DENTAL AGE ESTIMATION IN MALAYSIAN ADULTS BASED ON VOLUMETRIC ANALYSIS OF PULP/TOOTH RATIO USING CBCT DATA.

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FACULTY OF DENTISTRY
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DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF DENTAL SCIENCE

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Field of Study: Forensic Odontology, Oral Biology, Oro-maxillofacial radiology.

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ABSTRACT

Introduction

Secondary dentine formation is one of the vital physiological age-related change that occurs in the permanent dentition throughout life. This physiological age-related factor can be used for dental age estimation and identification of unknown deceased persons.

Aims

To investigate the strength of association between chronological age and pulp/tooth volume ratio among the Malaysian population and to find which amongst these 3 types (Maxillary right canines, maxillary left canines, maxillary right central incisors) of investigated monoradicular teeth had the highest strength of correlation.

Materials and Methods

Three hundred CBCT scanned data of 153 males and 147 females' belonging to either Chinese or Malay ethnicity, stored in the Oral and Maxillofacial Imaging Division, Faculty of Dentistry University of Malaya were selected according to the quality, image acquiring parameters and age of the patients at the time of registration. The subjects were divided into 5 age groups (10 years interval each group), ranging from 16 to 65 years to ensure balanced sample distribution across the 5 groups. Subsequently 100 maxillary left canines, 100 maxillary right canines and 100 maxillary right central incisors having no pathology and fully developed roots were selected from the database of 300 CBCT scans. Hence, one tooth per CBCT scan was selected. Maxillary right and maxillary left canines were selected for the study in order to investigate if any significant difference in the strength of correlation occurred between two similar types of teeth belonging to different

subjects. CBCT data was acquired using the i-CAT Cone Beam 3D Dental Imaging System (Imaging Sciences International, Hatfield, USA). The scans selected had exposure parameters of 120 KV, 18 mA and the scans were acquired using voxel size of 0.30 mm and scanning time of 20 sec. Volumetric analysis was performed with MIMICS software (Materialise NV, Belgium, version 16.0). Data was analysed using SPSS version 20.

Results

The Pearson correlation and regression analysis showed a significant inverse association between pulp/tooth volume ratio and chronological age for all the 3 types of investigated teeth ($p<0.01$). Results showed the highest coefficient of determination (R2) values for maxillary central incisor (R2=0.696) followed by maxillary right canine (R2=0.545) and maxillary left canine (R2=0.527). The strength of correlation for both male and female was found to be very good. Fisher Z test results showed no significant difference in the correlation coefficient values between genders for all the 3 types of investigated teeth and for the whole sample (Z critical 1.96 for $p < .05$). Fisher Z test indicated no significant difference in the correlation coefficient values between maxillary right and maxillary left canines (P=0.88), despite these teeth being selected from different individuals.

Conclusion

The present study reinforced that pulp/tooth volume ratio with age is a valuable indicator for dental age estimation for Malaysian population. Maxillary right central incisor has shown the highest coefficient of determination value among all 3 types of investigated teeth. Furthermore, the results have showed that this method of dental age estimation is indeed gender independent.

ABSTRAK

Pengenalan

Pembentukan dentin sekunder merupakan salah satu perubahan fisiologi penting yang berkaitan dengan usia. Ini berlaku dalam pergigian kekal sepanjang hayat seseorang. Faktor ini boleh digunakan untuk anggaran usia pergigian dan mengenal pasti si mendiang yang tidak dikenali.

Matlamat

Matalamt utama adalah untuk menyiasat kekuatan hubungan antara umur kronologi dan nisbah volumetrik pulpa / gigi di kalangan penduduk Malaysia. Tambah lagi, tujuan ini adalah untuk mencari yang mana antara 3 jenis gigi monoradikular yang diselidik mempunyai kekuatan korelasi tertinggi.

Bahan dan Kaedah

Tiga ratus data CBCT, iaitu sebanyak 153 laki-laki dan 147 perempuan (keturunan Cina atau Melayu), yang disimpan di Bahagian Imaging Mulut dan Maksiolofasial, Fakulti Pergigian Universiti Malaya telah dipilih untuk kajian ini. Faktor-faktor utama untuk pemilihan adalaah kualiti imej, kaedah-kaedah memperolehi imej yang sama dan umur pesakit pada masa pendaftaran. Subjek-subjek dibahagikan kepada 5 kumpulan (selang 10 tahun setiap kumpulan). Mereka adalah diantara 16 hingga 65 tahun dan jaminan telah dibuat untuk memastikan pengedaran sampel yang seimbang di dalam 5 kumpulan tersebut. Selepas itu, 100 kanin kiri maksila, 100 kanin kanan maksila dan 100 insisor sentral maksila yang tidak mempunyai apa-apa patologi dan akarnya telah membentuk dengan sempurna dipilih dari pangkalan data 300 CBCT tersebut. Dengan ini, satu gigi bagi setiap imbasan CBCT dipilih. Gigi

kiri dan kanan maksila telah dipilih untuk kajian ini untuk menyiasat jika terdapat perbezaan yang signifikan dalam kekuatan korelasi di antara dua jenis gigi yang sama, tetapi tergolong dalam subjek yang berbeza. Data CBCT diperolehi menggunakan Sistem Pengimejan Pergigian 3D i-CAT Cone Beam (Imaging Sciences International, Hatfield, Amerika Syarikat). Data imbasan dipilih mempunyai parameter pendedahan 120 KV, 18 mA dan menggunakan saiz voxel 0.30 mm dan masa pengimbasan sebanyak 20 saat. Analisis volumetrik dilakukan dengan perisian MIMICS (Materialize NV, Belgium, versi 16.0). Data dianalisis menggunakan SPSS versi 20.

Keputusan

Analisis korelasi dan regresi Pearson menunjukkan hubungan songsang yang signifikan di antara nisbah volumetrik pulpa dan gigi dengan umur kronologi untuk semua jenis gigi yang disiasat (p <0.01). Keputusan ini menunjukkan nilai koefisien penentuan tertinggi (R2) untuk incisor sentral maksila (R2 = 0.696) diikuti oleh kanin kanan maksila (R2 = 0.545) dan kanin kiri maksila (R2 = 0.527). Kekuatan korelasi untuk lelaki dan wanita didapati sangat baik. Hasil ujian Fisher Z tidak menunjukkan perbezaan yang signifikan dalam nilai koefisien korelasi di antara jantina untuk semua 3 jenis gigi yang dikaji (Z kritikal 1.96 untuk p <.05). Ujian Fisher Z tidak menunjukkan perbezaan yang signifikan dalam nilai koefisien korelasi di antara kanin kanan dan kanin kiri maksila (P = 0.88), walaupun gigi ini dipilih dari individu yang berbeza.

Kesimpulannya

Kajian ini memperkukuhkan bahawa nisbah volumetrik pulpa / gigi dengan umur adalah petunjuk penting untuk anggaran usia pergigian bagi penduduk Malaysia. Gigi insisor sentral maksila menunjukkan nilai koefisien penentuan tertinggi di antara semua 3 jenis gigi yang diselidik. Tambah pula, hasil kajian menunjukkan bahawa kaedah penilaian pergigian ini bebas daripada pengaruh jantina.

Dedicated to:

My father, Asif Ullah khan

My mother, Dilras Zafar

My beloved wife, Iqra

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In the name of Allah, most gracious, most merciful

First of all, I would like to thank the Almighty Allah for granting me the strength and knowledge to complete this research. I pray that the blessings of Allah upon me to continue throughout my life so that I can accomplish my future research projects for the wellbeing of humanity.

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LIST OF SYMBOLS AND ABBREVIATIONS

CBCT	: Cone Beam Computed Tomography
MIMICS	: Materialise Interactive Medical Image Control System
2D	: Two dimensional
3D	: Three dimensional
OPG	: Orthopantomogram
R2	: Coefficient of determination
R	: Correlation coefficient
Micro- CT	: Micro Computed Tomography
ROI	: Region of interest
SPSS	: Statistical package for the social sciences
ICC	: Intraclass correlation coefficient
PTV	: Pulp tooth volume
DICOM	: Digital imaging and communications in medicine
VRML	: Virtual reality modeling language
PLY	: Polygon file format

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

The importance of age estimation in clinical practice, forensic medicine and anthropology cannot be underestimated. Studies around the world have been conducted to find an accurate and simple method of dental age estimation (Bang & Ramm, 1970; Kvaal et al., 1995; Gustafson, 1950; Someda et al., 2009). The refugee crisis is rapidly increasing throughout the world due to global conflicts and terrorism. Dental age estimation is one of the most reliable method amongst other parameters for age estimation and identification of unknown deceased persons. The tooth is a highly-calcified tissue which can withstand mechanical, chemical and thermal forces in harsh conditions (Chun et al., 2014; Shekhawat & Chauhan, 2016; Merlati et al., 2004). This aspect is very crucial during natural or manmade disasters, where most of the bodies are mutilated or incinerated. Dental age estimation of unknown deceased bodies is a very significant step in the reconstruction of biological profiles of these dead persons.

The developmental pattern of human dentition is specific, and we can estimate the age of a growing individual by observing this pattern (Schour & Massler, 1940; Moorrees et al., 1963). But once the development of teeth is completed, physiological age-related changes in the adult human dentition e.g. secondary dentin deposition, root translucency, cementum apposition, root resorption can be used for age estimation (Paewinsky et al., 2005; Gustafson, 1950; Dalitz, 1963). Secondary dentine formation is one the reliable methods to estimate age as it forms throughout life once root formation is completed. Many two-dimensional radiographic techniques have been used to measure secondary dentine formation (Arora et al., 2016; Paewinsky et al., 2005). However, it is not appropriate to measure three-dimensional object with two-dimensional radiographs.

The introduction of CBCT and image enhancing softwares in the past decade has dramatically improved the techniques to measure various aspects of three-dimensional objects. These innovative image enhancing softwares are being introduced regularly, which can successfully measure three dimensional changes in the tooth. CBCT technology has improved with more effective greyscale thresholding and segmentation values. The use of CBCT for measuring secondary dentine formation to estimate age has been reported on different populations around the world (Vandevoort et al., 2004; Yang et al., 2006; Someda et al., 2009; Ge et al., 2015). Most of the studies have reported different regression equations and strength of correlation between chronological age and pulp/tooth volume ratio for their respective populations. Some studies have reported highest strength of correlation between chronological age and pulp/tooth ratio for permanent central incisors amongst the single-rooted teeth (Vandevoort et al., 2004; Someda et al., 2009; Agematsu et al., 2010; Star et al., 2011). In addition, volumetric study on multi-rooted teeth have also been reported (Ge et al., 2016). Maxillary second molar showed the higher strength of correlation among single and multi-rooted teeth, when only pulp chamber volumes were taken into consideration.

So far, different non-invasive methods to estimate the dental age among Malaysian adults have been reported (Marroquin Penaloza et al., 2016). However, no study has been reported on volumetric measurements of pulp/tooth volume ratio using CBCT images. They applied Kvaal's method of dental age estimation on the volumetric data acquired through CBCT scans. However, this methodology was time consuming as compared to the original approach of Kvaal's et al., (1995) method and the values obtained for standard error of estimates were beyond the acceptable range. Therefore, there is a need to find a simple and reliable method of dental age estimation. More research needs to be carried out to investigate the relationship between chronological age and pulp/tooth volume ratio among different populations with different types of teeth.

1.1 Statement of problem

Physiological age-related changes occur in the dental tissues throughout life. Once the development of the teeth and jaws is completed then we can estimate the dental age by observing these physiological age-related changes. Secondary dentine deposition is one of the physiological age-related parameter for dental age estimation. With increase in age, the volume of the pulp cavity decreases due to the deposition of secondary dentine. This change in the secondary dentine formation can be measured by observing the volumetric changes in the pulp cavity from CBCT images with image enhancing software like MIMICS. There is no evidence in the literature that volumetric study has been carried out on the Malaysian population for dental age estimation with the help of any 3-D imaging modality.

1.2 Rationale and Aim

Volumetric analysis using CBCT scans with the help of MIMICS software is currently one of the most accurate way to determine the pulp/tooth volume ratio. Since single rooted teeth proved more accurate in dental age estimation, it is worthwhile to study the pattern of physiological age changes employing pulp/tooth volume ratio of maxillary canines and central incisors for age assessment purposes. The aim of the study is to develop a regression equation for dental age estimation in a Malaysian population using pulp/tooth volume ratio in maxillary canines and maxillary right central incisors from CBCT scans with the help of MIMICS software.

1.3 Objectives of the study

The study will focus on the following specific objectives:

1. To investigate the linear association between chronological age and pulp/tooth volume ratio of the three investigated teeth viz; maxillary right canine, maxillary left canine and maxillary right central incisor.

2. To compare the strength of correlation between genders with respect to maxillary left canine, maxillary right canine and maxillary right central incisor.

3. To investigate which amongst the 3 types of investigated teeth has the highest strength of correlation between chronological age and pulp/tooth volume ratio.

4. To compare the strength of correlation values between maxillary right and maxillary left canines, each belonging to different subjects.

1.4 Research questions

1. Is there any association between volume of the pulp-tooth ratio with chronological age?

2. Is there any difference in the pattern of volumetric changes between genders?

3. Which among the 3 types of investigated teeth is showing highest strength of correlation between chronological age and pulp/tooth volume ratio?

4. Is there any significant difference in the pattern of volumetric changes with age between maxillary left and right canines?

1.5 Significance

This is the first local study on dental age estimation by volumetric analysis of pulp-tooth ratio on Malaysian population (Malays and Chinese). The present study focused on an easy, accurate and more likely inexpensive method of dental age estimation. This approach will aid in the identification of unknown deceased persons involved in mass disasters which can be natural or manmade, especially in the third world countries, where DNA profiling is not possible on such a large scale. This approach will be useful in identification and age estimation of unknown persons for the implementation of law in legal justice system. The results will give the regression equation particularly for the Malaysian population for dental age estimation, which will help in the age estimation and identification of unknown deceased persons in future. The findings will also indicate whether there is any gender variation for dental age estimation by this method among the Malaysian population. This method of dental age estimation is less-invasive.

CHAPTER 2: LITERATURE REVIEW

2.1 Dental age estimation methods in children

2.1.1 Specific pattern of development of teeth

The development or mineralisation of teeth is one of the most reliable indicators of chronological age, as the maturation of teeth is least influenced by environmental and nutritional deficiencies. The growth pattern of teeth and jaws is specific. By observing this specific pattern, dental age can be estimated at that particular phase of development. Different studies around the world have been carried out to estimate the age at the growing stages of teeth and jaws.

2.1.1.1 Tooth eruption chart comparisons

Observing total number and type of erupted teeth in the oral cavity is the simplest method of dental age assessment. From as early as 6 months to 20 years of age, the sequence of tooth eruption and maturation follows the specific chronological order and can be trusted to estimate dental age.

2.1.1.2 Atlas style and scoring systems for dental age estimation.

The mineralisation of deciduous dentition starts at 16th week of intrauterine life and around 30th week, mineralised cusp tips of A, B, C, D and E can be observed on radiographs. Just before birth the mesial cusp tip of the mandibular first permanent molar can be observed on radiographs. It is the only permanent tooth whose calcified mesial cusp tip can be visualised on radiograph just before birth. Radiographic dental age assessment methods or models have been developed in the past two centuries by observing pattern of teeth development and eruption (Schour &

Massler, 1940; Ubelaker & Grant, 1989; Moorrees et al., 1963; Demirjian et al., 1973)

According to Schour and Massler's (1940) model, dental age can be estimated from the 5th months of intrauterine life to 35 years of age with the help of diagrammatic representation of teeth development atlas. The developmental pattern on dental panoramic radiographs (OPG) were compared with these diagrammatic developmental phases in order to estimate age. This model has been applied on many different populations for dental age estimation (Cesário C, 2016; Eshitha et al., 2014). Schour and Massler's (1940)-method is simple and non-invasive, although the diagrammatic atlas does not give gender specific representation for comparison. However, this shortcoming was addressed in another study (Kahl & Schwarze, 1988) where they introduced gender specific diagrammatic representation.

