A THREE DIMENSIONAL AGE ESTIMATION USING CONE BEAM CT IN MALAYSIAN POPULATION

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ABSTRACT

Forensic odontology plays a vital role in the age estimation and identification process during criminal investigations. Forensic science in the past have mainly employed two-dimensional (2D) radiographic assessment of developmental and physiological age related dental parameters for age estimation. However, 2D radiograph doesn't represent the three-dimensional (3D) anatomical structures. Rapidly evolving CBCT imaging modality can be effectively employed in forensic sciences to overcome the limitations of 2D imaging. The current study aimed to develop a novel method of dental age estimation using 3D surface area analyses of root apices of the developing permanent maxillary canines and mandibular third molars among Malaysian population using CBCT data. Two new and validated dental age estimation regression equations were developed. The first regression equation was developed for Malaysian children aged 7 to 14 years by assessing the relationship between chronological age and surface area of the developing maxillary canine apex. Variations based on ethnicity (Malay/Chinese), gender and status of root development (open/closed) were also investigated in terms of fit to the age estimation model. The training sample of 191 permanent maxillary canines was selected from the CBCT images belonging to 100 Malays and 91 Chinese. Moreover, an independent validation sample of 96 permanent maxillary canines was also selected. Multiple linear regression analysis was used to derive an age estimation model using chronological age as a dependent variable and surface area of the maxillary canines apices, ethnicity, gender and status of the root development (open/closed apices) as predictor variables. A strong correlation ($r = 0.978$) was observed between chronological age and the predictor variables. 95.6 % of the variation in age was explained by the surface area of canine apex, gender and status of the root development (open/closed apices). However, ethnicity did not contribute to the fit of age estimation model. Mean absolute error (MAE) value of 0.30 was observed when tested on an independent validation sample. The second regression equation was developed for Malaysian juveniles and young adults aged 13 to 24 years by assessing the relationship between chronological age and surface area of the developing mandibular third molars apices. The training sample of 128 intact mandibular third molars was selected from the CBCT images belonging to 66 Malays and 62 Chinese. In addition, 55 mandibular third molars were selected as a validation sample. Multiple linear regression analysis was used to derive an age estimation model using chronological age as a dependent variable and surface area of the mandibular third molars apices, ethnicity, gender and status of the root development (open/closed apices) as predictor variables. A strong inverse correlation (r = 0.95) was observed between chronological age and all the predictor variables. The results showed that 89.6% of the variation in age can be explained by the predictor variables. MAE value of 0.822 was observed when the derived regression equation was tested on the independent validation sample. In conclusion, 3D surface area analysis of the developing root apices can be used as a reliable method for age estimation in Malaysians.

Keywords: CBCT; Age estimation; Three-dimensional; Malaysian.

ABSTRAK

Odontologi forensik memainkan peranan yang penting dalam mengenalpasti dan mengganggarkan usia semasa melakukan siasatan jenayah. Pada masa lalu, sains forensik biasanya akan menganggarkan umur berdasarkan kepada penilaian radiografi dua dimensi (2D) untuk menilai perkembangan dan umur fisiologi yang berkaitan dengan parameter pergigian. Walau bagaimanapun, radiografi 2D tidak dapat memaparkan struktur anatomi dalam tiga dimensi (3D). Mesin pengimejan CBCT yang berkembang pesat dapat digunakan secara berkesan dalam sains forensik bagi mengatasi kelemahan pengimejan 2D. Kajian ini bertujuan untuk menghasilkan kaedah baru untuk menganggar usia gigi dengan cara menganalisa luas permukaan secara 3D pada puncak akar gigi taring atas dan gigi geraham ketiga rahang bawah yang masih berkembang, di kalangan penduduk Malaysia menggunakan data CBCT. Dua persamaan regresi anggaran usia pergigian yang baru dan disahkan telah dihasilkan. Persamaan regresi pertama dihasilkan untuk menilai hubungan antara usia kronologi dan luas permukaan puncak akar gigi taring rahang atas yang sedang berkembang untuk kanak-kanak Malaysia yang berumur 7 hingga 14 tahun. Variasi berdasarkan etnik (Melayu / Cina), jantina dan status perkembangan akar (terbuka / tertutup) juga disiasat dari segi kesesuaian dengan model anggaran usia. Sebanyak 191 sampel latihan gigi taring kekal rahang atas telah diambil dari imej CBCT orang Melayu (100) dan orang Cina (91). Selain itu, sampel pengesahan bebas dari 96 gigi taring kekal rahang atas juga telah dipilih. Analisis regresi linier berganda digunakan untuk menghasilkan model anggaran usia menggunakan usia kronologi sebagai pemboleh ubah bersandar dan luas permukaan puncak akar gigi taring rahang atas, etnik, jantina dan status

perkembangan akar (terbuka / tertutup) sebagai pemboleh ubah ramalan. Korelasi yang kuat (r = 0.978) dapat diperhatikan diantara umur kronologi dan pemboleh ubah peramal. 95.6% variasi usia dapat dijelaskan oleh luas permukaan puncak gigi taring, jantina dan status perkembangan akar (terbuka / tertutup). Walau bagaimanapun, etnik tidak menyumbang kepada model anggaran usia yang sesuai. Nilai ralat mutlak (MAE) yang diperhatikan semasa menguji sampel pengesahan bebas ialah 0.30. Persamaan regresi kedua telah dihasilkan untuk remaja Malaysia dan dewasa muda yang berusia 13 hingga 24 tahun dengan menilai hubungan antara usia kronologi dan luas permukaan puncak akar gigi geraham ketiga rahang bawah yang sedang berkembang. Sebanyak 128 sampel latihan gigi geraham ketiga yang masih utuh telah dipilih dari imej CBCT orang Melayu (66) dan orang Cina (62). Sebagai tambahan, 55 gigi geraham ketiga rahang bawah telah dipilih sebagai sampel pengesahan. Analisis regresi linier berganda digunakan untuk mendapatkan model anggaran usia menggunakan usia kronologi sebagai pemboleh ubah bersandar dan luas permukaan puncak akar gigi geraham ketiga rahang bawah, etnik, jantina dan status perkembangan akar (terbuka / tertutup) sebagai pemboleh ubah ramalan. Korelasi terbalik yang kuat (r = 0.95) telah diperhatikan diantara umur kronologi dan semua pemboleh ubah peramal. Hasil kajian menunjukkan bahawa 89.6% variasi usia dapat dijelaskan oleh pemboleh ubah peramal. Nilai MAE ketika persamaan regresi diperoleh diuji pada sampel pengesahan bebas adalah 0.822. Kesimpulannya, analisis luas permukaan 3D dari puncak akar yang berkembang dapat digunakan sebagai kaedah yang boleh dipercayai untuk penganggaran usia di Malaysia.

Kata kunci: CBCT; Anggaran umur; Tiga dimensi; Malaysia.

Dedicated to:

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My mother, Dilras Zafar

My beloved wife, Iqra

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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/DPT	: Orthopantomogram/ Dental Panoramic Image
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

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CHAPTER 1: INTRODUCTION

The chronological age estimation is one of the important aspects of forensic odontology, medicine and anthropology. Forensic odontology plays a vital role in the identification and age estimation of victims (especially in mass disasters), criminals and undocumented individuals. Forensic odontologists apply the knowledge of dental science to achieve these objectives. Dental records comparison, radiographic assessment of teeth maturation, bite mark and dental deoxyribonucleic acid (DNA) analyses are some of the techniques employed by forensic odontologists in criminal and civil investigations to implement the legal justice system. Dental tissues are highly calcified and their ability to resist vigorous environmental conditions makes them one of the most reliable tools in forensic investigations (Chun et al., 2014; Shekhawat & Chauhan, 2016; Merlati et al., 2004). The human remains in mass disasters are mostly incinerated, mutilated or in a condition where other biological profiling techniques may not be useful. Thus, the expertise of forensic odontologists plays a vital role in the reconstruction of biological profiles of the deceased unidentified individuals. Moreover, the mineralisation or development of dental tissues is less affected by the nutritional deficiencies in comparison to other skeletal tissues.

