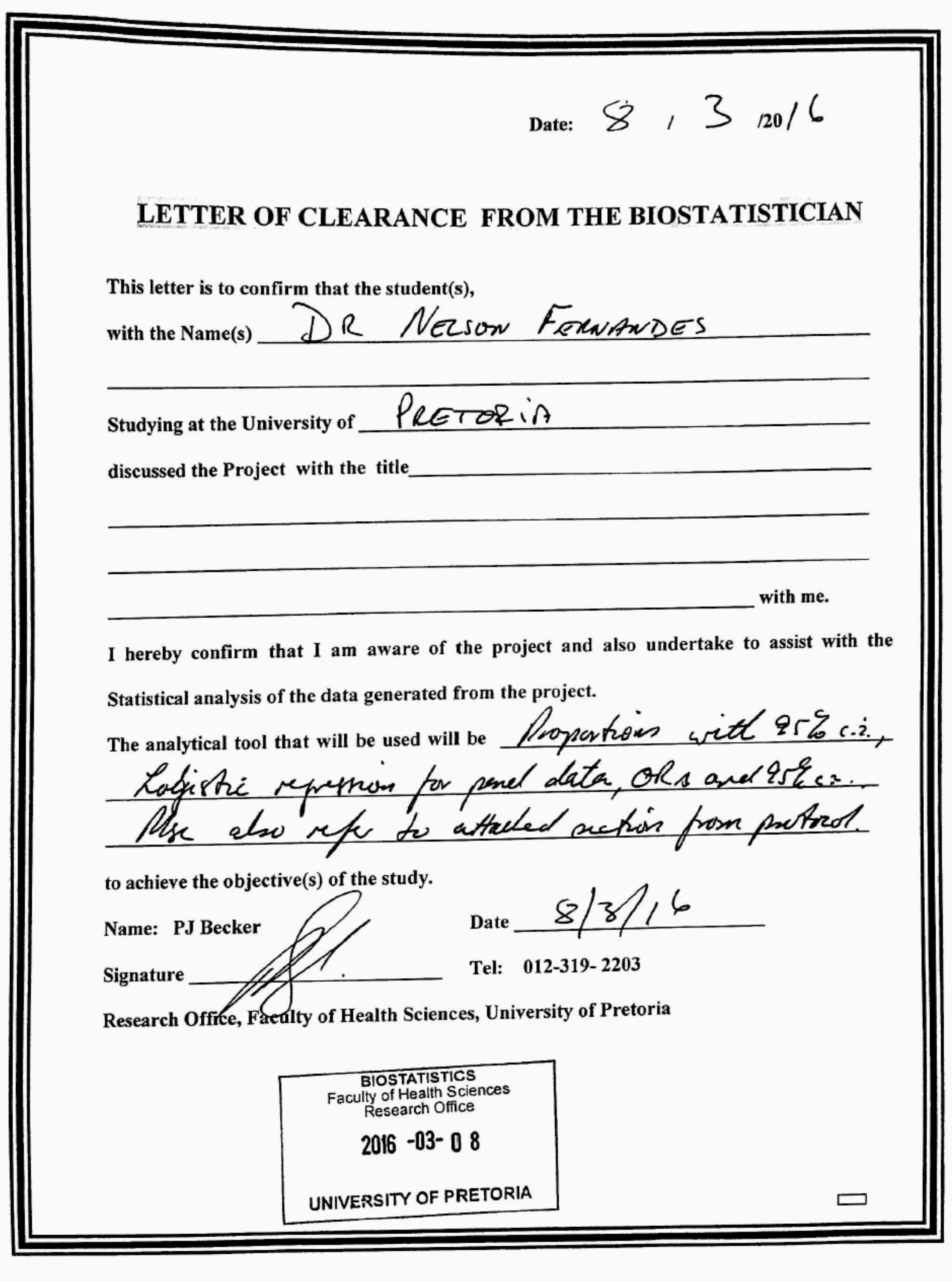
70. Carvalho TS, Lussi A. Age-related morphological, histological and functional changes in teeth. J Oral Rehabil. 2017; 44(4): 291-8.

71. Farman AG. ALARA still applies. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005; 100(4): 395-7.

CHAPTER 9

9.0 APPENDIX

9.1 Annexure A: Letter of clearance from the biostatistician



Date: 8 / 3 /2016

LETTER OF CLEARANCE FROM THE BIOSTATISTICIAN

This letter is to confirm that the student(s),

with the Name(s) DR NELSON FERNANDES

Studying at the University of PRETORIA

discussed the Project with the title

with me.

I hereby confirm that I am aware of the project and also undertake to assist with the Statistical analysis of the data generated from the project.

The analytical tool that will be used will be Proportions with 95% c.i., Logistic regression for panel data, ORs and 95% c.i. Plse also refer to attached section from protocol.

to achieve the objective(s) of the study.