Other methods involved scoring stages of tooth development and maturity scores at particular stage of teeth eruption (Moorrees et al., 1963; Demirjian et al., 1973). Demirjian standards used seven mandibular teeth for maturity scoring and his model consisted of eight stages of tooth development ranging from A to H. Moorrees et al. (1963) method was designed for maxillary incisors and eight mandibular teeth using written and pictorial methods for dental age estimation. Moorrees et al. (1963) developed maturity scores into fourteen stages. Both of these methods gave gender specific details of age estimation. Studies have been reported on different populations using Demirjian et al. (1973) method of dental age estimation such as, Australian (Blenkin & Evans, 2010), Turkish (Tunc & Koyuturk, 2008), Indian (Koshy & Tandon, 1998), South African (Phillips & van Wyk Kotze, 2009) and Belgian population (Chaillet et al., 2004). All of the above-mentioned studies revealed some degree of overestimation in chronological ages. It must be noted here that each population gave different results which showed that populations belonging to different race must have their own regression equations or models for dental age estimation. More validation studies were reported for Demirjian et al. (1973) method as compared to Moorrees et al. (1963) method of dental age estimation. However, as Demirjian et al. method is based on giving maturity scores to all seven mandibular teeth, any missing tooth will not allow this method to be used. In contrast to Demirjian et al. (1973) method, Moorrees et al. (1963) technique followed tooth specific scoring system which was more practical when compared to Demirjians et al., (1973). However, this method was based on graphical comparison rather than using proper normative data to be statistically analysed. Researchers introduced modified graphs of Moorrees, designed to be analysed statistically with mean ages of tooth eruption along with standard deviations (Harris & Buck, 2002). Alqahtani et al. introduced detailed atlas on the basis of teeth developmental pattern from twenty-eight weeks of intrauterine life to twenty-three years of age (Alqahtani et al., 2010). Their model was based on the modified Moorrees et al. (1963) method of assessing teeth development. However, this atlas does not provide statistically quantified data which is a huge disadvantage for dental age estimation in forensic sciences.

Modified Demirjian's model was introduced by Willems et al. (2001) and they introduced adapted scoring system. The scoring system was more accurate than the original model as the new model addressed the persistent overestimation in the chronological age (Willems et al., 2001).

2.1.1.3 Application of atlas style and scoring methods on Malaysian population

Demirjian's method is widely used for dental age estimation among children across the world. Moreover, this method has been reported on Malaysian population as well

(Abu Asab et al., 2011; Mani et al., 2008). Both studies have resulted in overestimation of dental age among Malaysian children.

2.2 Juvenile verses adult dental age estimation

The time of eruption of third molar is vital in accessing the age at adulthood. A lot of research has been carried out to find the relationship of third molar development with chronological age.

2.2.1 Radiographic evaluation of third molar for dental age estimation

Third molar development is vital in establishing the age of adulthood. Studies have been carried out to find a relationship between third molar development and chronological age (Orhan et al., 2007; Bolanos et al., 2003). Radiographic assessment of periodontal membrane in third molars were studied by Olze et al.(Olze et al., 2010). This study developed gender specific 4 stages and concluded that age of 18 years can be estimated with reasonably accuracy using these staging systems.

2.2.2 Relationship between chronological age and third molar development in Malaysian population

Relationship between chronological age and third molar development was reported among Malaysian population (Nambiar, 1995; Johan et al., 2012). Third molar development can be used for dental age estimation in Malaysian population. Males showed more advanced development and the pattern of root mineralisation was faster in males than females by 6 months (Johan et al., 2012). The development of teeth was scored according to the Demirjian's method from A to H (A-D for crown development and E-H for root development).

2.3 Pre-natal, neonatal and post-natal age estimation

The radiographic evidence of mineralisation of deciduous teeth starts at 16th week of intrauterine life. However, tooth germs can be seen as radiolucent areas even before mineralisation starts. Kraus & Jordan (1965) introduced 10 stages of early mineralisation in deciduous dentition and permanent first molar (Kraus & Jordan, 1965). Mesial cusp of permanent mandibular first molar is the only mineralised segment among all permanent teeth which can be seen on radiograph just before birth.

2.4 Physiological age-related changes after completion of teeth development

The pattern of development of teeth is specific and the development of teeth is completed up to 18-21 years. When the growth of the permanent teeth is completed then the age estimation is performed through observing different physiological age-related changes e.g. attrition, apical migration of periodontal ligament, deposition of secondary dentin, cementum apposition, root resorption and transparency of the root dentin. Point system was introduced for different physiological age-related structural changes to predict age (Gustafson, 1950). He reported six structural changes that take place as life progresses, which are as following:

1. Secondary dentine formation

2. Attrition.

3. Apical migration of periodontal ligaments.

4. Cementum apposition.

5. Root resorption.

6. Root transparency.

The point system introduced by Gustafson ranged from 0-3 scores depending on the physiological change with ageing. Gustafson added all the scores that he measured from each of the six physiological factors and a regression line was derived. These changes reported by Gustafson were very important findings but measuring these changes with reasonably accuracy was a big challenge for researchers at that time. The relationship between age and physiological changes reported by Gustafson were further tested by many researchers (Johanson, 1971; Dalitz, 1963; Bang & Ramm, 1970).

2.4.1 Criticism on Gustafson method of age estimation

Cementum formation, root resorption and apical migration of periodontal ligaments were difficult to score due to lack of any definite pattern at each stage in Gustafson point system. Maples (1978) reported that root resorption is the least reliable out of all the six-physiological age-related changes reported by Gustafson (1950) (Maples, 1978). He suggested that secondary dentine formation and transparent dentine together can gave more accurate results as compared to using all the six variables for dental age estimation. Another objection which was raised in Gustafson four-point system was the value of standard error (4.5 years) which was found to be lower than the actual values (Maples & Rice, 1979).

2.4.2 Modified Seven-staging system by Johanson

Johanson (1971) modified Gustafson's method to estimate age by multiple regression analysis and proposed a more accurate formula for age estimation. He gave a more comprehensive staging system by assigning each variable seven stages based on severity or extent of physiological change. Standard error reported by Johanson was 5.16 years.

2.4.3 Testing the relationship between chronological age and physiological age-related variables.

Gustafson method of age estimation by adding scores of all the six variables was further investigated by examining 146 anterior teeth of individuals ranging in age from 14 to 76 years (Dalitz, 1963). The new 5-point system suggested by Dalitz had greater accuracy than Gustafson model. Premolars and molars were not investigated in the 5-point system. Root resorption and secondary cementum related least with age in the study. Bang and Ramm (1970) conducted a microscopic study to investigate the changes in root dentine translucency with age. Root dentin translucency begins in the apical part of the root and increases in the coronal direction. However, the tooth needed to be sacrificed for preparation of the ground sections. Additionally, the standard errors reported in their study ranged from 7-13 years.

In another age assessment study, the researchers examined the association between tooth wear with aging (Kim et al., 2000). The strength of correlation between occlusal wear with age was significant. Age was estimated using this method with reasonably accuracy. The strength of correlation was relatively strong between tooth wear scores of all examined teeth (coefficient of determination, R2 = 0.4199 - 0.7465). Interestingly, the strength of correlation for molars was higher than bicuspids.

The relationship between cemental annulations and chronological age was investigated (Mallar et al., 2015). The study was designed to observe the annulations at the cross-sectional and longitudinal sections of the teeth at the middle of root portion. Cross sections were more feasible to count. However, the study reported underestimation in younger age group and overestimation in older age group. The study suggested that cemental annulations can be used for dental age estimation but it was more advisable to use it along with other age estimation modalities.

2.4.4 Study on age-related structural changes on Malaysian population

The concept of age-related structural changes for dental age estimation has been tested on different populations. Similarly, studies have been conducted on Malaysian population (Koh et al., 2017). The study investigated the relationship between chronological age with buccal alveolar bone level. The strength of correlation was found to be very good. This age related physiological change can be used for predicting dental age among Malaysian population.

2.4.5 Secondary dentine as a vital parameter to estimate dental age

Once the development of the teeth is completed, age can be estimated only based on the different physiological age-related changes in teeth. Among different parameters, secondary dentine formation is a significant physiological factor to estimate age. Different studies have been carried out to see the correlation between chronological age and secondary dentine formation. Secondary dentine formation starts after the completion of root development and it continues throughout life. Due to the formation of secondary dentine, size of the pulp cavity decreases. This change in the size of pulp cavity has been previously reported by many researchers using two-dimensional and three-dimensional radiographic modalities.

2.4.5.1 Two-dimensional radiographic methods for measuring secondary dentine deposition

Two-dimensional radiographic methods have been effectively used in the twentieth century for measuring reduction in the size of three-dimensional pulp cavity. During that time, three-dimensional radiographic modalities (CBCT) were not available to measure these age-related structural changes.

2.4.5.1.1 Relationship between chronological age and secondary dentine deposition on periapical X-rays

A study reported by Kvaal et al. (1995) was one of the earliest attempts to measure secondary dentine formation using periapical radiographs. Measurements on six different types of teeth were performed. Ratios of pulp/root length, pulp/tooth length, tooth/root length and pulp/root width were measured and statistical analysis showed strong correlation between all the measured ratios and chronological age, except for tooth/root length ratio. The coefficients of determination value ($R2=0.76$) showed that this method could be used with reasonable accuracy for estimating dental age. Canines showed weakest correlation among all the other types of teeth investigated in the study. A study on periapical radiographs was conducted to find the relationship between pulp/tooth area ratio with chronological age (Cameriere et al., 2007). They reported standard error of 5.4 years in maxillary and mandibular canines.

2.4.5.1.2 Relationship between chronological age and secondary dentine deposition on panoramic radiographs

Bosmans et al. (2005) conducted a study to investigate the effects of Kvaal et al. (1995) technique on panoramic radiographs to see the relationship between secondary dentine deposition with age (Bosmans et al., 2005). The results indicated no significant difference between two radiographic modalities. Paiwensky et al. (2005) reported another significant relationship between chronological age and reduction in the size of pulp cavity using orthopantomograms. However, the coefficient of determination value was higher for maxillary lateral incisors (R2=0.913).

Another study reported on underestimation of Kvaal et al. (1995) method and overestimation of Paewinsky et al. (2005) method using dental panoramic radiographs among Austrian population (Meinl et al., 2007). Regression equations derived from the above-mentioned studies were used to estimate dental age among individuals ranging in age from 13 to 24 years.

2.4.5.1.3 Relationship between chronological age and secondary dentine deposition by measuring ground sections of teeth.

Arora et al. (2016) conducted a study to investigate the reliability of Gustafson's qualitative method and Kedici's quantitative method of measuring ground sections of secondary dentine for dental age prediction among North Western adult Indians. Study reported ±10.4 years error in Gustafson's method while ±13 years error in Kedici's technique. The results showed that both methods were gender independent and there was no statistical difference in the strength of correlation between genders.

2.4.5.2 Use of Three dimensional radiographic modalities in observing reduction of pulp cavity volume

There are different radiological methods to measure the secondary dentine formation but to be more effective, the use of 3-dimensional techniques has been preferred over 2-dimensional techniques. It is not logical to measure 3-dimensional structures with 2-dimensional techniques. Thus far, no volumetric study has been reported on Malaysian population using CBCT technology for estimating dental age. Following are the studies which have been conducted to document pulp cavity volume reduction with age in the last few decades on different populations.

2.4.5.2.1 Pulp/tooth volumetric studies reported on Belgian population using 3D scans.

The first two studies reported on volumetric analysis of pulp cavity using 3D scans were carried on Belgian population (Vandevoort et al., 2004; Yang et al., 2006). Images used for the volumetric reconstruction of pulp/tooth volumes were acquired through X-ray micro focus computed tomography by Vandevoort et al. (2004). However, Yang et al. (2006) used CBCT images for volumetric analysis. Single rooted teeth were examined in the studies. Both of these studies showed weak strength of correlation between chronological age and pulp/tooth volume ratio as compared to the studies carried on other populations. As this technique was less-invasive it can be used on living individuals for identification and age estimation. So, these studies provided simple and a new concept of volumetric analysis among the forensic researchers.

The above-mentioned studies were pilot studies and involved small sample size. However, another study was reported on Belgian population using CBCT images with comparatively large sample size (n=111) (Star et al., 2011). This study involved single rooted teeth for volumetric analysis. Volumetric analysis of pulp cavity and tooth was performed using Simplant Pro software. Despite using adequate sample size, the strength of correlation (0.34) between chronological age and pulp/tooth volume ratio were similar to the previously reported studies on Belgian population (Vandevoort et al., 2004; Yang et al., 2006). The study reported no significant difference in the strength of correlation values between different single rooted teeth involved in the study (Incisors, canines, premolars). The study suggested future research to be carried out on larger sample size and on different types of teeth. Improvements in CBCT technology and image enhancing softwares with more reliable thresholding and segmentation will improve the volumetric analysis of pulp cavity and tooth.

2.4.5.2.2 Pulp/tooth volumetric studies reported on Japanese population using 3D scans.

This simple, non-invasive and more likely less expensive way of estimating age has drawn the attention of the researchers among forensic science experts from all over the world. Studies on Japanese populations were conducted to investigate the relationship between chronological age and pulp/tooth volume ratio (Someda et al., 2009; Agematsu et al., 2010; Aboshi et al., 2010; Sakuma et al., 2013).

2.4.5.2.2.1 Images acquired through Micro-CT for Volumetric analysis

Someda et al. (2009) reported a different approach of dental age estimation. The aim of the study was to investigate the strength of correlation between chronological age and volumetric changes in pulp/tooth ratio. The study also aimed to investigate significant difference in the strength of correlation between genders and to observe the association of age with enamel, dentin and pulp cavity. Images for volumetric analysis were acquired through micro-CT. Only mandibular central incisors were studied. Following ratios were analysed for dental age estimation.

1. Pulp cavity/ whole tooth including enamel.

2. Pulp cavity/ whole tooth excluding enamel.

3. Pulp cavity/ whole crown including enamel.

4. Pulp cavity/ whole crown excluding enamel.

5. Pulp cavity/ root region.

The highest correlation out of the above-mentioned segments was observed for the pulp cavity/ whole tooth excluding enamel (R2; Male=0.67, Female=0.76). This study suggested gender specific results.

Agematsu et al. (2010) also used images acquired through Micro-CT for volumetric analysis. The study used the method as suggested by Someda et al. (2009) by using the pulp/tooth ratio excluding enamel to investigate the relationship between chronological age and pulp/tooth volume ratio. In contrast to the study of Someda et al (2009) this study investigated two single-rooted teeth (mandibular central incisors and second premolars). Like Someda et al. (2009), this study also reported strong correlation between chronological age and pulp/tooth volume ratio for both the investigated teeth. However, the correlation was higher in mandibular central incisors (male, R2=0.67; female, R2=0.75) as compared to mandibular second premolars.

In another research from Japan, a dental age estimation technique was investigated by measuring the pulp cavity volumes using Micro-CT among first and second premolars (Aboshi et al., 2010). The pulp/tooth volumes at four levels (crown area, coronal one third, mid-root and apical one third of the root) were investigated. The coronal one third of the root showed the greatest strength of correlation between chronological age and pulp/tooth ratio. This study also reported strong correlation for both investigated teeth (first premolars=0.64, second premolars=0.70).

2.4.5.2.2.2 Images acquired through Multidetector Computed Tomography for Volumetric analysis

Sakuma at el. (2013) for the first time investigated the relationship between age and pulp/tooth volume ratio on dead bodies using MDCT. The investigated tooth was mandibular first premolar. The results indicated strong correlation between age and pulp/tooth volume ratio (R=0.76)(Sakuma et al., 2013).