The armed global conflicts, illegal immigrants, terrorism and human trafficking are those grim realities of the current world, which are forcing the researchers to develop a more reliable and simple age estimation methods. DNA profiling, finger printing and artificial intelligence are the advanced techniques currently being employed to identify undocumented persons. However, these advanced facilities may not be available in the third world or developing countries. Moreover, these profiling techniques are expensive and time consuming, and may not be feasible in mass disasters, where large numbers of casualties are expected. Therefore, a simpler and inexpensive identification and age estimation process should be developed.

Studies have been conducted to develop an accurate and simple method of dental age estimation (Bang & Ramm, 1970; Kvaal et al., 1995; Someda et al., 2009). However, most of the studies have employed two-dimensional (2D) radiographic modalities such as panoramic images. 2D radiographic dental age estimation methods can be stratified into age estimation of growing children, juveniles verses adults, and age estimation by analysing physiological age related dental parameters in adults e.g. secondary dentine, cementum annulations etc (Gustafson, 1950; Demirjian et al., 1973; Zandi et al., 2015). The teeth eruption and developmental pattern is specific with age. This could be the reason why dental age estimation methods in growing children have proved to be effective (Demirjian et al., 1973; Cameriere, Ferrante, & Cingolani, 2006). However, once the development of teeth is completed, then physiological age-related dental changes in the adult human dentition can be analysed to predict chronological age (Gustafson, 1950). The maturation of the permanent dentition is completed by the age of 15 to 16 years except third molars. The crown completion of third molar is around 13 to 15 years, the development continues till the age of 20 to 23 years. Third molars have been effectively used to ascertain juvenile verses adult age by employing 2D radiographic imaging modalities (Cameriere et al., 2008).

The 3D dental structures cannot be quantified accurately using 2D radiographs or images. The rapidly evolving cone beam computed tomography (CBCT) technology and image enhancing software's (MIMICS, 3-matics) can be used effectively to quantify dental tissues for age estimation in forensic sciences. The advantages of using CBCT in comparison to medical CT includes higher voxel resolution, lower cost, rapid scan time, small field of view (FOV) and lower radiation exposure (Scarfe & Farman, 2008).

Developing permanent dentition root apices can be used effectively for age estimation. Cameriere et al., (2006) introduced a new dental age estimation method by analysing the developing root apices in growing children using 2D panoramic images (Cameriere, Ferrante, & Cingolani, 2006). The results indicated a strong correlation between chronological age and changes in the linear measurements of developing root apices. The method proved to be effective and was successfully applied on different ethnic groups around the world (Cameriere, Ferrante, Scarpino, et al., 2006; Cameriere et al., 2007; Rai et al., 2010; Mazzilli et al., 2018). Similar 2D radiographic technique was also applied in measuring the developing third molars apices to ascertain juvenile's verses adults age (Cameriere et al., 2008; Olze et al., 2004; K. Zelic et al., 2016; Solari & Abramovitch, 2002; S. AlQahtani et al., 2017; Liversidge, 2008). Despite of promising results of Cameriere's dental age estimation method among children, juveniles, and young adults using panoramic images, the age estimation method can be further improved and the limitations of 2D radiographic images in measuring 3D anatomical structures can be nullified by using 3D CBCT images and models (Cantekin et al., 2013; Bassed et al., 2011). To the best of our knowledge, no study has been reported in the literature that has investigated the surface area of the developing roots apices for dental age estimation using 3D imaging modality such as CBCT. Therefore, there is a need to investigate the relationship between chronological age and surface area of the developing roots apices using CBCT images among different populations. This will help in developing a new 3D method of dental age estimation with greater accuracy and reliability.

1.1 Statement of problem and research questions

Dental age estimation along with DNA profiling, finger printing and artificial intelligence plays a vital role in the identification of unknown deceased individuals and criminal investigations to implement the legal justice system around the world. This is possible in growing children by assessing mineralisation or maturation of teeth and among adults by quantifying physiological age-related dental parameters such as attrition, secondary dentine formation etc. Cameriere et al. (2006) introduced the 2D radiographic dental age estimation technique by measuring permanent dentition root apices has proved to be effective in growing children, juveniles and adults (Cameriere, Ferrante, & Cingolani, 2006; Cameriere et al., 2008). However, measuring the linear distance of developing root apices using panoramic images can be difficult due to the inability of 2D images to assess any sectional information. Moreover, the lower image clarity and resolution, higher magnification and distortion of anatomical structures are some of the limitations of panoramic radiographs/images, which may lead to faulty biased measurements and analyses (Peker et al., 2008; Ladeira et al., 2012).

As stated earlier, there is no evidence of a study in the literature which has reported 3D analyses of the developing root apices for dental age estimation using advance imaging modalities like CBCT, MRI etc. It is important to develop a more reliable, accurate and simple dental age estimation method using advanced 3D imaging modalities among growing children, juveniles and adults.

Following are the research questions:

i. Can a CBCT image analysis be used for assessing surface area of the developing permanent dentitions roots apices for age estimation?
ii. Is there any association between chronological age and changes in the surface area of developing maxillary canine’s apices using 3D CBCT image analyses among Malaysian children (Malay and Chinese)?
iii. Is there any significant difference in the development of permanent maxillary canines’ apices among genders and investigated ethnic groups (Malay and Chinese)?
iv. Is there any significant effect of surface area of the maxillary canine apex, ethnicity, gender and status of the root development (open/closed apices) as predictor variables on the age estimation regression model for Malaysian children?

v. Can the newly developed dental age estimation model be applied on Malaysian children in future?

vi. Is there any association between chronological age and changes in the surface area of developing mandibular third molars apices using 3D CBCT image analyses among Malaysian (Malay and Chinese) juveniles and young adults?

vii. Is there any significant difference in the development of mandibular third molars apices among genders and investigated ethnic groups?

viii. Is there any significant effect of surface area of the mandibular third molars apices, ethnicity, gender and status of the root development (open/closed apices) as predictor variables on the age estimation regression model for Malaysian juveniles and young adults?

ix. Can the newly developed dental age estimation model be applied on Malaysian juveniles and young adults in future?

1.2 Rationale and Aim

Previously developed 2D radiographic techniques may have limited capabilities in assessing the root apices of the developing permanent dentition for age estimation. Tooth is a 3D structure and it's more logical to assess its development or mineralisation by 3D imaging modalities like cone beam computed tomography (CBCT).

The study aimed to develop a novel method of dental age estimation using 3D surface area analyses of root apices of the developing permanent maxillary canines and mandibular third molars among Malaysian children, juveniles and young adults using CBCT data.

1.3 Objectives of the study

The study was designed to develop two different dental age estimation regression equations/models based on the newly developed 3D surface area analysis of developing root apices. The first model has been developed for Malaysian children and the second model for Malaysian juveniles and young adults. Following are the research and specific objectives of the study:

1.3.1 Research Objective 1

To develop a novel and validated dental age estimation regression equation by investigating the relationship between chronological age and surface area of the developing maxillary canines apices using 3D models developed by employing CBCT images among Malaysian children (Malay and Chinese) aged 7 to 14 years.

1.3.1.1 Specific objectives

i. To investigate the correlation between chronological age and surface area of the developing maxillary canines apices among Malaysian children.

ii. To investigate and compare the strength of correlation (r) values between Malays and Chinese ethnic groups for each gender separately. In addition, ethnic based differences in the canine apex surface area measurements will also be compared.

iii. To investigate and compare the strength of correlation (r) values between male and female gender for each ethnic group separately. In addition, gender based differences in the canine apex surface area measurements will also be compared.

iv. To develop dental age estimation regression equation for Malaysian children using chronological age as a dependent variable and surface area of the maxillary canine's apices, ethnicity, gender and status of the root development (open/closed apices) as predictor variables.

v. To investigate the validity and accuracy of the newly developed age estimation model for Malaysian children on an independent CBCT validation sample.

1.3.2 Research Objective 2

To develop a novel and validated dental age estimation regression equation by investigating the relationship between chronological age and surface area of the developing mandibular third molars apices using 3D models developed by employing CBCT images among Malaysian (Malay and Chinese) juveniles and young adults aged 13 to 24 years.