Name: PJ Becker

Date 8/3/16

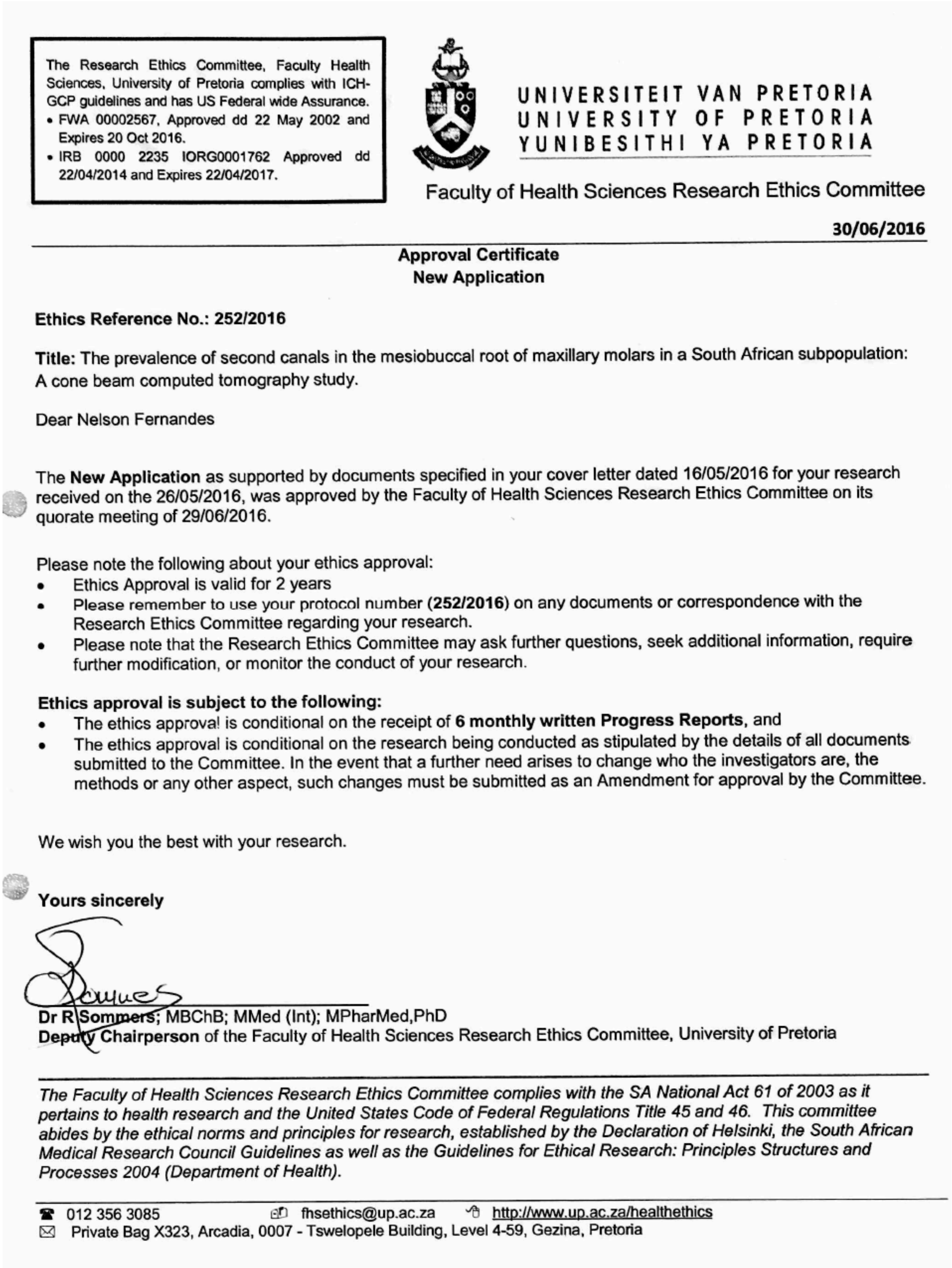
Signature

Tel: 012-319- 2203

Research Office, Faculty of Health Sciences, University of Pretoria

BIOSTATISTICS
Faculty of Health Sciences
Research Office
2016 -03- 0 8
UNIVERSITY OF PRETORIA

9.2 Annexure B: Research ethics committee approval



The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 20 Oct 2016.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 22/04/2017.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

30/06/2016

Approval Certificate
New Application

Ethics Reference No.: 252/2016

Title: The prevalence of second canals in the mesiobuccal root of maxillary molars in a South African subpopulation: A cone beam computed tomography study.

Dear Nelson Fernandes

The **New Application** as supported by documents specified in your cover letter dated 16/05/2016 for your research received on the 26/05/2016, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 29/06/2016.