2.4.5.2.3 Pulp/tooth volumetric studies reported on French population using 3D scans.

An age estimation method was investigated by measuring the volumes of pulp cavities in canines through CT images with the help of MIMICS software (Tardivo et al., 2011; Tardivo et al., 2014). Both studies were performed using same image enhancing software and investigated tooth. However, there was striking difference in the strength of correlation values between the two studies (study 1; R=0.59, study 2; R=0.95). The only difference between the two studies were the sample size (study 1; n=133, study 2; n=840).

2.4.5.2.4 Pulp/tooth volumetric studies reported on Italian population using 3D scans.

The relationship between chronological age and pulp/tooth volume ratio in maxillary canines was reported from Italy (De Angelis et al., 2015). The volumetric analysis was performed using images acquired with the help of CBCT. The correlation was moderate (R2=0.39) and significant in both genders where the female showed higher correlation than males. However, they also reported that this method of age estimation is gender independent.

Another study was reported on maxillary left central incisors (Pinchi et al., 2015). The study showed good correlation between pulp/tooth volume ratio and chronological age. This authors also found that this method of dental age estimation was gender independent. They concluded that the reduction in the volume of pulp cavity is a reliable method of age estimation.

2.4.5.2.5 Pulp/tooth volumetric studies reported on Chinese population using 3D scans.

For the first time, volumetric study on multi-rooted teeth was reported in 2015 for dental age estimation (Ge et al., 2015). But instead of investigating pulp/tooth volume ratio, this study used just pulp chamber volumes alone. Maxillary and mandibular first molars were investigated. The strength of correlation was very good between chronological age and pulp chamber volume (R2=0.56) and can be used with precision for dental age estimation.

Another recent study was conducted in China for age estimation based on pulp cavity/chamber volume of 13 types of teeth using CBCT scans (Ge et al., 2016). The goal of the study was to find which amongst the investigated teeth is showing the highest strength of correlation between chronological age and pulp/tooth volume ratio. Maxillary second molars had the largest coefficient of determination value (R2=0.498).

2.4.5.2.6 Pulp/tooth volumetric studies reported on Indian population using 3D scans.

Another study on Indian population was reported to observe pulp/tooth volume ratio with aging among mandibular canines (Jagannathan et al., 2011). Cone beam computed tomography scans of mandibular canines from 140 individuals ranging in age from 10 to 70 years were analysed to measure pulp and tooth volumes. Moderate correlation was established for Indian population (r = -0.63).

2.5 Importance of dental age estimation and identification in the legal justice system

To evaluate the methods for age estimation (due to the rise in the illegal immigrants) a group of specialists were asked to investigate at Berlin in the year 2000. They suggested that dental age estimation methods are effective for age estimation where age is unknown (Schmeling et al., 2001). Moreover, the Norwegian Dental Age Estimation Project is well recognised to estimate the dental age of the illegal immigrants who are less than 18 years of age (Solheim & Vonen, 2006).

Dental age estimation plays a vital role not only in the criminal investigations but also in the identification of the victims due to mass disasters or during immigrant crises. The forensic odontologist coordinates with the investigation team using their scientific and updated technological skills to estimate the dental age of suspects, victims or migrants (Nuzzolese & Di Vella, 2008). For the last few centuries dentists were providing their expertise in legal justice system. One of the initial cases was documented in Scotland in 1814 (Campbell, 1963). Dr. Granville Sheep Pattison and his two students were blamed for transferring the remains of Mrs. Mc Alister from the grave. It was the dentist (Dr. James Alexander) of Mrs. Alister who confirmed these allegations when he discovered the maxillary denture of Mrs. Alister in one of the heads in the dissecting room. A dentist also shared their expertise in one of the trials about the Caroline Walsh missing woman case in 1831 (Churchill, 1905).

In 1795 prince Louis XVII died at young age of 10 years and it was one of the early documented cases of forensic dental age estimation. Through dental evaluation it was concluded that the remains and skeleton in the coffin was aged above 16 years and therefore it was not the prince Louis XVII (Amoedo, 1898).

Nineteenth century was the era of rapid industrialization in Europe and thousands of children were hired as labourers in industries. Different methods of age assessment were introduced for hiring children for work. Edwin Saunders in 1837 suggested that teeth provided the most reliable guide to age when compared to height (Shamim et al., 2006).

2.6 Teeth can withstand extreme conditions

Dental structures play a very important role in the forensic investigations as it can withstand physical, thermal, chemical, nutritional deficiencies and dental hard tissues are the last to be destroyed under extreme conditions (Shekhawat & Chauhan, 2016). Tooth is the most highly-calcified tissue of the body.

A study was conducted in 2008 to analyse the effects of high temperatures on dental hard tissues (Fereira et al., 2008). One group of teeth were exposed to direct heat and another group was exposed to a gradual raise of temperature. Teeth which were exposed to direct heat showed more structural damage than those exposed to gradual raise of temperature. Although in both situations teeth were able to withstand high temperatures.

In 2004, another study was conducted to see the effects of high temperatures on the teeth having restorations and without restorations (Merlati et al., 2004). The changes in the pattern of these samples can be of great importance in forensic investigations and identification of unknown individuals.

The radiographic evaluation of dental structures plays a significant role in the forensic odontology. A study investigated the effects of high temperatures on different restorations and endodontically treated teeth. The results of the study showed that composite fillings can maintain its shape till 600-degree C, amalgam fillings can maintain its shape till 1000-degree C and endodontically treated teeth can withstand up to 1100-degree C (Savio et al., 2006).

In the period 1992-1996 a total of 89 unidentified bodies were found in Danish water (Kringsholm et al., 2001). The forensic experts identified 78% of the cases through dental examination. This study suggested that teeth can be a good tissue to rely on for identification and also age estimation even though they have been exposed to harsh and extreme conditions.

2.7 Role of forensic odontologist in mass disasters

Natural and manmade disasters are sudden, unexpected and involves large amount of loss of life and it is very difficult to manage with the help of local government resources. Thus, forensic odontologist plays a very important role in the identification of deceased in mass disasters. Mass disasters can be due to human errors like roadside accidents, aviation disasters or through sea route involving large amount of loss of life. Mass disasters can also be naturally caused like earth quakes, tsunamis, volcanic eruptions and landslides.

When large amount of loss of life is involved then it is very difficult to go for DNA profiling or finger printing to identify each victim as biometric fingerprinting records are not available in the third world countries. Moreover, it is tedious, time-consuming and requires accredited expensive laboratories. Thus, forensic odontologist provide more likely inexpensive and quickest way of identification in such events.

It is very challenging to identify burnt victims especially when the remains are badly incinerated. In Spain, 28 incinerated victims of bus accident were examined and it was possible to identify 57% of the cases by dental means, including age estimations (Valenzuela et al., 2000).

2.8 Comparison of ante mortem and post mortem records

Record keeping by the dentist is essential for the forensic implications and legal documentation of the patient. Patient records consists of demographics (including age), diagnosis, examination, investigations, previous and current treatments, anatomical discrepancies and prognosis of the patient. Tooth is a highly calcified tissue of the body and it's the last to be destroyed in the extreme fire or any aviation disasters. If ante mortem dental records of the unidentified remains of the individual are provided, then it can be compared with the post mortem records and assist in the identification process (Charangowda, 2010; Sarode et al., 2009). In 1988 Lockerbie air disaster, 209 victims of the 270 passengers were identified with the help of dental comparison methods (Moody & Busuttil, 1994). Subsequently, in 2002 Bali bombings, more than 60% of victims were similarly identified (Lain et al., 2003). In 2004, Boxing Day tsunami in Thailand, dental identification was the primary identifier in more than 75% of cases (James, 2005). The important role played by forensic odontology in the identification protocols in mass disasters cannot be ignored. Apart from comparison of dental records for this identification process, the other important scope of the dental tissues is the ability to perform age estimation.

CHAPTER 3: RESEARCH METHODOLOGY

3.1 Introduction

The research study was designed to investigate the relationship between the chronological age and ratio of the pulp/tooth volume among the Malaysian population (Malays and Chinese). The teeth included in the study were maxillary right central incisors, maxillary right canines and maxillary left canines. The study was designed to investigate which among the 3 types of investigated teeth are showing the highest strength of correlation and whether, this method of dental age estimation is gender dependent. This study was also aimed to compare the strength of correlation values between maxillary right and maxillary left canines, each belonging to different subjects.

3.2 The materials of the study

3.2.1 Cone-Beam Computed Tomography (CBCT)

Cone-Beam Computed Tomography (CBCT) scans stored in the Division of Oral Radiology, Faculty of Dentistry, University of Malaya, were used for the present study using i-CAT Cone Beam 3D Dental Imaging System (version 3.1.62 supplied by Imaging Sciences International, Hatfield, USA). The scans selected had exposure parameters of 120 KV, 18 mA and the scans were acquired using voxel size of 0.30 mm and scanning time of 20 sec. The required data was selected and then saved in the external hard disk for the purpose of this study. The data was then transferred to the MIMICS software for the volumetric measurements of the maxillary right central incisors and canines. The cases selected for the study dated back from January 2004 to June 2016. Latest CBCT images were given the priority for the selection process.

3.2.2 MIMICS Software

MIMICSs (Materialise Interactive Medical Image Control System) is an image enhancing software for 3D reconstruction and modelling, manufactured by Materialize NV, a Belgian company specialized in additive manufacturing software and technology for medical, dental and additive manufacturing industries ("3D Medical Image Processing Software; Materialise Mimics", 2016). Images from CBCT data can be further enhanced with MIMICS (Materialise Interactive Medical Image Control System) software. MIMICS with the assistance of 'Image segmentation module' calculates the volume of the three-dimensional models from stacked image data provided by CBCT. MIMICS calculate and create images in the XZ (coronal) and YZ (sagittal) direction. This enables a more comprehensive 3D feel of the 2D data. The region of interest (ROI), selected in the segmentation process is converted to a 3D surface model using an adapted marching cube algorithm that takes the partial volume effect into account, leading to very accurate 3D models. The 3D files are represented in the STL format. Other formats are VRML, PLY and DXF. Commonly used input format is DICOM. This software is capable of creating 3D models for the pulp cavity and calcified tooth. Caution must be expressed here that the software is not able to demarcate small structural details, particularly in the radicular portion of the tooth. As a consequence, manual intervention is required in the radicular portion during 'multiple slice editing phase' of the software.

3.3 The subjects of the study

3.3.1 Sample size calculation.

Sample size was calculated using G Power 3.1.9.2 software (Faul et al., 2009). This calculation is based on the correlational study previously reported by Ge ZP et al., (2016). The strength of correlation reported between chronological age and pulp cavity volume change was (R) 0.316 for maxillary canines in their study. Following are the details of the values and the type of test used by the software for sample size calculations.

Exact - Correlation: Bivariate normal model

Options: exact distribution

Analysis: A priori: Compute required sample size

Input:	Tail(s)	= Two
	Correlation ρ H1	= 0.3162278
	α err prob	= 0.05
	Power (1-β err prob)	= 0.80
	Correlation ρ H0	= 0
Output:	Total sample size	= 76
	Actual power	= 0.8042273

Seventy-six was the sample size calculated for each type of investigated tooth and overall ‘228’ sample size was calculated for all the 3 types of investigated teeth included in the study. However, 300 sample size was selected for this comprehensive research study.

3.3.2 Sampling method

This is the retrospective study. Three hundred CBCT scanned data of 153 males and 147 females' belonging to either Chinese or Malay ethnicity, stored in the Oral and Maxillofacial Imaging Division, Faculty of Dentistry University of Malaya were selected according to the quality, image acquiring parameters and age of the patients at the time of registration. The subjects were divided into 5 age groups (10 years interval each group), ranging from 16 to 65 years to ensure balanced sample distribution across the 5 groups (Table 3.1) (Figure 3.1 & 3.2).

Table 3.1: Grouping of the sample based on the age

Group 1	16 years to 25 years
Group 2	26 years to 35 years
Group 3	36 years to 45 years
Group 4	46 years to 55 years
Group 5	55 years to 65 years

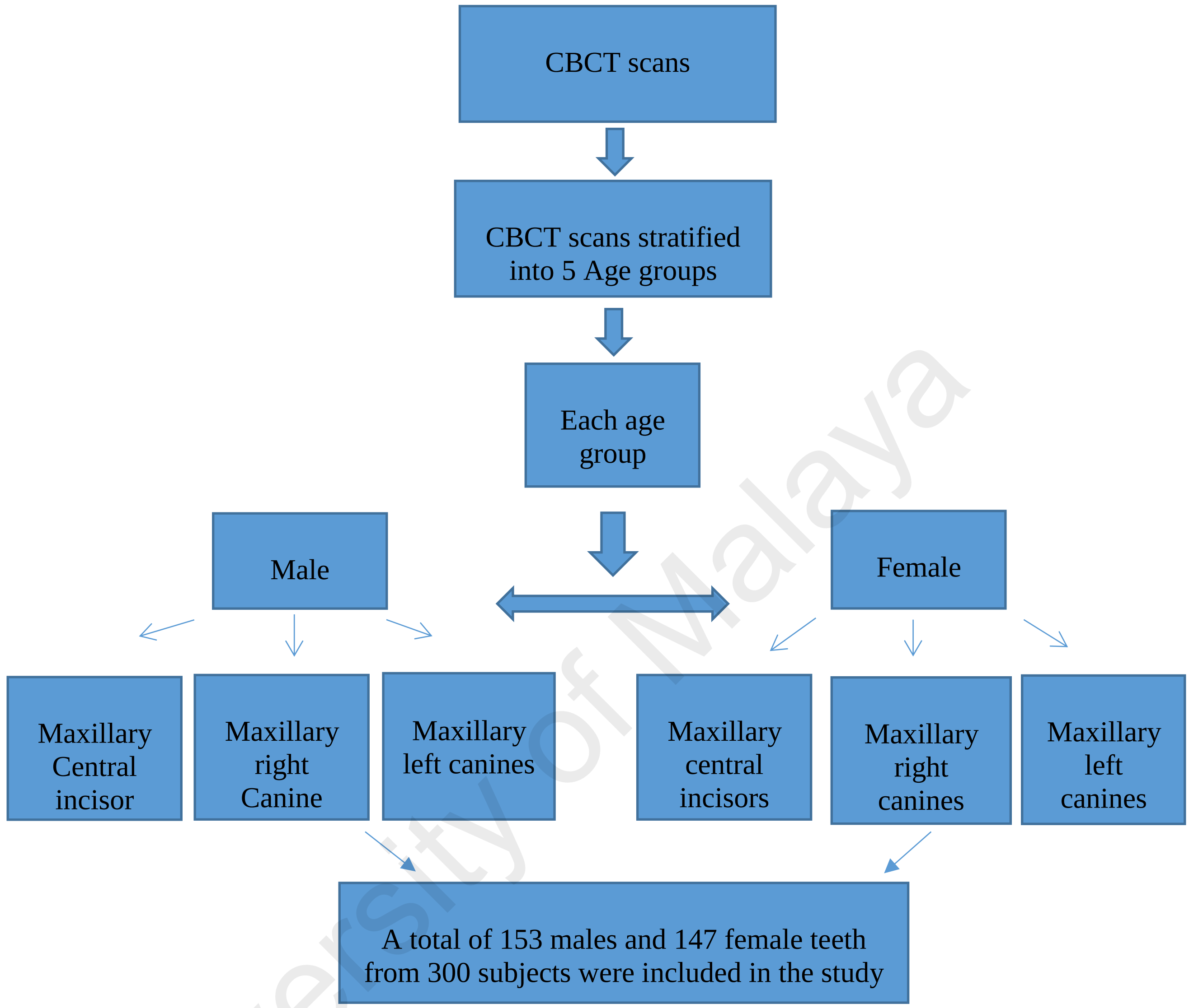

Figure 3.1: Process for sample size distribution

3.3.3 Selection criteria of the sample

The study sample was chosen according to the inclusion and exclusion criteria as follows:

3.3.3.1 Inclusion criteria:

1. CBCT scans having exposure parameters of 120KV,18Ma and scans with voxel size of 0.30mm, available in the database of Oral and maxillofacial Imaging Division, Faculty of Dentistry University of Malaya.
2. Scans of Malaysians belonging to the Malay and Chinese race, as recorded at the time of registration.
3. Patients aged 15 to 65 years as recorded at the time of registration.
4. No evidence of caries or pathology associated with maxillary canines and maxillary right central incisors and completely developed roots on the radiographic images.