1.3.2.1 Specific objectives

i. To investigate the correlation between chronological age and surface area of the developing mandibular third molars apices among Malaysian juveniles and young adults.

ii. To investigate and compare the strength of correlation (r) values between Malays and Chinese ethnic groups for each gender separately. In addition, ethnic based differences in the mandibular third molars apices surface area measurements will also be compared.

iii. To investigate and compare the strength of correlation (r) values between male and female gender for each ethnic group separately. In addition, gender based differences in the mandibular third molars apices surface area measurements will also be compared.

iv. To develop dental age estimation regression equation for Malaysian juveniles and young adults using chronological age as a dependent variable and surface area of the mandibular third molars apices, ethnicity, gender and status of the root development (open/closed apices) as predictor variables.

v. To investigate the validity and accuracy of the newly developed age estimation model for Malaysian juveniles and young adults on an independent CBCT validation sample.

1.4 Alternative hypothesis

i. There is a significant association between chronological age and surface area of the developing permanent maxillary canine apex among Malaysian children.
ii. There is a significant association between chronological age and surface area of the developing mandibular third molars apices among Malaysian juveniles and young adults.

1.5 Null hypothesis

i. There is no significant association between chronological age and surface area of the developing permanent maxillary canine apex among Malaysian children.
ii. There is no significant association between chronological age and surface area of the developing mandibular third molars apices among Malaysian juveniles and young adults.

CHAPTER 2: LITERATURE REVIEW

2.1 Dental age estimation and identification in legal justice system

Dental age estimation is one of the least invasive methods in forensic or criminal investigations. Dental age estimation and identification plays an integral part in the implementation of judicial system around the world. Forensic odontologists through their expertise in the field of dental science discover the facts surrounding the criminal acts for the administration of law. The applicability of dental age estimation methods are useful because dental tissues can withstand harsh or extreme environmental conditions and are less susceptible to destruction in disasters (Chun et al., 2014; Thevissen et al., 2006; Kringsholm et al., 2001; Fereira et al., 2008). Forensic odontologists besides other biological profiling methods employ their scientific and technological skills to estimate the dental age of suspects, victims or migrants (Nuzzolese & Di Vella, 2008).

2.1.1 Earlier evidence of dental identification

The services of forensic experts have been acquired for centuries to identify and estimate the age of unknown deceased individuals in criminal proceedings. One of the early and famous documented case of forensic dental age estimation and identification was of Prince Louis XVII in 1795, who died at the age of 10 years. After thorough dental assessment it was confirmed that the skeletal remains in the coffin belong to a deceased aged above 16 years of age. Finally, it was concluded that the skeletal remains found were not of the prince Louis XVII (Amoedo, 1898).

Europe was the hub of rapid industrialization in the Nineteenth century. Children were hired illegally in large numbers as labourers in many industries. Various age estimation methods were proposed to prevent illegal employment of children in industries. Height measurement was used as one of the tools to estimate age but Edwin Saunders in 1837 proposed to the British parliamentarians that teeth can provide more reliable guide for age estimation as compared to height (Shamim et al., 2006). This proposed method later proved to be effective as compared to height evaluation in illegal child labour prevention.

Howard Carter was the British Egyptologist and he discovered the mummy of Pharaoh King Tutankhamun of the ancient Egypt in 1922. Carter and Derry in 1925 performed the first autopsy in 1925 and found 3 partially erupted third molars (Gerloni et al., 2009). They concluded that the King may have died at the age of around 18 to 22 years based on the dental evaluation.

In 2002 and 2004, three mummies were found in Korea. The age at death of these mummies were determined based on the amount of dental attrition observed using 3D reconstructed CT images (Jeong et al., 2008). The estimated dental ages of the mummies were 51.01, 64.45 and 23.57 years.

2.1.2 Importance of dental age estimation in Malaysian justice system

Forensic Odontology is an emerging speciality in Malaysia and many population specific advance research in dental age estimation have been reported in the last few decades (M. K. Asif et al., 2019; Asif et al., 2018; Johan et al., 2012; Cugati et al., 2015). By law, the age of adulthood in Malaysia is 18 years and the punishment for a crime involving suspects below 18 years of age is different. Moreover, Rape is a serious crime and in Malaysia the suspect can be imprisoned up to thirty years. According to the Malaysian law, teenagers below 16 years of age are not allowed to undergo sexual intercourse, with or without her consent ("Law of Malaysia, Penal Code Rape Law", 2006). Sex before 16 years can be charged as rape in Malaysia. Thus, developing reliable dental age estimation methods for juveniles and adults may prove to be effective for the implementation of the Malaysian legal justice system.

According to Criminal Procedure Code 1976 corporal punishment by caning is lawful as a sentence for serious crimes in Malaysia. However, caning is spared for convicted females and also males above 50 years ("Laws of Malaysia Criminal Procedure Code 1976; Sentences and the carrying out of it. ", 1976). Therefore, developing more reliable and accurate Malaysian specific dental age estimation models may become useful in the future for those sentenced persons who are above 50 years and their age cannot be verified with any legal documents.

The majority of Malaysian studies developed age estimation models based on 2D radiographic modalities to ascertain the age of adulthood (Johan et al., 2012). Similarly, dental age estimation methods in children have mainly employed 2D panoramic radiographs (Cugati et al., 2015; Kumaresan et al., 2016; Ismail et al., 2018; Mani et al., 2008). However, research in age estimation by employing 3D imaging modalities is limited for Malaysian population and majority of the recently developed 3D dental age estimation methods are based on pulp/tooth volume ratio (Asif et al., 2018; M. K. Asif et al., 2019).

2.2 Reliability of forensic dental age estimation

According to the Interpol, dental identification is one of the primary identifiers along with DNA profiling and biometric fingerprinting in identifying suspects or victims. A group of forensic experts belonging to various countries (Austria, Germany, Switzerland and Norway) evaluated different methods of age estimation in Berlin due to the rapid increase in illegal immigrants in the year 2000. They introduced age estimation guidelines in criminal investigations among living persons. According to their guidelines dental age estimation is one of the most suitable methods for age estimation (Schmeling et al., 2001). In addition, the Norwegian Dental Age Estimation Project is well recognised in reporting the dental age of the illegal immigrants who are less than 18 years of age (Solheim & Vonen, 2006).

Dental age estimation assists in other methods of identification like DNA profiling and finger printing. Moreover, dental age estimation methods are less invasive and cost effective as compared to other techniques. Many countries rely on DNA (deoxyribonucleic acid) analysis for fast, reliable and sensitive identification of unknown individuals. The DNA differences between two individuals are an extremely useful tool. However, identification can be a challenge if the crime scene is contaminated with DNA from different individuals. It must be borne in mind that the advanced biological profiling methods like DNA profiling or fingerprinting may not be available in underdeveloped countries. Moreover, when large numbers of casualties are involved then it becomes very difficult to do DNA profiling on such a large scale as commingling of human anatomical parts occur.

2.2.1 Teeth are resistant to extreme conditions

Human tooth is the highly-calcified tissue of the body. Dental tissues play an important role in the forensic or criminal investigations due to their ability to withstand chemical, physical, thermal, nutritional deficiencies for a longer duration of time (Chun et al., 2014; Thevissen et al., 2006; Kringsholm et al., 2001; Fereira et al., 2008). It has been reported that dental tissues are the last to be destroyed under extreme conditions (Shekhawat & Chauhan, 2016). There are many evidences in the literature indicating the highly resistant nature of the dental tissues to extreme environmental conditions.

A study investigated the effects of high temperatures on dental tissues (Merlati et al., 2004). The investigated teeth were exposed to the temperatures ranging from 200 to 1100 degree Celsius. The results indicated that unrestored and restored teeth can withstand extreme temperature. However, the changes in the pattern of the restored teeth in comparison to the unrestored teeth can be of great importance in forensic investigations and identification of unknown individuals.

Another study was conducted to assess the effects of extreme temperatures on dental hard tissues (Fereira et al., 2008). One set of teeth were exposed to direct heat and another set was exposed to a gradual increase in temperature. In both groups, dental tissues were able to withstand intense heat. However, teeth exposed to direct heat showed more structural damage as compared to those teeth which were exposed to gradual rise in temperature.