Please note the following about your ethics approval:

- Ethics Approval is valid for 2 years
- Please remember to use your protocol number (**252/2016**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:

- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

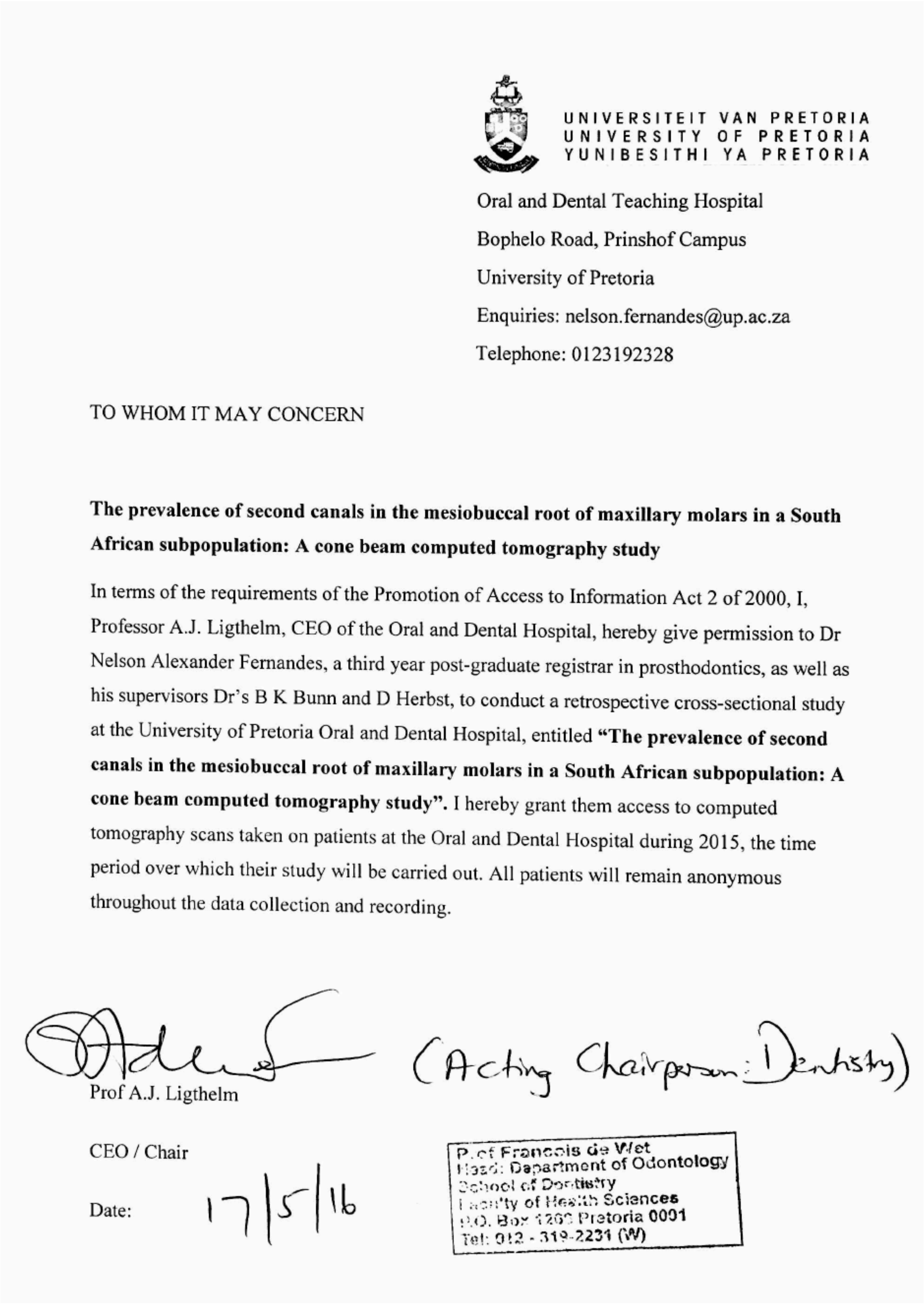
Yours sincerely

Dr R Sommers; MBChB; MMed (Int); MPharMed,PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

☎ 012 356 3085 fhsethics@up.ac.za http://www.up.ac.za/healthethics
✉ Private Bag X323, Arcadia, 0007 - Tswelopele Building, Level 4-59, Gezina, Pretoria

9.3 Annexure C: Consent letter from hospital manager



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Oral and Dental Teaching Hospital
Bophelo Road, Prinshof Campus
University of Pretoria
Enquiries: nelson.fernandes@up.ac.za
Telephone: 0123192328

TO WHOM IT MAY CONCERN

The prevalence of second canals in the mesiobuccal root of maxillary molars in a South African subpopulation: A cone beam computed tomography study

In terms of the requirements of the Promotion of Access to Information Act 2 of 2000, I, Professor A.J. Ligthelm, CEO of the Oral and Dental Hospital, hereby give permission to Dr Nelson Alexander Fernandes, a third year post-graduate registrar in prosthodontics, as well as his supervisors Dr's B K Bunn and D Herbst, to conduct a retrospective cross-sectional study at the University of Pretoria Oral and Dental Hospital, entitled **"The prevalence of second canals in the mesiobuccal root of maxillary molars in a South African subpopulation: A cone beam computed tomography study".** I hereby grant them access to computed tomography scans taken on patients at the Oral and Dental Hospital during 2015, the time period over which their study will be carried out. All patients will remain anonymous throughout the data collection and recording.

(Acting Chairperson: Dentistry)

Prof A.J. Ligthelm

CEO / Chair

Date: 17/5/16

Prof Francois de Wet
Head: Department of Odontology
School of Dentistry
Faculty of Health Sciences
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Tel: 012 - 319-2231 (W)