3.3.3.2 Exclusion criteria

1. Patients aged below 15 and above 65 years.
2. Non-Mongoloid population.
3. Caries or any pathology associated with maxillary left and right canines and maxillary central incisors.
4. Roots of the investigated tooth not fully developed on the images.

3.4 Methodology

3.4.1 Methods

This ethical approval for this study was obtained from the institutional Medical Ethics Committee (Ref. No: DF OS1606/0022(L) (Appendix A). 300 CBCT images of Mongoloid (Malay and Chinese) patients stored in the Oral and Maxillofacial Imaging Division, University of Malaya were stratified into 5 age groups as explained in the 'sampling method section'. Each scan was selected following the strict inclusion and exclusion criteria's. Intact 100 maxillary left canines, 100 maxillary right canines and 100 maxillary right central incisors were selected from the 300 CBCT scans (Table 3.2). Only one tooth per subject was selected to avoid bias in data acquired from the same patient. Maxillary right and maxillary left canines were selected for the study to find any significant difference in the strength of correlation between two similar types of teeth belonging to different subjects.

Table 3.2: Sample size distribution

Age	**Maxillary right canine**		**Maxillary central incisor**		**Maxillary left canine**		**Total**	
Groups	Male	Female	Male	Female	Male	Female	Male	Female
16-25	14	10	12	07	14	10	40	27
26-35	08	12	10	09	08	12	26	33
36-45	12	11	12	13	12	11	36	35
46-55	10	09	11	12	10	09	31	30
56-65	06	08	08	06	06	08	20	22
Total	50	50	53	47	50	50	153	147

The selected CBCT data in DICOM files were then imported to the MIMICS software (Materialise NV, Belgium, version 16.0) for the analysis of pulp-tooth volume ratio (Figure 3.3). The images were first oriented properly in axial, coronal and sagittal planes. New masks were created for the pulp cavity and tooth after setting different grayscale threshold values for each of the investigated tooth (Figure 3.4). The masks were cropped in all 3 planes to separate the tooth from the surrounding structures. In the ‘multiple slice editing phase’, masks were manually checked slice by slice for segmentation and separation from the surrounding structures, taking more care in the radicular portion of the tooth. Areas of the pulp cavity and calcified tooth structures, which were not automatically selected by the software were manually checked and edited in the required area (Figure 3.5). After editing the mask in the multiple slice editing phase the pulp cavity and tooth were grown in the ‘Region growing phase’ of the software (Figure 3.6 and 3.7). Three dimensional models of the pulp tissue and tooth were created (Figure 3.8). The software then automatically calculated the volumes (mm^3) of the pulp cavity and tooth.

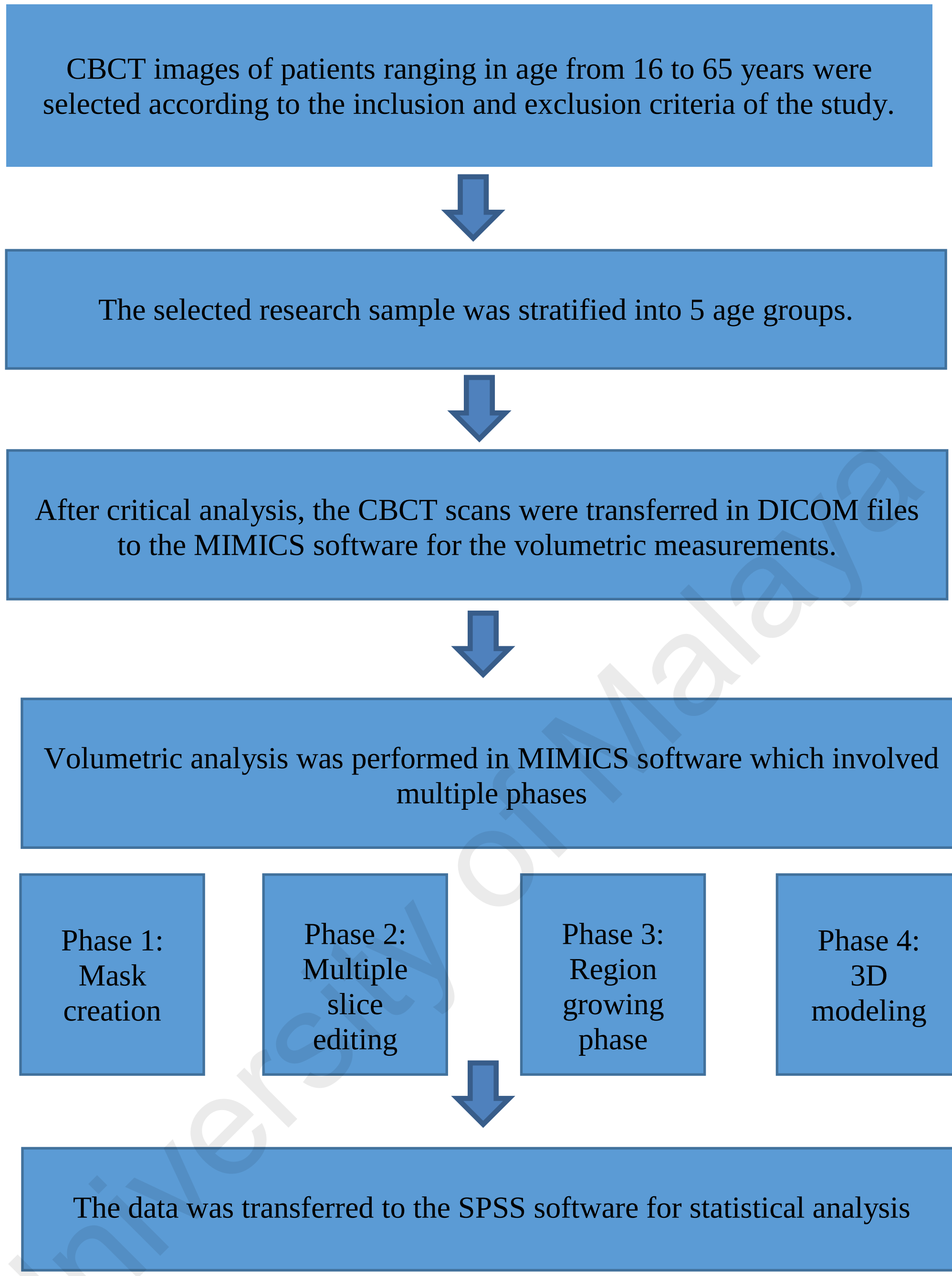

Figure 3.2: Flow chart of the methodology

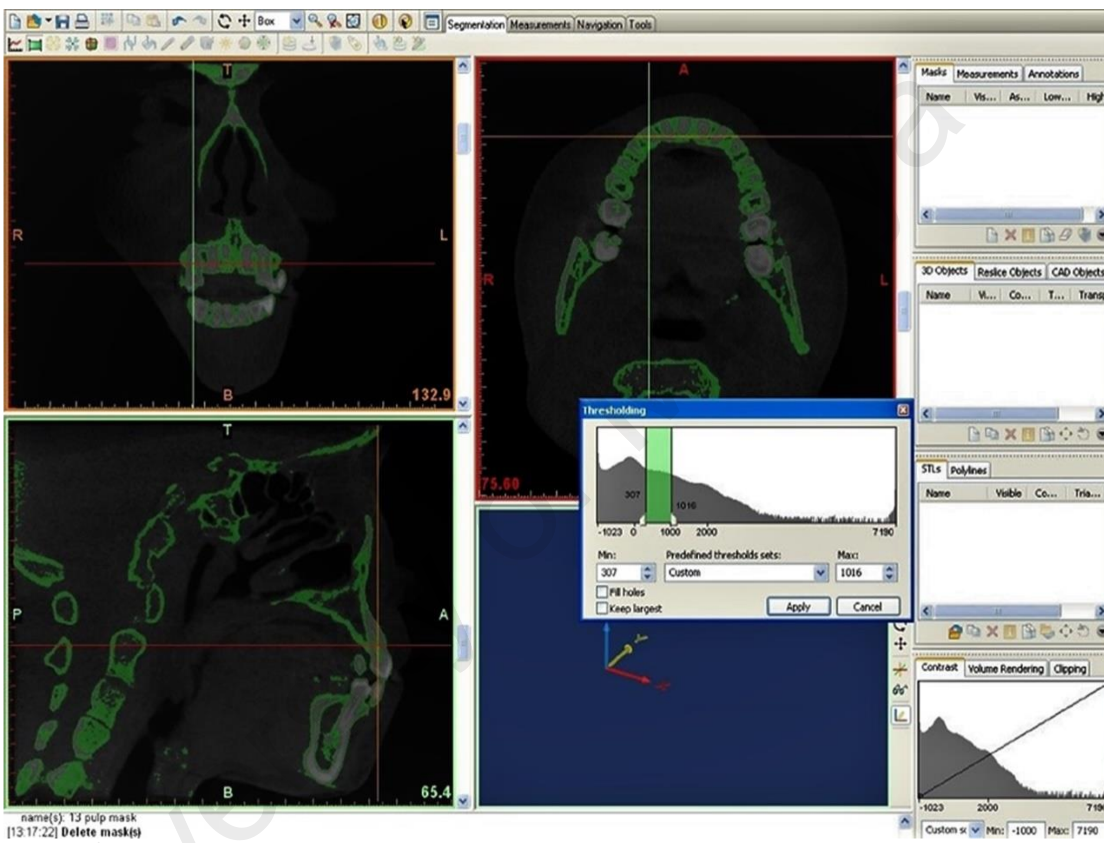

Figure 3.3: Pulp "tissue thresholding or new mask creation phase" in sagittal, axial and coronal view of maxillary right canine (13).

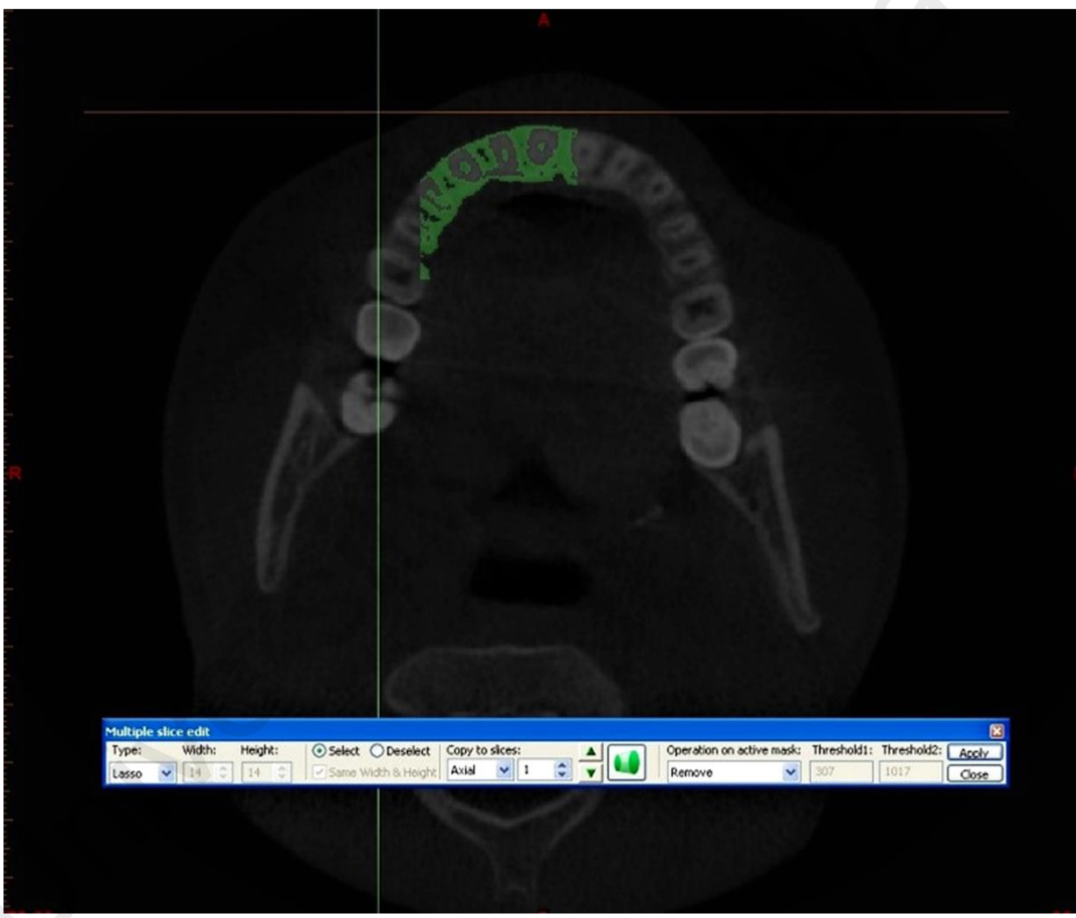

Figure 3.4: "Multiple slice editing phase" for separation and segmentation of pulp cavity in the maxillary right canine on axial view (13).

Figure 3.5: Tooth 'Region growing phase' of maxillary right central incisor on sagittal view.

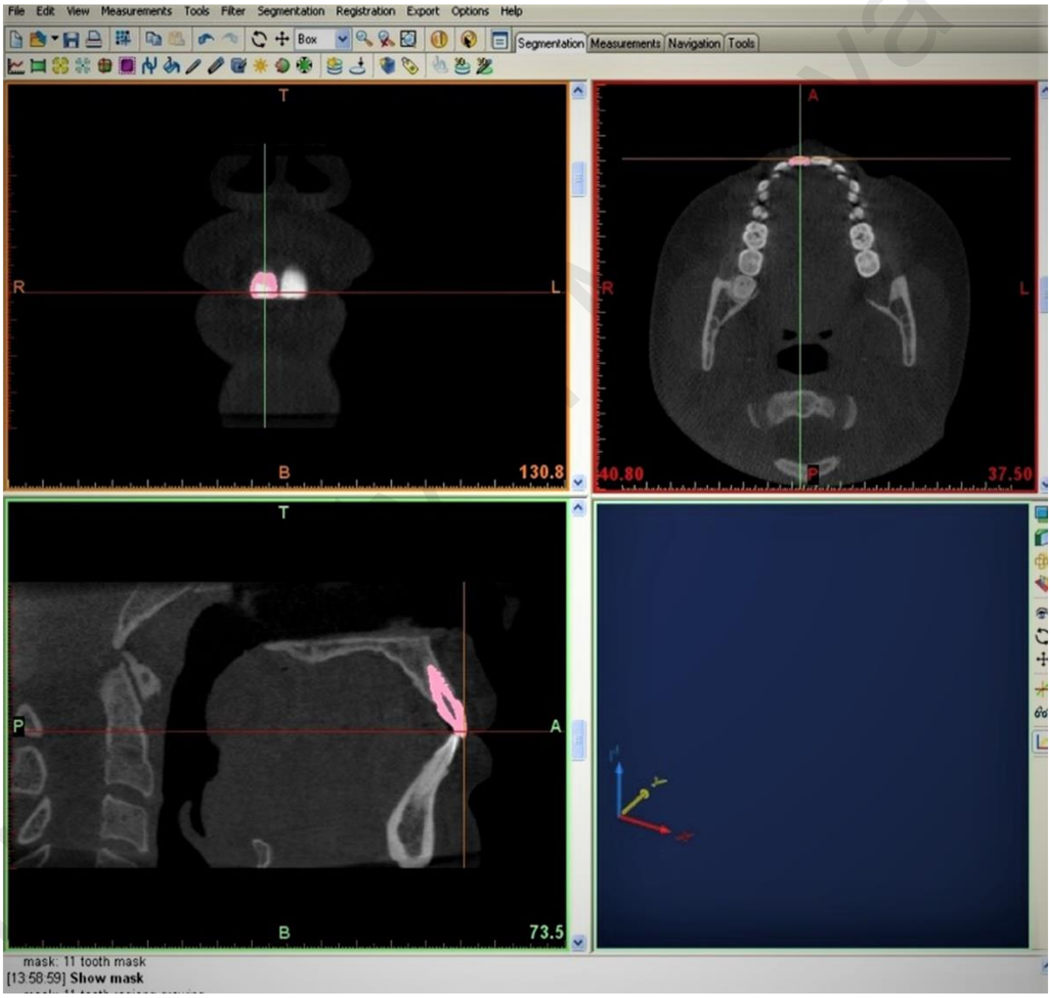

Figure 3.6: Tooth 'Region growing phase' on sagittal, axial and coronal view of the maxillary central incisor (11).