The radiographic evaluation of dental structures plays a significant role in the science of forensic odontology. A study investigated the radiographic effects of high temperatures on different restorations (amalgam & composite) and endodontically treated teeth (Savio et al., 2006). Periapical radiographs of all the investigated teeth before and after exposing to intense thermal stresses were assessed and the differences were recorded. The results of the study indicated that composite fillings can maintain its shape till 600-degree Celsius, amalgam fillings up to 1000-degree Celsius and endodontically treated teeth up to 1100-degree C.

Eighty nine unidentified bodies were recovered from Danish water during a period of 1992 to 1996 (Kringsholm et al., 2001). The forensic experts identified 78% of the cases through dental examination. Even though the unidentified bodies found in Danish waters were exposed to extreme environmental conditions, the study suggested that dental age estimation and identification can be used as a reliable and useful method of identification along with other specialities of forensic sciences.

2.2.2 Comparison of ante mortem and post-mortem dental records in mass disasters

Documenting and securing patient's record by the dentist is essential to be used for the legal testimony in courts and forensic implications. Patient records consist of demographics (including age), examination, diagnosis, investigations, anatomical discrepancies, previous and current treatments and prognosis of the patient. If ante mortem dental records of the unidentified remains of the deceased are available, then it can be compared with the post-mortem records and it can assist in the identification process (Charangowda, 2010; Sarode et al., 2009).

Tooth is a highly calcified tissue of the body and it's most likely the last to be destroyed in the extreme fire or any aviation disasters. It is very challenging to identify burnt victims especially when the remains are badly incinerated. In Spain, 28 incinerated victims of traffic accident were examined by the forensic odontologists and 57% of the cases were identified by dental record matching (Valenzuela et al., 2000). Similarly, Lockerbie air disaster was one of the history's famous aviation disasters in the year 1988. 209 victims of the total 270 passengers were identified with the help of dental records comparison methods (Moody & Busuttil, 1994).

In 2002 Bali bombings, 202 people were killed that included 38 Indonesians, 23 Britishers, 88 Australians and people of more than 20 other nationalities. More than 60% of victims were identified by dental records comparison (Lain et al., 2003). Similarly, the 2004 Boxing Day tsunami with an epicentre off the west coast of northern Sumatra killed around 227,898 people in 14 countries. Dental identification was the primary identifier in more than 75% of cases in Thailand (James, 2005).

The importance of forensic odontology in the age estimation and identification protocols in mass disasters cannot be ignored. Apart from comparison of dental records, the other developmental and physiological age-related dental parameters can also be investigated to develop more precise, easy and reliable age estimation techniques.

2.3 Dental age estimation

The development or maturation of teeth is one of the most reliable indicators of chronological age. The pattern of teeth eruption and maturation is specific with age and the development of dental tissues are least influenced by nutritional deficiencies and environmental factors. Dental age can be predicted by observing the specific pattern of teeth eruption at the particular phase of human development. Dental age estimation in growing individuals is more reliable as compared to the age estimation methods once the development of teeth is completed. This is due to the fact that in children and juveniles' development phases of permanent dentition are shorter and fairly constant. However, once the development of permanent dentition is completed, physiological age-related dental parameters can be considered for age estimation (Gustafson, 1950; Koh et al., 2017; Star et al., 2011).

2.3.1 Dental age estimation methods in children

Various dental age estimation methods have been developed successfully in growing children. Following are some of the pre-natal, neonatal and post-natal dental age estimation parameters.

2.3.1.1 Mineralisation of deciduous dentition

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 the radiographs. However, tooth germs can be seen as radiolucent areas even before mineralisation starts. Even some of the histological techniques can detect early mineralization up to 12 weeks before being seen on the radiographs. Kraus & Jordan (1965) introduced 10 intrauterine stages of early mineralisation in deciduous dentition and permanent first molars (Kraus & Jordan, 1965). They reported their findings by recording the development of teeth in 95 fetuses. The study also concluded that the mesial cusp tip of the mandibular first permanent molar is the only permanent teeth which can be observed on radiograph just before birth. This finding can also be used to ascertain whether a foetus was born alive or had still-birth during forensic criminal investigations.

2.3.1.2 Dental age assessment through neonatal lines

Neonatal lines can be used as a reference to estimate the survival period of an infant in peri-natal period (Janardhanan et al., 2011). The rate of enamel formation in deciduous enamel is 2.5 - 4.5 micron / day (Birch & Dean, 2009). Daily incremental depositions of enamel are seen across enamel rods as cross striations (Risnes, 1998). Counting these cross striations from neonatal line can be used as one of the accurate methods to estimate the age of new born babies (Miles, 1958; Janardhanan et al., 2011). One limitation of estimating age through neonatal lines is that at least a couple of days of survival of the newborn baby is necessary for the maturation of neonatal lines to be identified. However, it must be noted that some of the studies have reported that the absence of the neonatal line is not always an indicator of still birth (Ciapparelli L, 1992).

2.3.1.3 Age assessment from the dry weight of the developing dentition

Method of age estimation was developed by measuring the dry weight of the mineralized tooth cusps (Miles, 1958). According to this method at 6 months of intra uterine development, the weight of the mineralised tooth cusp should be around 60 mg. In newborn, the weight should be around 0.5 grams which can increase up to 1.8 grams after 6 months of birth.

2.3.2 Correlation between dental and skeletal maturity with age

Studies have investigated the correlation between dental and skeletal maturity and its effects on the teeth eruption pattern with age. Svendsen and Björk reported that delayed third molar maturation and advanced or early skeletal development in the mandible can lead to the impaction of third molar (Svendsen & Bjork, 1988). There is also a strong agreement among the oral biologists that a strong correlation exists between eruption time and dental maturity. The teeth normally erupt when they have reached 2/3 root length

(Haavikko, 1970). Moreover, combining other skeletal and dental age assessment methods can further improve the accuracy and precision of age estimation (Vilma Pinchi et al., 2016)

2.4 Two-dimensional radiographic dental age estimation methods

Forensic investigations in the past have mainly focussed on 2D radiographic assessment of developmental and physiological age related dental parameters for age estimation (Gustafson, 1950; Maples & Rice, 1979; Demirjian et al., 1973; Schour & Massler, 1940; Mincer et al., 1993). This could be due to the fact that 3D imaging techniques were not available commercially in the 20th century. Following are some of the radiographic methods used to estimate dental age in growing foetus, children, juveniles and adults.

2.4.1 Tooth eruption chart comparisons

Human studies have investigated different aspects of the teeth eruption sequence and development. However, studies have mainly focussed on the clinical and radiological aspects rather than histological factors. Dental age estimation by assessing total number and type of erupted teeth in the oral cavity is the simplest and one of the non-invasive methods of age estimation (Lysell, 1962; Parner et al., 2002; Helm & Seidler, 1974; Svanholt & Kjaer, 2008). The timing and sequence of tooth eruption and maturation follows the specific chronological order from 6 months to 21 years of age and has been successfully used to estimate dental age for centuries. However, majority of the studies have reported that the tooth development is a more reliable indicator of dental age than tooth eruption (Demirjian et al., 1973; Willems et al., 2001; Emilia, 2011).

2.4.2 Atlas style and scoring systems

2D radiographic dental age estimation techniques or models have been developed in 20th century by introducing atlas or scoring stages of tooth development and maturation

(Schour & Massler, 1940; Ubelaker & Grant, 1989; Moorrees et al., 1963; Demirjian et al., 1973). Moreover, recently population specific schematic/diagrammatic charts of dental maturation have been introduced to develop more reliable and simple age estimation method (M. Blenkin & Taylor, 2012)

2.4.2.1 Schour and Massler age estimation model (1940)

Schour and Massler (1940) introduced an atlas of diagrammatic representation of deciduous and permanent dentition development (Schour & Massler, 1940) (Appendix A). The atlas is used to estimate dental age from the 5th months of intrauterine life to 35 years of age by comparing the teeth development patterns observed on panoramic image (DPT) with the diagrammatic teeth development representation developed by Schour and Massler. This model has been successfully applied on many different populations for dental age estimation (Cesário C, 2016; Eshitha et al., 2014). Schour and Massler (1940) method is less-invasive and simple. However, the atlas does not provide gender specific diagrammatic representation of teeth development for age estimation. This limitation was addressed in another study by Kahl and Schwarze in the year 1988 (Kahl & Schwarze, 1988). The gender specific charts were developed by observing the differences in the development of the permanent dentition and resorption of the deciduous dentition using dental panoramic images. They also reported a slight delay in the development of the permanent dentition in comparison to the study reported by Schour and Massler in 1941.

2.4.2.2 Nollas method of dental age estimation (1960)

Nollas method was based on the 2D radiographic assessment of teeth maturation from the crypt formation to the apical closure of single and multi-rooted permanent dentition. He developed 11 stages (0-10) of permanent dentition mineralisation (Appendix B). This method proved effective because it can be applied on a single quadrant of the upper or lower jaw, or even the complete arch, either including or not including the third molars.

Each tooth has an assigned stage, represented by punctuation; these punctuations are added and scores (points) are obtained, which are then transformed into dental age (DA) by means of reference tables for each gender (Nolla, 1960).

2.4.2.3 Moorrees age estimation model (1963)

Written and pictorial method of dental age estimation was introduced by Moorrees et al. (1963) using maxillary incisors and 8 mandibular teeth (Moorrees et al., 1963). They reported gender specific 14 stages of tooth maturation. Anderson et al. in 1976 further refined the Moorrees et al. (1963) model by incorporating all permanent teeth for age estimation including third molars.

Alqahtani et al. (2010) also introduced a comprehensive and detailed atlas of teeth developmental pattern from twenty-eight weeks of intrauterine life to twenty-three years of age (S. J. Alqahtani et al., 2010) (Appendix C). Their model was based on the modified Moorrees et al. (1963) method of assessing teeth development. This atlas also considered the eruption pattern of teeth relative to the alveolar bone level. The study also reported that teeth maturation is less variable in infancy and most variable after the age of 16 years. However, this atlas did not provide statistically quantified data which is a huge disadvantage for dental age estimation in forensic sciences. In addition, gender differences were also not addressed in the atlas.

2.4.2.4 Demirjian age estimation model (1973)

Demirjian et al. (1973) introduced gender specific 8 stages (A to H) of tooth development using only 7 left mandibular teeth from central incisor to second molar (Demirjian et al., 1973). Each investigated tooth was assigned maturity scores based on the stages of tooth development (A-H). The maturity scores for all the investigated teeth were then added to get the dental age.

The Demirjian method was originally applied on a French Canadian population. This method was later tested on different populations around the world such as Turkish (Tunc & Koyuturk, 2008), Australian (M. R. Blenkin & Evans, 2010; Chiam et al., 2016), Indian (Koshy & Tandon, 1998), Belgian (Chaillet et al., 2004), Malaysian (Mani et al., 2008), South African (Phillips & van Wyk Kotze, 2009), Sri Lankan (Ranasinghe et al., 2019), Italian (Vilma Pinchi et al., 2012). These studies have reported a consistent overestimation in age.

Willems method which is a modification of the Demirjian method was originally developed on a Belgian population. They introduced an adapted Demirjian's scoring system expressed in years and this method was more accurate as it addressed the overestimation reported in Demirjian's originally developed model.

Although Demirjian's method of age estimation is the most widely used and tested technique but it has some limitations. This method cannot be used if any of the seven left mandibular teeth is missing. In contrast to Demirjian et al. (1973) method, Moorrees et al. (1963) technique employed tooth specific scoring system which was more applicable when compared to Demirjian et al., (1973) method. However, Moorrees et al. (1963) method was based on graphical comparison rather than using proper normative data to be statistically analyzed. Modified graphs of Moorrees were designed by future researchers to be analyzed statistically with mean ages of tooth eruption along with standard deviations (Harris & Buck, 2002).

2.4.2.5 Validation of Demirjian's method on Malaysian population for age estimation

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; Nik-Hussein et al., 2011; Bunyarit et al., 2017). Mani et al. (2008) investigated the applicability of Demirjian (1973) and Willems (2002) methods for estimating dental age in a Malay population. 428 panoramic radiographs were analysed for dental age estimation based on the maturity scores and tables proposed by Demirjian and Willems. Both methods resulted in overestimation of age in both gender among Malaysian children (Mani et al., 2008). The results were similar to the previous population specific studies which also reported overestimation using Demirjian's method.

Nik-Hussein et al. (2011) also reported overestimation of chronological age among Malaysian children using Demirjian method (Nik-Hussein et al., 2011). They concluded that the Willem's method is more accurate as compared to Demirjian's method for estimating dental age among Malaysian Children.

Another validation study of Demirjian's method on Kelantanese Malay children also reported overestimation of the chronological age by 1.20 years in female and 1.23 years in male (Abu Asab et al., 2011). Their study showed that dental development in Kelantanese Malay female and male was more advanced as compared to French-Canadian children.

Recently, Bunyarit et al., 2017 also reported overestimation of chronological age among Malay children using Demirjian's method (Bunyarit et al., 2017). Moreover, they developed gender-specific age prediction model using Artificial Neural Networks (ANN) analysis to develop new and more accurate maturity scores for Malay children.

2.4.3 Radiographic assessment of developing root apices as a tool for age estimation in children

In 2006, a new method of dental age estimation was introduced by Cameriere et al. (2006) for Italian children based on 2D linear measurements of developing root apices using dental panoramic images (Cameriere, Ferrante, & Cingolani, 2006). This was one of the first reported studies that indicated a good correlation between chronological age and changes in the linear measurements of the root apices. Seven left permanent mandibular root apices were evaluated and linear distance between the inner margins of the open apices was measured (Figure 2.1). To nullify the differences in magnification and angulations associated with panoramic radiographs, the linear measurements between the inner margins of the open apex was divided by the tooth length (Figure 2.1). The results indicated that gender, second premolar, the sum of the normalized open apices (s), the number (N0) of teeth with root development complete and the first order interaction between 's' and 'N0' contributed significantly to the fit of the age estimation model (Equation 2.1). 83.6% of the variations in estimated age were explained by these variables.

Equation 2.1: $Age = 8.971 + 0.375\ g + 1.631 \times 5 + 0.674\ N0 - 1.034\ s - 0.176\ s \cdot N0$

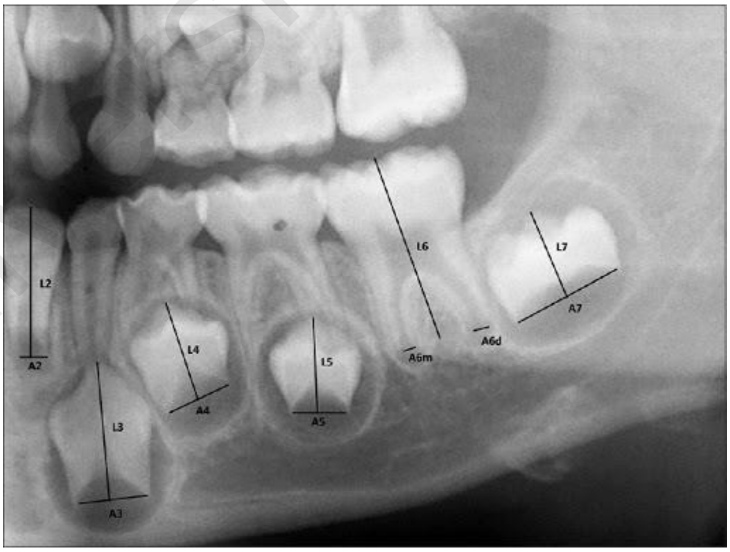

Figure 2.1: The linear measurements of the developing roots apices in Cameriere's technique

Cameriere and his co-researchers further tested the newly developed 2D age estimation method on different European Caucasian children belonging to Germany, Croatia, Italy, Kosovo, Slovenia, UK and the Spain (Cameriere, Ferrante, Scarpino, et al., 2006; Cameriere et al., 2007). The results indicated no significant influence of the population belonging to various European regions on the age estimation model parameters. European model explained 86.1% (R2=0.861) of the variance in age by gender (g), sum of normalized open apices (s), the number of teeth with root development complete (N0), second premolar and the first-order interaction between s and N0 (Cameriere et al., 2007). It must be noted that the European model indicated slightly higher coefficient of determination value (R2 = 86.1) in comparison to the originally developed model on Italian children (R2 = 83.6) (Cameriere, Ferrante, & Cingolani, 2006). In another Italian study, Cameriere method showed more accuracy in estimating the age of 14-year in comparison to Haavikko, Demirjian and Willems method (V. Pinchi et al., 2016).