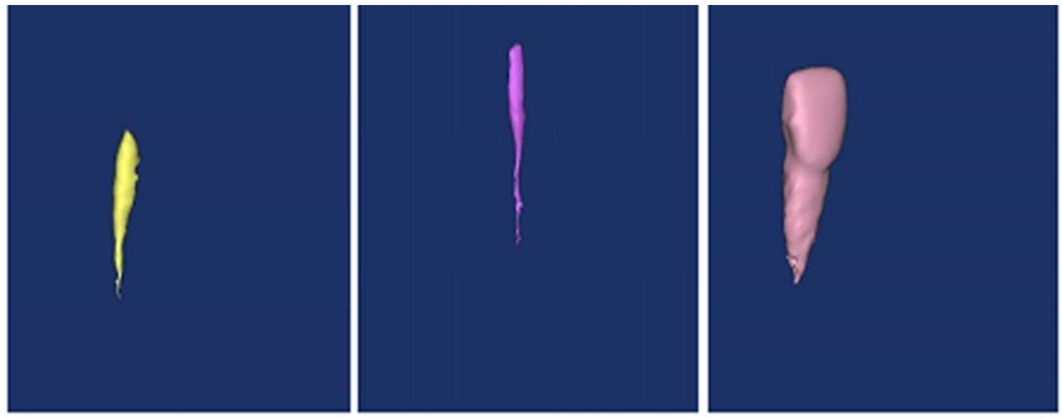

Figure 3.7 a: 3D view of the maxillary right canine pulp cavity; b: 3D view of maxillary central incisor pulp cavity; c: 3D view of maxillary central incisor.

3.4.2 Reliability of the measurements

As this research involved quantitative data and there was manual intervention during multiple slice editing phase, the intraexaminer and interexaminer reliability of the measurements were evaluated.

3.4.2.1 Intraexaminer reliability

The intraclass correlation coefficient (ICC) analysis was used to determine the consistency in tooth volume ratio before and after three months. Pulp/tooth volumetric analyses on 30 teeth from the sample was investigated twice 3 months apart for intraexaminer reliability. The analysis was performed using SPSS version 20 (IBM, 2011).

3.4.2.2 Interexaminer reliability

The intraclass correlation coefficient (ICC) analysis was used to determine the consistency in the measurement of pulp/tooth volume ratio between two different examiners. The second examiner (postgraduate dental student) carried out volumetric analysis to test for the interexaminer reliability on the same 30 teeth from the sample. The analysis was performed using SPSS version 20 (IBM, 2011).

3.5 Data analysis

All the measurements and the data was analysed by using SPSS statistical software (version 20) (IBM, 2011)The intraclass correlation coefficient (ICC) analysis was used to measure intra and interexaminer reliability. Squared Pearson correlation coefficient analysis was performed to find the association between the chronological age and pulp/tooth volume ratio for each type of the investigated teeth and for the whole research sample. Linear regression analysis was performed by using chronological age as a dependent variable and pulp/tooth volume ratios as predictor. Independent t-test analysis was used for the comparison of mean values of pulp/tooth volume ratio between genders. Fishers Z test was used to find the significant difference in the correlation coefficient values between, gender and for the maxillary right and left canines belonging to different subjects.

CHAPTER 4: RESULTS AND DATA ANALYSIS

4.1 Introduction.

This study investigated the relationship between the chronological age and pulp/tooth volume ratio in maxillary right and left canines and maxillary right central incisors. The results of the study are presented in different sections as follows:

4.1.1 Intraexaminer and interexaminer reliability.

4.1.2 Relationship between pulp/tooth volume ratio and chronological age for the maxillary left canines.

4.1.3 Relationship between pulp/tooth volume ratio and chronological age for the maxillary right canines.

4.1.4 Relationship between pulp/tooth volume ratio and chronological age for the maxillary right central incisors.

4.1.5 Relationship between pulp/tooth volume ratio and chronological age for the left canines based on genders.

4.1.6 Relationship between pulp/tooth volume ratio and chronological age for the right canines based on genders.

4.1.7 Relationship between pulp/tooth volume ratio and chronological age for the maxillary right central incisors based on genders.

4.1.8 Comparison in the mean values of pulp/tooth volume ratios between genders for the whole research sample.

4.1.9 Fisher Z-test to investigate significant difference in the coefficient of correlation values.

4.1.9.1 Comparison of the coefficient of correlation values between genders.

4.1.9.2 Comparison of the coefficient of correlation values between maxillary left and maxillary right canines.

4.1.1 Intraexaminer and interexaminer reliability.

The intraclass correlation coefficient (ICC) analysis was performed to determine the intraexaminer and interexaminer reliability. Volumetric analyses on 30 randomly selected teeth from the sample was performed in a span of 3 months for intraexaminer reliability. Similarly, a second examiner (postgraduate dental student) carried out volumetric analysis to test for the interexaminer reliability on the same sample. The obtained ICC value was 0.945 for intraexaminer reliability and 0.968 for the interexaminer reliability. The results suggested that the consistency value was almost perfect.

4.1.2 Relationship between pulp/tooth volume ratio and chronological age for the maxillary left canines.

The Pearson correlation analysis showed that there was significant inverse association between maxillary left canine pulp/tooth volume ratio and chronological age ($p<0.01$) (Table 4.1) (Appendix B). The strength of correlation was found to be very good to perfect correlation ($r=-0.73$).

Table 4.1: Pearson correlation coefficient between chronological age and pulp/tooth volume ratio for maxillary left canines. (p<0.01)

Investigated tooth (FDI notation)	Pearson correlation coefficient (r)		
	Male	**Female**	**Whole sample**
23	0.77	0.68	0.73

The simple linear regression analysis was done to determine the relationship between maxillary left canine pulp/tooth volume ratio and chronological age as the outcome (Figure 4.1). The relationship was found to be inversely significant ($p<0.01$). The derived Regression equation for dental age estimation of maxillary left canines was as following: Age = 66.19- (748.29 x PTV ratio).

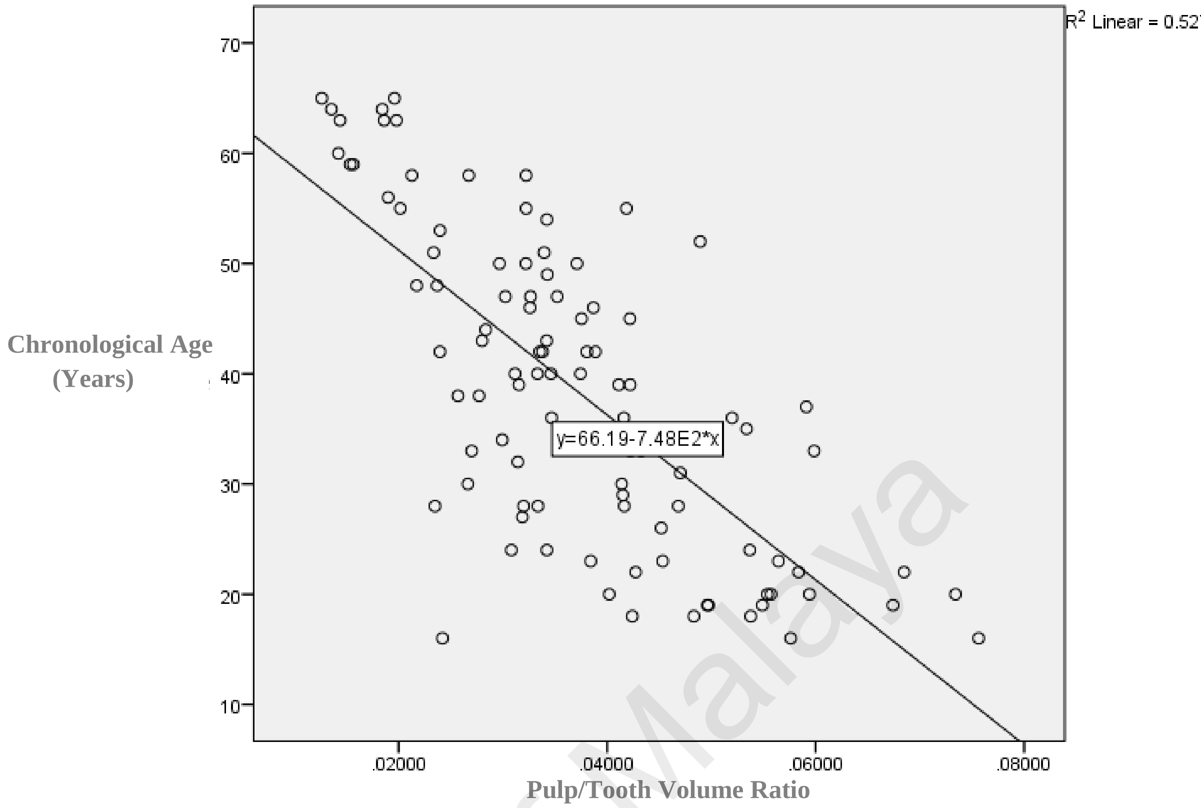

Figure 4.1 The relationship between chronological age and pulp/tooth volume ratio for the maxillary left canines.

4.1.3 Relationship between pulp/tooth volume ratio and chronological age for the maxillary right canines.

The Pearson correlation analysis showed that there was significant inverse association between maxillary right canine pulp/tooth volume ratio and chronological age ($p<0.01$) (Table 4.2) (Appendix B). The strength of correlation is found to be very good to perfect ($r=0.74$).

Table 4.2: Pearson correlation coefficient between chronological age and pulp/tooth volume ratio for maxillary right canines. ($p<0.01$)

Investigated tooth (FDI notation)	Pearson correlation coefficient (r)		
	Male	**Female**	**Whole sample**
13	0.74	0.73	0.74

The simple linear regression analysis was done to determine the relationship between maxillary right canine pulp/tooth volume ratio and age as the outcome (Figure 4.2). The relationship was found to be inversely significant ($p<0.01$). The derived Regression equation for dental age estimation of maxillary right canines was as following: Age=67.07-(732.97 x PTV ratio).

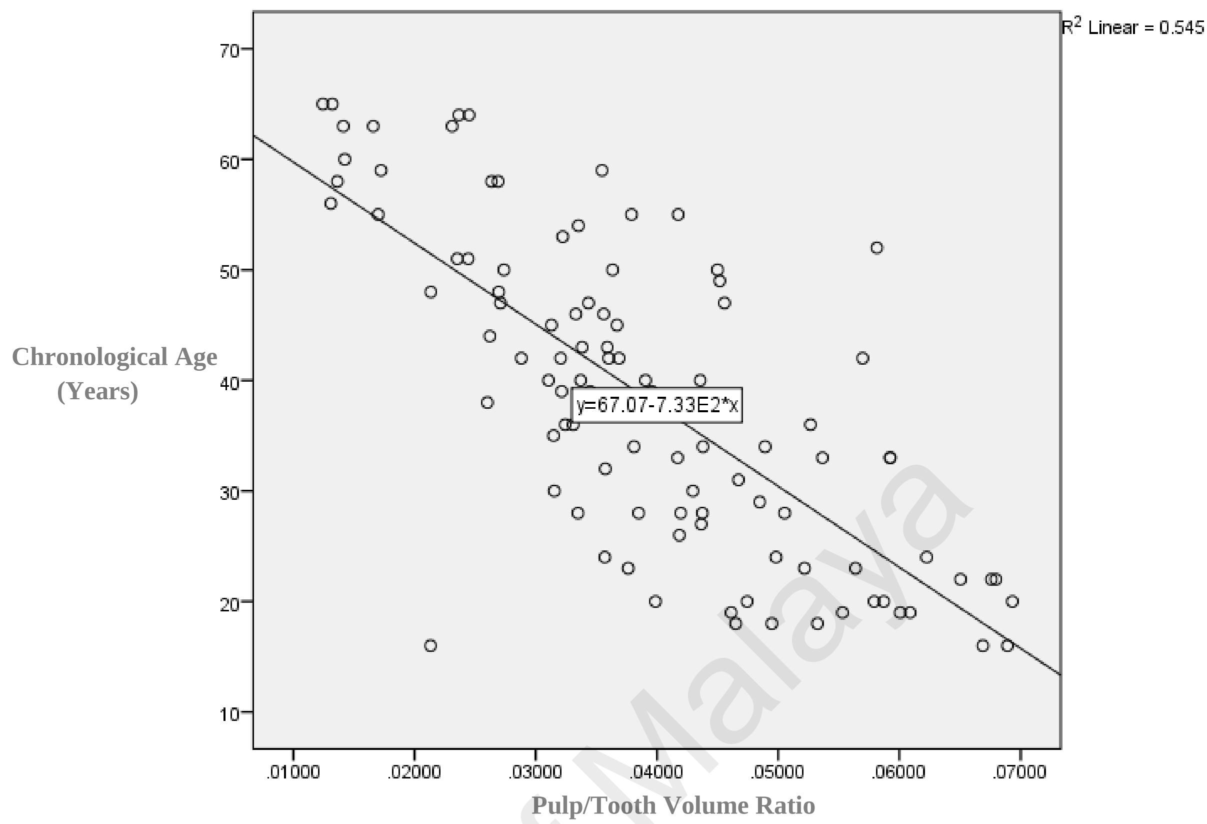

Figure 4.2 The relationship between chronological age and pulp/tooth volume ratio for the maxillary right canines.

4.1.4 Relationship between pulp/tooth volume ratio and chronological age for the maxillary right central incisors.

The Pearson correlation analysis showed that there was significant inverse association between the maxillary right incisor pulp/tooth volume ratio and chronological age ($p<0.01$) (Table 4.3) (Appendix B). The strength of correlation was found to be very good to perfect correlation ($r=-0.834$).