Gender dimorphism in dental parameters has been reported in many morphometric studies (Schwartz & Dean, 2005; Prabhu & Acharya, 2009; Ling & Wong, 2007). Teeth eruption pattern and dental maturation may vary due to different ethnic, environmental and hormonal factors during sexual development. Gender variances were also investigated in Cameriere's 2D radiographic method and majority of the studies showed a significant contribution of gender variable to the fit of the age estimation model (Cameriere, Ferrante, & Cingolani, 2006; Cameriere et al., 2007; Cameriere, Ferrante, Scarpino, et al., 2006; Cugati et al., 2015; Mazzilli et al., 2018; Talal Halilah et al., 2018; Guo et al., 2015). However, some studies reported no significant contribution of gender variance to the fit of the age estimation model (Rai et al., 2010). It must be noted that most of the studies investigated gender variable in terms of fit to the age estimation model and did not investigate the difference in the strength of correlation (r) values between chronological age and the predictor variables among genders.

2.4.3.1 Validation of Cameriere's age estimation method on Malaysian children

Cameriere's 2D radiographic age estimation method has been tested on Malaysian children. Cugati et al. (2015) developed Malaysian specific age estimation regression model based on the relationship between chronological age and 2D linear measurements of the open apices using Cameriere's method (Cugati et al., 2015). The study also investigated variations based on the Malay, Chinese and Indian ethnic groups. The results of their study showed that the variables gender (g), sum of normalized open apices (s), number of teeth with completed root formation (N0) and the first order interaction between 's' and 'N0' contributed significantly to the fit of the age estimation model. However, the variable 'ethnicity' did not fit to the age estimation model. This was an important finding because all the investigated populations (Malays, Chinese, Indian) despite belonging to different ethnic groups, did not contribute to the fit of the age estimation model (Cugati et al., 2015). Study showed good correlation between chronological age and 2D linear measurements of the open apices among Malaysian children. It must be noted that some of the variables that showed significant contribution in the originally developed European age estimation model did not contribute significantly in the Malaysian population specific model (Cameriere et al., 2007). Moreover, gender distribution was not equal in each ethnic group (Malay: male 61, female 94; Chinese: male 53, female 76; Indian: male 62, female 75). Moreover, sample was not evenly distributed among different age groups (5 to 15 years) which could result in biased statistical results specifically in the multiple linear regression analysis.

Alghali et al. (2016) compared the applicability and reliability of Malay specific Cameriere's formula developed by Cugati et al., (2015) and Malay specific Demirjian's method among Malaysian Kepala Batas population (Noorazma. S et al., 2009). The study indicated that Malay specific Demirjian's method is more reliable than Cameriere's method (Alghali et al., 2016). However, the conclusion was based on combining the data of male and female gender together. Gender specific results in their study indicated that Cameriere's method was more accurate in males and Demirjian's method in females. One reason for the Demirjian's method being better in combined data could be due to the fact that more girls (77) were analysed compared to the boys (49) in their study.

Ramesh et al., 2014 investigated the reliability and validity of Demirjian's, Nolla's, Willems, Cameriere's and Haavikko's 2D radiographic age estimation methods among 426 Malaysian children ranging in age from 5 to 15 years (Kumaresan et al., 2016). The results of their study showed that Cameriere's method (-0.41 years) is the most reliable followed by Willems (+0.54 years), Demirjian's (+0.54 years), Nolla's (=0.97 years) and Haavikko's (-1.31 years) method.

2.4.3.2 Validation of Cameriere's age estimation method on different populations among children

Cameriere's 2D radiographic method was initially developed on Italian children (Cameriere, Ferrante, & Cingolani, 2006) and since then population specific age estimation models have been developed for Malaysian (Cugati et al., 2015), Indian (Rai et al., 2010), Brazilian (Mazzilli et al., 2018), German (Talal Halilah et al., 2018), Chinese (Guo et al., 2015), South African (Angelakopoulos et al., 2019), Serbian (Ksenija Zelic et al., 2020) and Turkish (Gulsahi et al., 2015) populations. The results showed good correlation of chronological age with root apices development. It has been found that the correlation coefficient (r) value varied among different populations and ethnic specific formulas are more reliable and accurate. Moreover, variations based on the predictor variables which originally fitted in the Cameriere's European age estimation model were also observed among different populations (Rai et al., 2010; Angelakopoulos et al., 2019; Guo et al., 2015; AlShahrani et al., 2019; Talal Halilah et al., 2018; Sharma et al., 2016).

Following are some of the validation studies of Cameriere's 2D radiographic age estimation method on different populations.

2.4.3.2.1 Application of Cameriere's method on South African children

Cameriere's originally developed European formula (Cameriere et al., 2007) was tested on 970 (girls 491, boys 479) black and 974 white (girls 493, boys 481) South African children (Angelakopoulos et al., 2019). The Cameriere's European formula has shown overestimation in younger and underestimation in older children, so a new age estimation model was developed. The model explained 76% of the variation in age by the predictor variables among black female and 78% in the black male. Similarly, 76% of the variation in age by the predictor variables among white female and 80% in white male. The mean absolute error of the residuals ranged from 0.718 to 0.769 years. The study concluded that Cameriere's method is applicable on South African black and white population. However, developing population specific age estimation models can give more accurate and precise results.

2.4.3.2.2 Application of Cameriere's method on Turkish children

The validation studies conducted among Turkish population resulted in underestimation of chronological age by employing Cameriere's method (Gulsahi et al., 2015; Apaydin & Yasar, 2018; Ozveren et al., 2019).

Gulsahi et al. (2015) reported underestimation of age using Cameriere's method among both gender (male: -0.47 years; female: -0.24 years) among Turkish children. Moreover, the differences between chronological and dental age increased in the older age groups. In another study, Apaydin et al. (2018) compared the applicability and reliability of Demirjian's, Willems and Cameriere's method (Apaydin & Yasar, 2018). The results suggested that Willems method is more reliable and precise, followed by Demirjian's and

Cameriere's method. However, contrasting results were reported recently in a comparative study between Willem's and Cameriere's method (Ozveren et al., 2019). The results indicated that Cameriere's method is more reliable as compared to Willems method among Turkish children.

2.4.3.2.3 Application of Cameriere's method on Indian children

Rai et al. (2010) developed an Indian formula for age estimation by employing Cameriere's radiographic method on 480 Indian children (Rai et al., 2010). Besides other variables reported in Cameriere's European formula (Cameriere et al., 2007), the effects of Indian children belonging to different parts of India (north, center and south) were also investigated. All the variables reported in Cameriere's European formula significantly fitted with the Indian population specific age estimation formula except gender and second premolar. They reported that the contrasting results with respect to gender could be due to fact that girls in Indian subcontinent are malnourished and the maturation of both genders occurs at the same phase of life. The results of their study also indicated that children belonging to different parts of India have a significant effect on the age estimation model. The study indicated population specific age estimation model should be used among Indian population as compared to the originally developed model on European population. Moreover, higher strength of correlation (r = 0.947) between chronological age and predictor variables was observed.

Sharma et al. 2016 also reported good reliability and applicability of Cameriere's method in North Indian population (Sharma et al., 2016). But in contrast to the originally developed Cameriere's formula (Cameriere, Ferrante, & Cingolani, 2006), the central incisor (x1), first premolar (x1) and molar (x6) also contributed significantly to the age estimation model in addition to the other predictor variables such as gender, sum of normalized open apices (s), number of tooth with completed root formation (N0). The study suggested 88.5 % of variation can be explained by the morphological variables and this method can be used effectively among Indian population for age estimation.

Balla et al. (2016) in another study investigated the applicability of Willems (Willems et al., 2001), Cameriere's (Cameriere et al., 2007) and Acharya's (Acharya, 2011) age estimation methods on the South Indian children (Balla et al., 2016). The results indicated that Acharya's (male: 0.41, female: 0.47) and Willems methods (male: 0.41, female: 0.18 years) had overestimated age. However, Cameriere's method underestimated age by −0.62 years in boys and −0.54 years in girls.