Table 4.3: Pearson correlation coefficient between chronological age and pulp/tooth volume ratio for maxillary right central incisors. (p<0.01)

Investigated tooth (FDI notation)	Pearson correlation coefficient (r)		
	Male	Female	Whole sample
11	0.84	0.83	0.83

The simple linear regression analysis was done to determine the relationship between the maxillary right incisor pulp/tooth volume ratio and chronological age as the outcome (Figure 4.3). The relationship was found to be inversely significant (p<0.01). The derived Regression equation for dental age estimation of maxillary right incisors is as following: Age= 73.24- (1010.71 x PTV ratio)

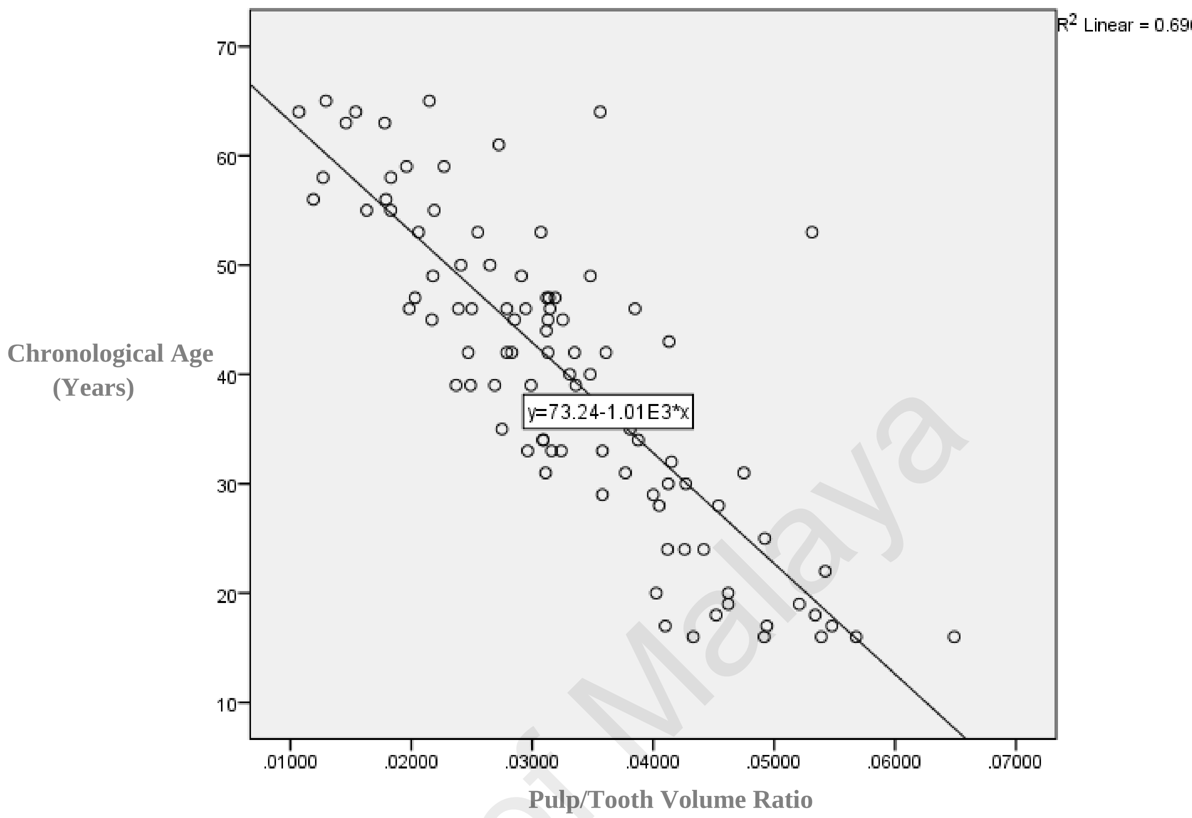

Figure 4.3 The relationship between chronological age and pulp/tooth volume ratio for the maxillary right incisors.

4.1.5 Relationship between pulp/tooth volume ratio and chronological age for maxillary left canines based on genders.

The Pearson correlation analysis showed that there was significant inverse association between the maxillary left canine pulp/tooth volume ratio and chronological age ($p<0.01$) based on genders (Figure 4.4) (Appendix C). The strength of correlation for both male (r=-0.769) and female (r=0.682) was found to be very good.

The simple linear regression analysis was done to determine the relationship between the maxillary left canine pulp/tooth volume ratio and chronological age as the outcome based on the gender. The relationship for both genders were found to be inversely significant ($p<0.01$). The derived regression equations for dental age estimation of maxillary left canines based on genders were as follows:

Male; Age=65.86-(723.38 x PTV ratio).

Female; Age=67.54-(804.65 x PTV ratio).

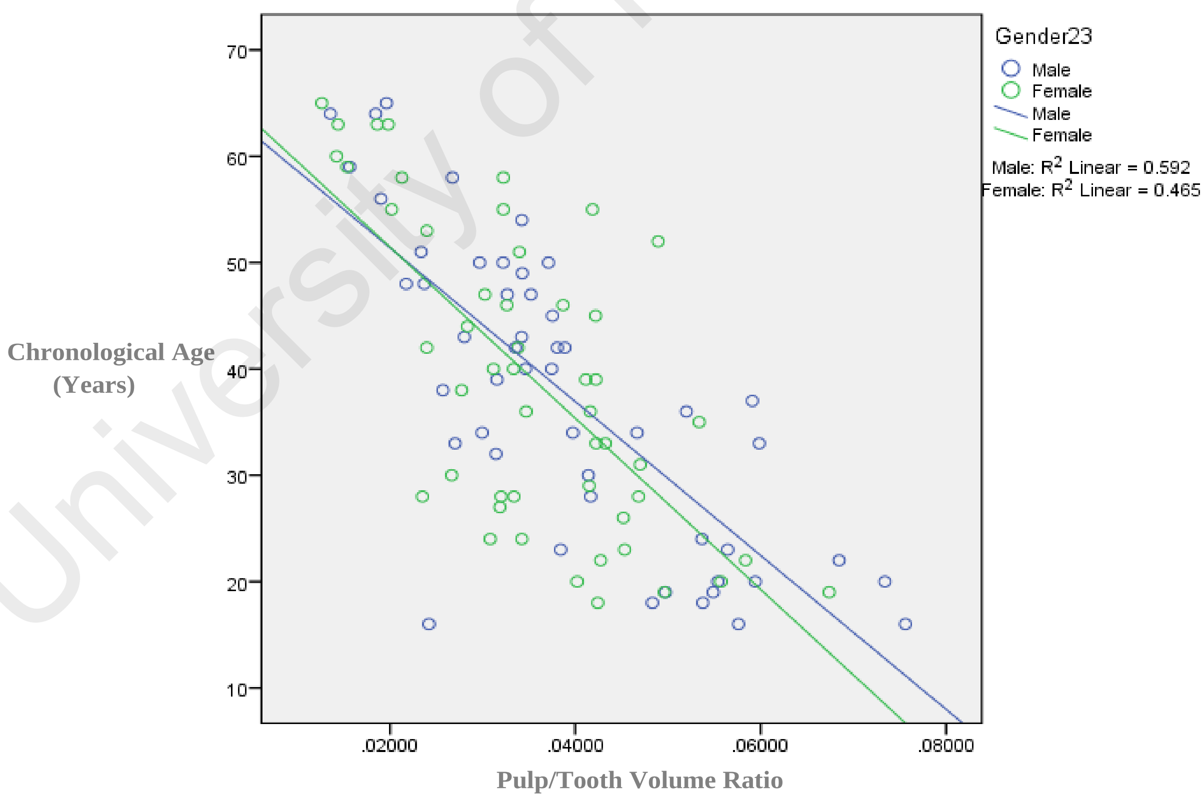

Figure 4.4 The relationship between age and pulp/tooth volume ratio for the maxillary left canine based on gender.

4.1.6 Relationship between pulp/tooth volume ratio and chronological age for maxillary right canines based on genders.

The Pearson correlation analysis showed that there was significant inverse association between the maxillary right canine pulp/tooth volume ratio and chronological age ($p<0.01$) based on genders (Figure 4.5) (Appendix C). The strength of correlation for both male ($r=-0.744$) and female ($r=0.731$) was found to be very good.

The simple linear regression analysis was done to determine the relationship between the maxillary right canine pulp/tooth volume ratio and chronological age as the outcome based on the gender. The relationship for both genders was found to be inversely significant ($p<0.01$). The derived regression equations for dental age estimation of maxillary left canines based on genders are as following:

Male; Age=67.45-(732.81 x PTV ratio).

Female; Age=66.93-(739.43 x PTV ratio).

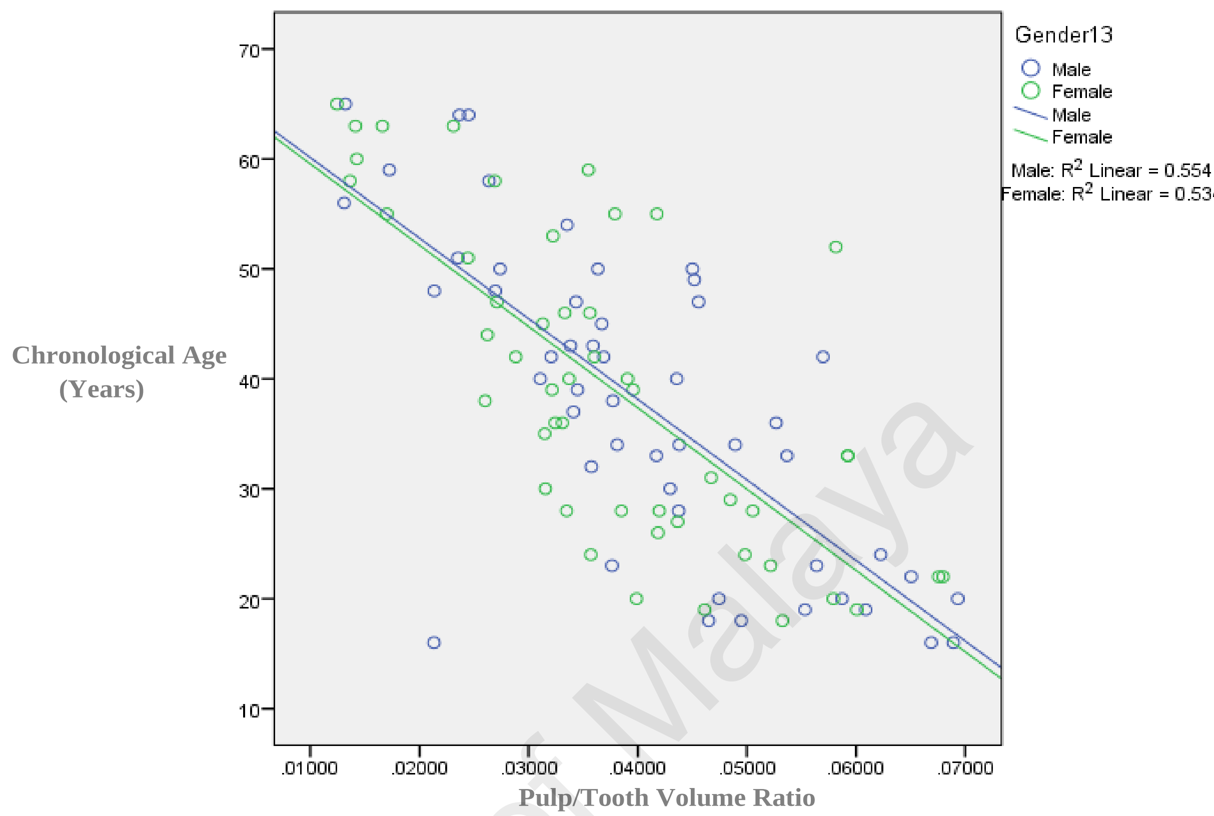

Figure 4.5: The relationship between age and pulp/tooth volume ratio for the maxillary right canine based on genders.

4.1.7 Relationship between pulp/tooth volume ratio and chronological age for maxillary right central incisors based on genders.

The Pearson correlation analysis showed that there was significant inverse association between the maxillary right central incisor pulp/tooth volume ratio and chronological age (p<0.01) based on genders (Figure 3.6) (Appendix C). The strength of correlation for both male (r=-0.840) and female (r=0.827) was found to be very strong.

The simple linear regression analysis was done to determine the relationship between maxillary right central incisor pulp/tooth volume ratio and chronological age as the outcome based on the gender. The relationship for both genders was found to be inversely significant ($p<0.01$). The derived regression equations for dental age estimation of maxillary right central incisors based on genders are as following:

Male; Age=71.84-(969.22 x PTV ratio).

Female; Age=75.47-(1079.94 x PTV ratio).

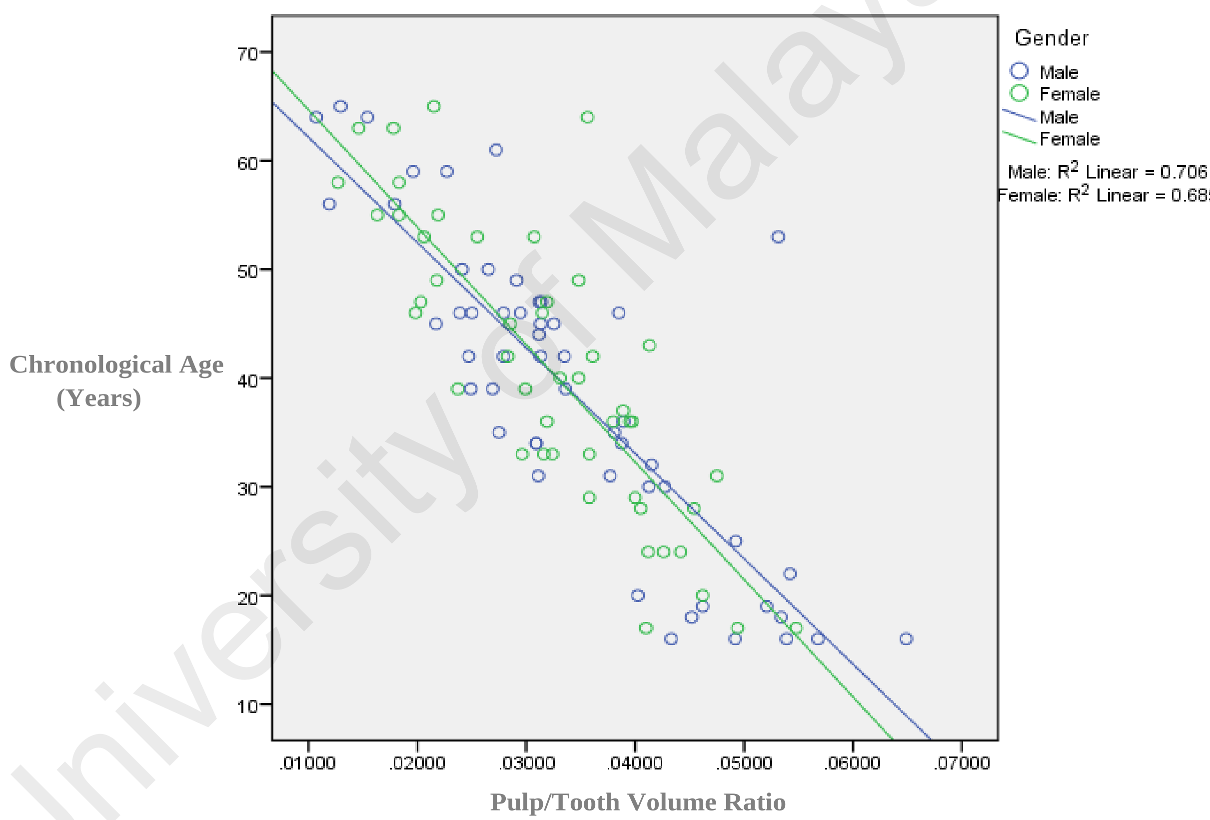

Figure 4.6 The relationship between age and pulp/tooth volume ratio for the maxillary right central incisor based on genders.

4.1.8 Comparison in the mean values of pulp/tooth volume ratios between genders for the whole research sample.

From independent t-test, there was no significant difference in the mean values of pulp/tooth volume ratios between genders (Table 4.4) (Appendix D).

Table 4.4: Comparison in the mean values between genders for the whole research sample using independent t-test.

Variables	Mean(SD)		t stat	p-value
	Male	Female		
Whole sample	0.038(0.014)	0.035(0.012)	1.82	0.070

4.1.9 Fisher Z-test to test the significant difference in the coefficient of correlation values.

Fishers Z test was used to find the significant difference in the correlation coefficient values between genders and for the maxillary right and left canines belonging to different subjects. First, the coefficient of correlation (*r*) values were calculated using SPSS software (version 20) for genders and for maxillary right and left canines. Then the (r) values were converted to 'z' scores using Fisher Z transformation test (Fisher, 1921).