Various studies on the applicability of Cameriere's method for age estimation on Indian population suggested that this method is useful and effective in estimating dental age (Rai et al., 2010; Balla et al., 2016; Sharma et al., 2016). However, variations were observed in the predictor variables that fitted with the age estimation model among Indian studies. This could be due to the fact that each of these studies employed populations belonging to different parts of India. This further emphasizes to develop population specific dental age estimation models because teeth maturation may vary among people belonging to different regions and ethnic groups.

2.4.3.2.4 Application of Cameriere's method on Brazilian children

Multiple Brazilian age estimation validation studies were reported on the reliability and accurancy of Cameriere's method among Brazilian children (Fernandes et al., 2011; Mazzilli et al., 2018). Recently, Luz et al., (2019), compared the reliability and applicability of four different age estimation methods namely Cameriere's [CAM] (Cameriere, Ferrante, & Cingolani, 2006), Liliequist and Lundberg's [LLH] (Liliequist & Lundberg, 1971) and Nolla (Nolla, 1960) without third molars [NOL7] or with third molars [NOL8]) in a Brazilian and Croatian populations (da Luz et al., 2019). The results of the study indicated that Liliequist and Lundberg's [LLH] method was the most reliable.

Cameriere's method showed more accuracy in younger as compared to the older age groups. However overall, the Cameriere's method was the least reliable among all four investigated methods.

In another study, Fernandes et al. (2011) also investigated the reliability of Cameriere's method (Cameriere, Ferrante, & Cingolani, 2006) among 160 Brazilian children ranging in age from 5 to 15 years. The results suggested no significant difference between chronological and estimated age ($p = 0.603$). However, on investigating the variance among different age groups, significant overestimation in age was observed in 5 to 10 years and underestimation in 11 to 14 years age groups. Moreover, the technique was less accurate among 13 to 14 years age group and gender dimorphism was not observed. The reason for less accuracy could be due to fact that the originals Cameriere's technique was developed on Italian population. However, recently a Brazilian population specific age estimation formula was developed by Mazzilli et al. (2018). They compared the effectiveness of the newly developed Brazilian population specific formula with Cameriere's European formula (Cameriere et al., 2007). The original Cameriere's formula showed underestimation of chronological age by -1.24 years as compared to 0.038 years of Brazilian formula. Population specific linear model explained 91.2 % of variation in chronological age with standard error of 0.91 year. The results showed that although Cameriere's original formula is applicable on Brazilian population but developing population specific formula can be more reliable, precise and accepted by the law enforcing agencies.

2.4.3.2.5 Application of Cameriere's method on Chinese children

Guo et al., (2015) developed Chinese specific age estimation formula based on Cameriere's 2D radiographic method and compared its accuracy with the originally developed European formula (Guo et al., 2015; Cameriere et al., 2007). Panoramic images of 785 Chinese children (397 female and 388 male) ranging in age from 5 to 15 years were analysed to develop and validate Chinese specific age estimation formula. Contrary to the original Cameriere's formula which was developed on Italian population, the variables second premolar (x5) and the sum of the normalized open apices (s) did not contribute significantly to the Chinese population specific age estimation model (Equation 2.2). However, canine (x 3), first premolar (x 4), second molar (x 7) contributed significantly to the model in addition to the other predictor variables such as gender (g), number of teeth with root development complete (N0), and the first-order interaction between s and N0. Moreover, when the Chinese population specific and original Cameriere's formulas was applied on an independent Chinese test sample, the results indicated -0.23 year of underestimation in chronological age using original Cameriere's European formula in comparison to -0.04 year using Chinese population specific formula. In addition, Chinese specific formula resulted in higher strength of correlation value (r = 0.955) as compared to the originally developed Cameriere's formula.

Equation 2.2: $Age = 10.202 + 0.826\, g - 4.068x3 - 1.536x4 - 1.959x7 + 0.536N0 - 0.219\, s \cdot N0$

2.4.3.2.6 Application of Cameriere's method on Saudi Arabian children

Cameriere's dental age estimation formula (Cameriere, Ferrante, & Cingolani, 2006) was tested on Saudi children (6-16 years) and resulted in a significant underestimation of chronological age (AlShahrani et al., 2019). As a result, new Saudi specific age estimation regression formula was developed using stepwise linear regression analysis. This newly developed formula indicated that all the investigated teeth showed significant contribution to the fit of the age estimation model except lateral incisors. Variables gender (g) and number of teeth with root development complete (N 0) also showed significant contribution to the model (Equation 2.3). However, the newly developed formula for age estimation was not tested on an independent separate sample.

Equation 2.3: Age = 9.7498 + 0.3031(g) + 0.3895 (N0) - 3.6270 (x central incisor) - 0.3810 (x canine) - 0.3802 (x 1st premolar) - 0.9546 (x 2nd premolar) - 2.6357 (x 1st molar) - 0.8302 (x 2nd molar).

Alsudairi & AlQahtani (2019) in another study compared the applicability of Cameriere's method and AlQahtani's London Atlas among Saudi children ranging in age from 6 to 15 years (Cameriere, Ferrante, & Cingolani, 2006; S. J. Alqahtani et al., 2010; Alsudairi & AlQahtani, 2019). The studied sample was divided into 10 age groups (1-10), each with an age range of 1 year. The results showed that both methods resulted in underestimation of chronological age. However, AlQahtani's London Atlas resulted in less underestimation (- 0.59 years) as compared to Cameriere's method (- 0.89 years). Moreover, there was no significant difference in the gender-based results for both methods.

2.4.3.2.7 Application of Cameriere's method on German children

Cameriere's European formula (Cameriere et al., 2007) was also tested on North German children aged 5–16 years belonging to Caucasian ethnicity and population specific age estimation formula was developed (Talal Halilah et al., 2018). Cameriere's European formula resulted in an underestimation of chronological age in both gender (male: - 0.56, female: - 0.32). The newly developed German population specific age estimation formula resulted in a significant contribution of all predictor variables except second premolar and the first-order interaction between the sum of normalized open apices and number of teeth with root development complete (N 0) (Equation 2.4). In addition, the first-order interaction between the normalized apex width of the canine (x3) and N0 contributed significantly to the fit of the newly derived German model (Talal Halilah et al., 2018).

84.1 % of variance in age was explained by the newly developed model. Following is the German population specific regression formula:

Equation 2.4: $DA = 9.829 + 0.686\, g + 0.632\, N0 - 1.037\, s - 1.582\, (N0 \times x3)$

German population specific regression formula was further applied on a validation sample of 200 panoramic images. The result suggested that the newly developed formula was more accurate and reliable as it underestimated the chronological age in male by - 0.04 years and - 0.08 years in female.

In another study, Wolf et al. (2016) reported the effectiveness and accuracy of Demirjian's method (Demirjian et al., 1973) over Cameriere's 2D radiographic age estimation formula (Cameriere, Ferrante, & Cingolani, 2006; Wolf et al., 2016). The results showed that Cameriere's method resulted in overestimation of chronological age in younger age groups, which was consistent with recently published research on German population (T. Halilah et al., 2018).

2.4.3.2.8 Application of Cameriere's method on Mexican children

Cameriere's European formula was tested on Mexican population aged 5 to 14 year's children (De Luca et al., 2012). Contrary to the studies reported on other populations, the results suggested that Cameriere's original European formula (Cameriere et al., 2007) can be used with high accuracy among Mexican children. The mean difference between estimated age and chronological age among boys was 0.00. However, girls showed slightly overestimation of dental age by 0.10 years. Authors mentioned that the high accuracy of Cameriere's European formula could be due to the migration of people from European origin in Mexico City. It was also observed that the dental age among younger girls were overestimated and underestimated among older age groups. This pattern was consistent with previously reported studies employing Cameriere's 2D radiographic age estimation method on other populations (Angelakopoulos et al., 2019; Fernandes et al., 2011; Talal Halilah et al., 2018; Wolf et al., 2016).