Transformation of the two correlation coefficients to z score:

$$r = 1/2 \ln [1+r / 1-r]$$

("ln" is the natural logarithm function)

("r" is the correlation coefficient)

After conversion of R values to z scores, following formula is used to get 'Z' observed.

The Z-test is computed as:

$$Z\ (obs)=Z_1-Z_2 / [1 / (n1-3) + 1 / (n2-3)]$$

(Z-critical is 1.96 for $p < .05$).

(Z1 and Z2 correspond to the correlation coefficients (r)

(n1 and n2 correspond to sample sizes).

The significance of Z observed value can be assessed by comparing it with the critical value. Since the level of significance was set at 0.05 in this study it indicates that critical value is ± 1.96. If the observed Z value is beyond this range, then the difference between the two correlation values is significant.

4.1.9.1 Comparison of the coefficient of correlation values between genders.

To test the statistical significance in the correlation values between genders, Fisher z test was performed. In the present study, the strength of correlation between chronological age and pulp/tooth volume ratio was stronger for males than for females (Table 4.5). However, Fishers Z test results showed that there was no significant difference in the coefficient of correlation values between genders in all the 3 investigated teeth and the whole sample together.

Table 4.5: Pearson correlation coefficient, and Fishers Z test for all 3 investigated teeth and whole sample, based on gender. ($p<0.01$), (Z critical 1.96 for $p < .05$).

Investigated teeth (FDI notation)	Pearson correlation coefficient (r)		Fishers Z-test	
	Male	**Female**	**Z-value**	**P-value**
23	0.77	0.68	0.93	0.352
13	0.74	0.73	0.11	0.912
11	0.84	0.83	0.31	0.872
Whole sample	0.77	0.73	0.79	0.429

4.1.9.2 Comparison of the coefficient of correlation values between maxillary left and maxillary right canines.

To find the statistical significance in the correlation values between left and right maxillary canines, Fisher z test was performed. The results showed no statistical significant difference in the correlation values between right and left maxillary canines belonging to different individuals (Table 4.6) (Appendix E).

Table 4.6: Pearson correlation coefficient, Fishers Z test for maxillary right and left canines. ($p<0.01$), (Z critical 1.96 for $p < .05$).

Pearson correlation coefficient (R)		Fishers Z test	
Maxillary right canines	**Maxillary left canines**	**(Z observed values)**	**P-values**
0.738	0.726	0.15	0.880

CHAPTER 5: DISCUSSION

5.1 Rational for choice of the study topic.

This study was designed to derive a regression equation for dental age estimation by obtaining pulp/tooth volume ratio in maxillary canines and maxillary right central incisors with the help of CBCT data and Mimics software. In addition, maxillary right and maxillary left canines were selected for the study to investigate any significant difference in the strengths of correlation between two similar types of teeth belonging to different subjects. The study was further designed to explore whether there is any statistical difference in the correlation coefficient values between genders.

The selection of this topic was based on the fact that to date, there is no data available on dental age estimation by volumetric analysis on maxillary canines and maxillary central incisors pulp cavity for the Malaysian population. The findings of the pattern of physiological age changes in the canines and central incisors pulp cavity would be beneficial for dental age estimation of adult Malaysians in the future and also to compare the strength of correlation values with the studies carried on other populations.

Previous studies reported different strength of correlations between chronological age and pulp/tooth volume ratio for single rooted teeth (Yang et al., 2006; Agematsu et al., 2010; Aboshi et al., 2010; Star et al., 2011; Jagannathan et al., 2011; Pinchi et al., 2015; Tardivo et al., 2011). This variations in the strength of correlations between different populations compelled us to investigate the relationship between chronological age and pulp/tooth volume ratio exclusively for the Malaysian population.

Another reason for selection of this topic was the dramatic increase in the frequency of natural disasters such as earth quakes, aviation disasters and tsunamis (Moody & Busuttil, 1994). Natural disasters are unexpected, sudden and involves large amount of loss of life so it becomes very important to find a simple and reliable method of dental age estimation to assist in human identification. Although, the other primary identifiers, namely DNA profiling and fingerprints matching are available. When large number of loss of lives are involved it may prove difficult and time-consuming to carry out DNA investigations on such a large scale. Moreover, biometric fingerprinting records are not available in most of the third world countries.

The rapid increase in the illegal immigrants and terrorist attacks has also drawn the attention of the worldwide researchers to find the reliable and easy method of age estimation and identification especially in case of incinerated bodies. Most often these attacks involve large number of casualties and forensic odontologist plays a very vital role in such events for dental age estimation and identification (Lain et al., 2003; James, 2005).

This study involves a less-invasive method of age estimation. In this method, CBCT is used as the imaging technique which is less expensive as compared to other CT modalities and emits low radiations. CBCT has been used in different specialties of dentistry for implant placements, third molar surgical extractions and endodontics (van Vlijmen et al., 2009; Haney et al., 2010; Kamburoglu et al., 2009; Worthington et al., 2010; Ahmad et al., 2012).

5.2 Specimen selection

This study involved 300 sample size of single-rooted teeth (Maxillary canines and maxillary right central incisors), which were selected based on gender, ethnicity, radiological parameters employed and age groups. Racial groups belonging to Malays and Chinese aged between 16 to 65 years with no pathology associated with the selected teeth were included in this study. Permanent canines and permanent central incisors among single rooted teeth were selected for the study since previous studies reported that most often these 2 types of teeth provided excellent correlation values with chronological age (Someda et al., 2009; Agematsu et al., 2010; Star et al., 2011; Tardivo et al., 2014). Thus, it was useful to include these 2 types of teeth to compare the results of strength of correlations with previously reported studies. Secondly permanent maxillary canine is the longest tooth in the oral cavity. Employing this tooth is ideal as the longevity of this tooth benefits identification of deceased persons in mass disasters.

CBCT scans for each selected tooth were thoroughly examined to eliminate teeth with any associated pathology, since they may have impeded growth and development. In cases of trauma on teeth, there is a possibility of lack of apical closure, total or partial obliteration of the root and even root fracture.

5.3 CBCT data and MIMICS software

The sample size for this study was selected from the records stored in the Division of Oral Radiology, Faculty of Dentistry, University of Malaya. As this study did not involve direct interaction of the examiner with the patients, exclusion and inclusion criteria were strictly based on information associated with the CBCT scans. One of the positive aspects of this study is that the CBCT images were chosen as an imaging technique due to less exposure time to radiation and reduced cost of CBCT as compared to other imaging modalities (Kumar et al., 2015; Dawood et al., 2009; Ziegler et al., 2002; Scarfe et al., 2006). Some studies have shown that CBCT is more accurate and has a higher resolution than CT, despite having less X-ray radiation exposure (Cotton et al., 2007).

MIMICS software was used for the volumetric analysis of the pulp cavity and calcified tooth structures in this study. This software has the advantage of altering the segmentation process and the ability to do the volumetric analysis in axial, coronal, sagittal and 3D views. It allows the investigator to add or remove mask in the area of concern, where the software is not able to automatically demarcate minute structural details. It also allows to accurately measure distances, angles, diameters and densities either on 2D images or directly on the 3D model. Another distinct advantage is that this software allows the operator to scroll through the entire volume with simultaneous viewing of axial, coronal and sagittal sections. Even complex measurements like ellipses, centrelines, multi-planar re-slice and curve planar re-slice can be measured ("3D Medical Image Processing Software; Materialise Mimics", 2016). Simulating surgical interventions on patient data can be performed with this software. This allows deriving optimal surgical plans by evaluating outcomes of various approaches or validating custom-made implants in advance.

5.4 CBCT scans acquired with voxel size of 0.30mm.

The CBCT scans selected for this study had exposure parameters of 120 KV, 18 mA and the scans were acquired using voxel size of 0.30 mm and scanning time of 20 sec. The CBCT scans with same voxel size of 0.30 mm were used for this study to nullify partial volume averaging effect of voxels on volumetric measurements (van

Vlijmen et al., 2009). Surprisingly, in another study by Tardivo et al. (2011) CBCT images with different voxel sizes were used. This is inappropriate because CBCT scans acquired with different voxel sizes have different volumetric results for the same anatomical structures (Whyms et al., 2013). Therefore, our study was strictly restricted to CBCT scans acquired with only 0.30 mm voxel sizes.

5.5 Selection of one tooth per subject.

Only one tooth per subject was selected for this study as opposed to previous studies reported by Tardivo et al. (2011 & 2014). This is to avoid selecting two samples that have similar developmental origin. Two teeth of similar type and having same developmental origin will have same pattern of physiological age-related changes. Thus, to avoid bias in the results only one tooth per subject was selected. Additionally, it was not statistically appropriate to include more than one sample per subject for regression analysis.

5.6 Pulp cavity volume alone verses pulp/tooth volume ratio.

Secondary dentine formation occurs throughout the life with age once the root formation is completed. This study included the pulp/tooth volume measurements instead of measuring the volume of the pulp cavity alone to nullify the effects of variations in tooth sizes amongst different individuals (Star et al., 2011). This approach is more scientifically acceptable and the findings are unbiased as compared to measuring the pulp cavity volumes alone.

5.7 Highest strength of correlation between chronological age and pulp/tooth volume ratio among all the 3 types of investigated teeth.

The Pearson correlation and regression analysis showed the significant inverse association between pulp/tooth volume ratio and chronological age ($p<0.01$) for all the 3 types of investigated teeth. Maxillary right central incisors have shown the strongest coefficient of determination values ($R2= 0.70$). This is similar to the results reported by other researchers in their respective studies involving incisors (Kvaal et al., 1995; Paewinsky et al., 2005; Star et al., 2011; Agematsu et al., 2010). Maxillary right canine ($R2=0.55$) and maxillary left canines ($R2=0.53$) followed maxillary right central incisor in the strength of correlation values. Star et al. (2011) also reported the same in their study as permanent canine's strength of correlation was less than permanent incisors.

5.8 Comparison with previously reported studies.

The aim of the present study was to find an association between chronological age and pulp/tooth volume ratio among Malaysian population (Malays and Chinese). The strength of correlation values for all the 3-investigated teeth was compared with other studies carried out on other populations. This section discusses the results of the present study with the previous studies.

5.8.1 Comparison with studies reported on the Belgian population.

A pilot study was reported on 43 single rooted teeth (30 incisors, 8 canines and 5 premolars) using microfocus computed tomography (micro CT) to estimate dental age (Vandevoort et al., 2004). This study was one of the first, which used volumetric analysis for dental age estimation using micro CT. The coefficient of determination value for the Belgian population was 0.31 for an association between chronological age and pulp/tooth volume ratio, which is far less than our study (R2 ranged 0.53 to 0.70) (Table 5.1). The study on Belgian population did not investigate any significant difference between different types of single rooted teeth involved in the study nor between the genders. Findings similar to our study were noted by Vandevoort et al. (2004), that suggested that maxillary right central incisors have shown highest strength of correlation as compared to maxillary canines and dental age estimation method is gender independent.

Yang et al. (2006) reported in their study that moderate correlation exists between chronological age and pulp/tooth volume ratio in single rooted teeth (R2=0.29). Their study included 28 single rooted teeth scans of 19 individuals acquired with CBCT. However, their study sample size included only 1 premolar, 12 canines and 15 incisors. The strength of correlation values (R2=0.70,0.53,0.55) in our study was stronger than that reported in their study (Yang et al., 2006). The reason for such a difference in strength of correlation may be attributed to low sample size and grouping of 3 different types of investigated single rooted teeth collectively in a regression analysis instead of measuring strength of correlation for each type of single rooted tooth separately.

Yang et al. (2006) further reported that the periodontal ligaments space was not detectable by the 'iDixel software (J Morita corporation, Kyoto Japan) during the volumetric analysis. Thus, they used 'Microsoft Paint' to draw the contours of the periodontal ligaments space before segmentation procedure. In contrast, MIMICS software allows the operator to do any manual segmentation and drawing the contours of the periodontal space during the segmentation phase in slice by slice 'multiple slice editing phase'. Therefore, for 3D measurements, it is advisable to use scientifically acceptable 3-dimensional manual manipulating software instead of relying on 2D software like 'Microsoft Paint' for manual interventions.

A weak correlation was reported between pulp/tooth volume ratio and chronological age in 111 single rooted teeth on the Belgian population (Star et al., 2011). Their study included 64 incisors, 32 canines, and 15 premolars from 54 male and 57 female CBCT scans. Volumetric analysis was performed using Simplant software

(Version 11.0 on Windows; Materialise Dental NV, Leuven, Belgium). Their results showed that the strongest coefficient of determination (R2) values for incisors was 0.41, followed by premolars 0.23, and canines 0.07. Interestingly, the strength of correlation values for all the 3 investigated types of teeth in our study was much higher although, they were one of the earliest to report on manual intervention during segmentation and separation phase of the volumetric analysis (Table 5.1). This was important as the software was not able to detect minute structural demarcations in the apical root portion. In addition, similar to the results reported by Star et al. (2011), our study also indicated that this method of dental age estimation is gender independent

Our study indicated higher coefficient of determination values as compared to all studies carried on Belgian population (Yang et al., 2006; Star et al., 2011; Vandevoort et al., 2004) (Table 5.1). This showed that the strength of correlation between pulp cavity volume change with age varies among different populations and each population belonging to different countries or race must have their own regression equation for dental age estimation.

Table 5.1: Coefficient of determination values reported on Belgian population.

SNO	Research work	Coefficient of determination values (R2)	Year of study	Study population
01	Vandevoort et al., 2004	0.31	2004	Belgian
02	Yang et al., 2006	0.29	2006	Belgian
03	Star et al., 2011	0.34	2011	Belgian
04	Present study	Maxillary right central incisors R2=0.70, Maxillary right and left canines R2=0.55,0.53.	2017	Malaysian

5.8.2 Comparison with studies reported on the Japanese population.

Someda et al. (2009) reported that mandibular central incisor pulp cavity volume can be used for dental age estimation. Samples were scanned with micro-CT and 3D measurements were recorded with the help of TRI 3D-BON software. Their study showed strong relationship between chronological age and pulp/tooth volume ratio (Male, R2=0.67; Female, R2=0.76) (Someda et al., 2009). However, the coefficient of determination value (R2=0.70) for maxillary right central incisor in our study was higher than the study carried on mandibular central incisors on Japanese population As opposed to this present study their results showed significant difference in the mean values between genders (Table 5.2). Although, Someda et al. (2009) did not report any statistical test regarding comparison of difference in the strength of correlation values between genders and they relied on 95% confidence and 95% prediction intervals to conclude that age estimation is gender dependent. The present study showed that there was no significant difference in the strength of correlation values between genders, although males had higher values than females unlike the findings of their study.

Table 5.2: Comparison in the coefficient of determination, standard error of the estimate and regression equations between two studies. (PTV ratio= Pulp/Tooth volume ratio).

Study	Type of Teeth	Coefficient of Determination (R2)	Std. Error of the estimate	Regression equation for dental age estimation 'y'
Present study	Maxillary right central incisors	0.70	7.603	Y=73.24-(1010.71 x PTV ratio)
Someda et al. 2009	Mandibular central incisors	Male, R2=0.67; Female, R2=0.76	Male=10.09, Female=8.09	Male, Y=-967.58 PTV ratio+74.62, Female, Y=-1060.90 PTV ratio+73.02

Another researcher followed the same methodology of Someda et al. (2009) by measuring the pulp/tooth volume ratio excluding enamel to determine the relation between pulp/tooth volume ratio and chronological age (Agematsu et al., 2010). Mandibular central incisors and mandibular second premolars were included in the study. The results of the study were similar to that reported by Someda et al. (2009) where females had higher correlation than males. But the coefficient of determination values reported by Agematsu et al. (2010) were comparable for mandibular central incisors with the present study on maxillary right central incisors. (Table 5.3). The reason for these comparable values may be attributed to the studied population belonging to the Mongoloid origin. Agematsu et al. (2010) reported higher coefficient of determination values for female compared to males but did not carry out any statistical tests for establishing statistical difference between genders. The coefficient of determination values for the mandibular second premolars were slightly higher than for maxillary canines in the present study (Table 5.3).

Table 5.3: Comparison in the coefficient of determination values between studies.

Study	Type of Teeth	Coefficient of Determination (R2)
Present study	Maxillary right central incisors, Maxillary canines	Maxillary right central incisors = 0.70, Maxillary right and left canines =0.55,0.53.
Agematsu et al., 2010	Mandibular central incisor, mandibular second premolar.	Mandibular central incisor (Male=0.67, Female=0.75), Mandibular second premolar (Male=0.56, Female=0.58).

Aboshi et al. (2010) reported on the relationship between age and pulp/tooth volume ratio in mandibular first and second premolar using micro CT. They observed this relationship at four different levels (Crown area, coronal one third of the root, midroot and apical third of the root). The highest correlation was observed for coronal one third of the root. This study however did not mention on gender differences. Interestingly, the coefficient of correlation values in this study for the maxillary right central incisors were similar to second mandibular premolars reported on the Japanese population (Aboshi et al., 2010)(Table 5.4). Again, higher values of coefficient of determination values for both studies may be due to the studied population belonging to same Mongoloid race.

Table 5.4: Coefficient of determination values between studies

Study	Type of Teeth	Coefficient of Determination (R2)
Present study	Maxillary right central incisors, Maxillary canines	R2=0.70 R2=0.55,0.53.
Aboshi et al., 2010	Mandibular 1st premolars, Mandibular 2nd premolars.	R2=0.63 R2=0.70

Sakuma et al. (2013) carried out a study on 136 mandibular premolars. The sample for their study was obtained from the dead bodies using MDCT to evaluate the relationship between pulp/tooth volume ratio with chronological age. The results were different from the previous studies due to marked differences in the coefficient of determination values between males and females (Male=0.596, female=0.186). However, they did not justify with statistical tests, the statistical difference in values of the strength of correlation between genders (Sakuma et al., 2013) (Table 5.5). Another factor for such a low value of coefficient of determination values for females in their study may be due to less number of female's sample (n=31) as compared to males (n=105) (Sakuma et al., 2013). Their result showed that the strength of correlation values for males were higher as compared to females which although similar to the present study but was not statistically significant. Our study incorporated CBCT scans, which has an advantage over MDCT data in terms of slice interval and small field of view.

Table 5.5: Coefficient of determination values based on genders.

Study	Type of teeth (FDI notation)	Coefficient of determination values (R2) (FDI notation)
Sakuma et al., 2013	Mandibular 1st premolars	Male=0.596, female=0.186
Present study	11 13 23	11; Male=0.71, female=0.69, 13; Male=0.55, female=0.53, 23; Male=0.59, female=0.47.

5.8.3 Comparison with studies reported on the French populations.

Tardivo et al. (2011 & 2014) reported that permanent canines pulp/tooth volume ratio can be used to estimate dental age. In both of their studies, they used more than one tooth per subject to estimate dental age (Table 5.6). The sample size included 58 CBCT scans for the analysis of 133 canines (Tardivo et al., 2011) and 210 CBCT scans for the analysis of 840 canines (Tardivo et al., 2014). There was a marked difference in the coefficient of determination values between the two studies which were carried within the span of 4 years' (Table 5.6). The reason behind such a big variation between the strength of correlation may be attributed to the large difference in sample size and bias in the results due to the selection of more than one tooth per subject having similar developmental origin. Unlike in the present study, biased data due to inclusion of teeth having similar developmental origin could have occurred.

Although the coefficient of determination values obtained from the present study were higher than reported by Tardivo et al. (2011) but the values were lower for canines (Tardivo et al. 2014) despite using the Mimics software (Table 5.6). The CBCT scans with same voxel size of 0.30 mm were used in the present study to nullify partial volume averaging effect of voxels on volumetric measurements (Maret et al., 2012) but this was not followed by Tardivo et al. As stated earlier, this is necessary because CBCT scans acquired with different voxel sizes have different volumetric results for the same anatomical structures (Whyms et al., 2013).

Table 5.6: Comparison between types of teeth, sample size, Coefficient of correlation (r) and coefficient of determination (R2) values between studies.

Study	Type of teeth (FDI notation)	Total number of subjects	Total number of teeth derived from the subjects	Coefficient of correlation (r) and coefficient of determination (R2) values. (FDI notation)
Tardivo et al., 2011	Permanent canines	58	133	r = 0.591
Tardivo et al., 2014	Permanent canines	210	840	R2 = 0.91
Present study	Maxillary right central incisor, maxillary right and left canines	300	300	11, R2 = 0.70; 13, R2 = 0.55; 23, R2 = 0.53.

5.8.4 Comparison with studies reported on the Italian population.

A pilot study was reported on maxillary left central incisor to estimate dental age by volumetric analysis of pulp/tooth volume ratio with age using CBCT images (Pinchi et al., 2015). The results of the study were in line with our study as their study also reported no significant difference in the strength of correlation values between genders (P=0.769). The coefficient of determination value reported in their study was 0.58. The present study showed higher strength of correlation value (R2=0.70) for maxillary right central incisor as compared to their study on maxillary left central incisors (Table 5.7).

Table 5.7: Comparison in the coefficient of determination values between two studies.

Study	Type of teeth	Coefficient of correlation values (R2)
Pinchi et al., 2015	Maxillary left central incisors	R2=0.58
Present study	Maxillary right central incisors	R2=0.70

Angelis et al. (2015) reported that pulp chamber/tooth volume ratio with aging can be used for dental age estimation. They conducted study on 91 maxillary right canines. Their study selected one tooth per subject to avoid bias in the results, similar to the methodology in our study. The coefficient of determination value (R2) reported in their results was lower than the results observed in our study. (Table 5.8). They also reported that dental age estimation method is gender independent, which was similar to our results. However, their study did not report on any manual segmentation during the segmentation procedure, which is an important protocol in volumetric studies.

Table 5.8: Comparison in the coefficient of determination values between studies.

Study	Type of investigated teeth. (FDI notation)	Coefficient of determination values (R2)
Danilo De Angelis et al., 2015	13	R2=0.39
Present study	11, 13, 23.	11, R2=0.70; 13, R2=0.55; 23, R2=0.53.

5.8.5 Comparison with studies reported on the Chinese population.

Zhipu et al. (2015) reported that volume of the pulp chamber of first molars can be used for dental age estimation using CBCT scans. CBCT scans of mandibular and maxillary first molars from different quadrants were obtained for their study. Unlike the present study, they measured just the pulp chamber volume (tooth crown) instead of measuring the complete pulp cavity. When we compared our study with the study carried on multi-rooted teeth, the strength of correlation (R2=0.70) for maxillary central incisor in the present study was much higher than reported for first molars (Table 5.9). Nevertheless, the result of maxillary canines is comparable with their results. Their study reported that there was a significant difference in the strength of correlation values between maxillary and mandibular first molars. They also indicated significant difference in the strength of correlation values between genders, unlike in the present study. Therefore, they derived separate regression equations for each gender intended for maxillary and mandibular first molars (Table 5.9). Moreover, their study used independent sample t-test to conclude that there was a statistical difference in the mean values between genders.

Table 5.9: Comparison of coefficient of determination values and regression equations.

Study	Type of investigated teeth.	Coefficient of correlation value (R2).	Regression equation for dental age estimation.
Zhe Pu Ge et al., 2015	Maxillary 1st molars, mandibular 1st molars.	Male maxillary first molars R2=0.544	Age = 118.456 – 25.67 ×ln (pulp chamber volume).
		Male mandibular first molars R2=0.562	Age = 118.398 – 26.756 ×ln (pulp chamber volume).
		Female maxillary first molars R2=0.684	Age = 131.455 – 30.685 ×ln (pulp chamber volume.
		Female mandibular first molars R2=0.612	Age = 119.519 – 28.182 ×ln (pulp chamber volume
Present study	Maxillary right central incisors.	R2=0.70	Age =73.24-(1010.71 x PTV ratio)
	Maxillary right canines.	R2=0.55	Age =67.07-(732.97 x PTV ratio)
	Maxillary left canines.	R2=0.53	Age=66.19-(748.29 x PTV ratio)

In a subsequent research, Ge ZP et al. (2016) reported that maxillary second molars have the highest coefficient of determination value (R2=0.498) amongst the 13 different types of single-rooted and multirooted teeth studied (Table 5.10). Except for the mandibular first molars (p=0.102), all the other types of investigated teeth showed significant difference in the volumes between genders. As reported earlier, our results showed no significant difference in the values between genders (Table 5.10). Similarly, in concordance to the present study, their study reported maxillary canines showed the lowest values of coefficient of determination (R2=0.108) and maxillary central incisors showed the highest value (R2=0.323) among all the maxillary single-rooted teeth. Furthermore, although this is the most recent study, their study did not report on the need of undertaking manual segmentation during the segmentation phase of the volumetric analysis for improved measurements.

Table 5.10: Comparison in the coefficient of determination values between studies based on genders and pooled sample.

Study	Type of investigated teeth (FDI notation)	Coefficient of correlation values (R2)		
		Pooled sample	Male	Female
Zhi Pu Ge et al., 2016	11,21	0.323	0.391	0.290
	12,22	0.311	0.406	0.285
	13,23	0.108	0.198	0.117
	15,25	0.305	0.323	0.386
	31,41	0.253	0.334	0.206
	32,42	0.201	0.284	0.162
	33,43	0.167	0.252	0.269
	34,44	0.330	0.413	0.351
	35,45	0.344	0.404	0.393
	16,26	0.489	0.481	0.554
	36,46	0.434	0.457	0.458
	17,27	0.498	0.491	0.642
	37,47	0.487	0.458	0.614
Present study	11	0.70	0.706	0.685
	13	0.55	0.554	0.534
	23	0.53	0.592	0.465

5.8.6 Comparison with study reported on the Indian population.

A study on mandibular canines was reported to estimate dental age by volumetric analysis using CBCT images (Jagannathan et al., 2011). One hundred and eightyeight intact mandibular canines from 140 individuals (ranging in age from 10 to 70 years) were included in the study. They reported that age estimated by using formula carried out on Belgian population showed errors of more than ten years in almost 86% of the study sample. When they applied the regression equation to an independent control group (n=48), which was obtained for an Indian population, Age = 57.18+ (- 413.41 x pulp/tooth volume ratio), the results were significantly lower than Belgian formula and mean absolute errors of 8.54 years was reported. In addition, their study reported a coefficient of correlation value of 0.63, which is lower than our results for maxillary canines (R=0.74,0.73). Their study also did not report on the manual segmentation and any significant difference between genders.

5.9 Comparison between right and left maxillary canines

In the present study, Fishers Z test was used to find if there was any significant difference in the correlation coefficient values between maxillary right and left canines- each tooth belonging to different subjects. It was important to know if similar type of teeth belonging to different quadrants in the oral cavity and different individuals showed similar or different pattern of secondary dentine formation. The results have shown no significant difference in the strength of correlation values between maxillary right and left canines (P=0.880) (Table 5.11)

Table 5.11: Pearson correlation, Fishers Z-test for maxillary right and left canines. ($p<0.01$), (Z critical 1.96 for $p < .05$).

Pearson correlation coefficient (R)		**Fishers Z-test**	
Maxillary right canines	Maxillary left canines	(Z observed values)	P-values
0.738	0.726	0.15	0.880

5.10 Limitations of the study

It must be borne in mind that following are some of the limitations involved in this study. This study does not involve direct contact of the researcher with the patients rather inclusion of the sample is from CBCT data stored in the Oral and maxillofacial Imaging Division, Faculty of Dentistry University of Malaya. Thus, the exclusion of any pathology associated with the investigated tooth is ruled out merely by observing the CBCT scans. This process of sample selection may miss some of the pathologies or previous traumatic history associated with the investigated tooth, which cannot be detected on CBCT images alone.

The segmentation procedure in this study involved some minor manual interventions. In the apical portion of the root and pulp tissue, the software was not able to automatically detect the minute structural demarcations. Areas of the pulp cavity and calcified tooth structures, which were not automatically selected by the software were manually checked and edited in the required area in ‘multiple slice editing phase’ of the software. In future with advancements in the CBCT technology and improvement of image enhancing software’s, this phase of the ‘segmentation and multiple slice editing’ can be improved which can lead to less manual interventions and minimal chances of human error in the methodology.

CHAPTER 6: CONCLUSION

6.1 Introduction

The study investigated the relationship between the secondary dentine formation with age, to derive a regression equation for dental age estimation. Following are the conclusions, clinical applications of the study and recommendations for future studies.

6.2 Research outcomes

(1) The results indicated a strong relationship between chronological age and pulp/tooth volume ratio for all the 3 types of investigated teeth in the study. Thus, secondary dentine formation with age is the valuable indicator for dental age estimation in Malaysian population.

(2) Among all the 3 types of investigated teeth maxillary right central incisor has shown the strongest coefficient of determination value as compared to maxillary canines.

(3) This method of dental age estimation is gender independent as there was no statistically significant difference in the coefficient of correlation values between genders.

(4) There was no statistically significant difference in strength of correlation between chronological age and pulp/tooth volume ratio for maxillary canines belonging to different subjects and quadrants.

6.3 Clinical applications

(1) Results of the present study clearly showed the difference in the strength of correlation values for Malaysian adults as compared to the previous studies carried on other populations. Thus, the regression equation for dental age estimation derived from this study can be used specifically for Malaysian adults with greater accuracy and precision.

(2) This method of dental age estimation can be helpful in the implementation of laws in the legal justice system in Malaysia. e.g. According to Criminal Procedure Code 1976, corporal punishment by caning is lawful as a sentence for serious crimes in Malaysia ("Laws of Malaysia", 1976). However, caning is spared for convicted females and also males above 50 years. Therefore, this method of age estimation may become useful in future for those sentenced persons who are indeed above 50 years and their age cannot be verified with any legal documents.

(3) This method of dental age estimation can be used in mass disasters (earthquakes, tsunami's, aviation disasters) for dental age estimation and identification of unidentified remains of the deceased. Dental identification is inexpensive, but this technique of age estimation takes about 3 to 4 hours for the volumetric analysis of each tooth.

(4) X-ray beam limitation, rapid scan time, dose reduction and accuracy of the CBCT images are the features of the CBCT technology, which makes this method of dental age estimation more favourable for clinical application as compared to other invasive modalities (Patcas et al., 2012; Scarfe et al., 2008). The volume of the CBCT data can be reoriented according to the patient's anatomical features because it is acquired with voxel resolutions that are isotropic. Images from CBCT

data can also be further enhanced with MIMICS (Materialise Interactive Medical Image Control System) software, as performed in this study.

6.4 Recommendations for further research

(1) Another pulp/tooth volume ratio study on a new set of samples should be performed by applying the regression equation derived from the present study. This will ascertain if the dental age estimation corresponds to the chronological age of the individual or further modifications of the equation is necessary for the Malaysian population. Furthermore, the results can be compared with the previous studies carried out on other populations around the world to determine if the results are similar or if the variations are large.

(2) Studies can also be carried out using the same pulp/tooth volume ratio technique to determine juvenile verses adults dental age in legal circumstances (eighteen years old being the threshold).

(3) Future studies can be performed using the same method involving different types of single-rooted and multi-rooted teeth to analyse which tooth in the oral cavity is showing the highest strength of correlation between chronological age and pulp/tooth volume ratio among Malaysian population.

(4) With regards to the segmentation procedure in this study, it required some minor manual interventions. At the apical portion of the root and pulp tissue, the software was not able to detect minute structural demarcations. It took approximately 3 to 4 hours during ‘multiple slice editing phase’. This protocol may need to be improved with further advancements in the MIMICS software technology.

(5) To overcome limitation of the present software not being able to detect the minute structural details, future studies can be conducted to investigate only the pulp

chamber volumes rather than including the root canal too. This can further improve the accuracy in the measurements and results as manual interventions is not necessary.

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