2.4.3.2.9 Application of Cameriere's method on Bosnian-Herzegovian children

Galic et al. (2010) compared the accuracy of Cameriere (Cameriere et al., 2007), Haavikko (Haavikko, 1970) and Willems (Willems et al., 2001) radiographic dental age estimation methods among Bosnian-Herzegovian Children (Galic et al., 2011). 1089 panoramic images were analysed belonging to Serbian, Bosnian and Croatian children aged 6 to 13 years. Cameriere's European formula showed the maximum accuracy (0.09 year in female, - 0.02 in male) in predicting chronological age followed by Haavikko (−0.29 year in female, - 0.09 year in male) and Willems methods (0.24 year in female, 0.42 year in male). It was also reported that Cameriere's method resulted in overestimation of chronological age in younger age groups and underestimation in older age groups among male. However, female showed overestimation of chronological age in all age groups (6-13 years) except 10 and 13 years.

2.4.3.2.10 Application of Cameriere's method on Egyptian and American children

The validation studies on Egyptian (A. A. El-Bakary et al., 2010) and American (Santana et al., 2017) children also resulted in underestimation of chronological age in both genders. These results were in line with majority of the Cameriere's validation studies conducted on Turkish (Apaydin & Yasar, 2018), Indian (Balla et al., 2016), Brazilian (Mazzilli et al., 2018), Chinese (Guo et al., 2015), Saudi Arabian (Alsudairi & AlQahtani, 2019) and German (Talal Halilah et al., 2018) populations.

2.4.4 Dental age estimation among juveniles and young adults by assessing third molars development

Evaluation of legal age of adulthood also known as the age of majority, has become increasingly important over the years. It is a well-established fact that dental age estimation among children and juveniles is more precise and reliable until 16 years of age. This is because multiple teeth are developing at the same time with shorter developmental phases in young children and teeth maturation is less susceptible to extreme environmental factors and nutritional deficiencies (Chun et al., 2014; Kringsholm et al., 2001; Fereira et al., 2008). The development of third molars continue till the age of 20 to 23 years and they are the last teeth to erupt in the oral cavity (Orhan et al., 2007; Zandi et al., 2015). Moreover, they are the only developing teeth during early adulthood and adolescent. Despite of variations in the development of third molars, they can be used effectively for age estimation among juveniles and adults (Johan et al., 2012; Corradi et al., 2013).

Developing third molars can be used in legal or criminal investigations for age estimation. Most of the justice systems around the world consider 18 years of age, as the age of adulthood. However, this varies among different countries according to their religious practices and civil laws. In Indonesia, Myanmar and Yemen the age of adulthood is 16 years and according to the Saudi Arabian law, the age of adulthood is based on physical signs of puberty (bulugh), with age 15 as the upper limit. Similarly, United Kingdom, Kuwait, Cambodia, Cuba, Palestine, Kyrgyzstan and Vietnam have set the age of majority of 16 years.

Studies have been carried out to find the relationship between third molars development and chronological age (Orhan et al., 2007; Bolanos et al., 2003). Two-dimensional radiographic studies showed favorable correlation of third molars development with age

(Jung & Cho, 2014; Orhan et al., 2007; Zandi et al., 2015; Mincer et al., 1993). Olze et al. (2010) reported 2D radiographic evaluation of periodontal membrane in third molars for age estimation (Olze et al., 2010). They introduced gender specific 4 stages and concluded that the age of 18 years can be estimated with reasonably accuracy. 2D studies have also investigated gender-based variations in third molars development and reported no statistically significant difference in the rate of maturation of third molars between genders (Cameriere et al., 2008; Soares et al., 2015; Dardouri et al., 2016). However, these results were contrary to the studies reported on other populations in analyzing third molars development (Johan et al., 2012; Jung & Cho, 2014; Mincer et al., 1993; Solari & Abramovitch, 2002; Kasper et al., 2009; Uys et al., 2018). Moreover, ethnic or population-based variations in the third molars development have also been reported in 2D radiographic age estimation studies (Solari & Abramovitch, 2002; Kasper et al., 2009; Uys et al., 2018; Blankenship et al., 2007; Olze et al., 2007).

Despite showing promising results, the method of assessing third molars maturation can further be improved by employing advanced imaging modalities. Recently, Cantekin et al. (2013) successfully employed CBCT images to assess third molars development for age estimation based on the Demirjian et al. (1973) staging system (Cantekin et al., 2013).

2.4.4.1 Relationship between chronological age and third molar development in Malaysian population by employing 2D radiographic modalities

2D radiographic studies have investigated the relationship between chronological age and third molar development among Malaysian population (Nambiar, 1995; Johan et al., 2012). Johan et al. (2012) employed Demirjian’s staging system (A-D for crown development and E-H for root development) to score mandibular third molars maturation among Northeast Malaysian population. They concluded that third molars development can be used to assess the chronological age among Malaysian population. 71.1% of variance in age was explained by gender and mandibular right third molars development. Moreover, faster development of third molars was reported in males as compared to females. These gender specific results were similar to the previously reported studies on different populations and ethnic groups (Jung & Cho, 2014; Mincer et al., 1993; Lee et al., 2009; Zeng et al., 2010; Prieto et al., 2005; Solari & Abramovitch, 2002; Kasper et al., 2009; Uys et al., 2018). It must be noted that due to multicollinearity problem between the teeth 38 and 48, only tooth 48 was included in the final age estimation model. However, gender-based variance was not investigated in terms of the strength of correlation values between chronological age and third molars development.

In 1995, an interesting age estimation case study of a murdered Malaysian male was reported (Nambiar, 1995). It was observed that the third molar crown development was complete, but the roots were still in the developmental phase corresponding to the stage 'F' of Demirjian's staging system (Mincer et al., 1993). The estimated dental age was l7 .5 years ± 2.1, which was later confirmed by the fingerprint analysis and identification documents of the deceased.

It is evident that a limited 2D radiographic studies have been reported among Malaysian population for age estimation by investigating third molars development. However, to the best of our knowledge no 3D study has reported the relationship of third molars development with chronological age among Malaysian population. Due to the variations reported in the third molar development among various populations, it is important to develop a more reliable age estimation method for Malaysian juveniles and adults by employing advanced 3D imaging modalities such as CBCT.

2.4.5 Cameriere's 2D radiographic age estimation method to differentiate minors from majors

Age estimation by evaluating the eruption pattern and mineralization of third molars have been employed for many years using 2D radiographic modalities such as panoramic images. These methods were mostly based on Demirjian's tooth developing staging system (Orhan et al., 2007; Zandi et al., 2015; Mincer et al., 1993). This proved to be effective; however, they showed high percentage of false positive results in differentiating juvenile from adults. Cameriere et al., (2008) developed a cut off value or maturity index (I_{3M} =0.08) for estimating the age of adulthood among Caucasian population by measuring the linear distance of third molars root apices using panoramic images (Cameriere et al., 2008). The method for measuring the linear distance between the open apices was same as was reported by Cameriere et al. (2006) in an age estimation study among Italian children (Figure 2.2) (Cameriere, Ferrante, & Cingolani, 2006). The sum of the distance between the open apices was divided by the tooth length of third molars to asses' maturity index (I_{3M}). The logistic regression analyses showed a good relationship between the chronological age and third molar maturity index. However, gender did not contribute to the fit of the logistic regression model. This indicated that the relationship between chronological age and third molar maturity index is gender independent. The sensitivity of this test (the proportion of individuals being 18 years of age or older whose test is positive) was 70% and its specificity (the proportion of individuals younger than 18 whose test is negative) was 95% (Cameriere et al., 2008). The proportion of correctly classified individuals was 83%. This technique proved to be more precise and accurate as compared to the previously used 2D radiographic maturity staging systems (Orhan et al., 2007; Jung & Cho, 2014; Zandi et al., 2015). Cameriere's third molar maturity index (I_{3M}) was further successfully investigated on other Italian samples with high accuracy (Cameriere, Pacifici, et al., 2014; De Luca et al., 2014). However, it must be noted that the aim of the study was to assess whether an individual is above or below 18 years of age or to differentiate minors from majors. The study did not aim to develop a multiple regression equation which can be used on a larger age range to estimate the age among juveniles and young adults.

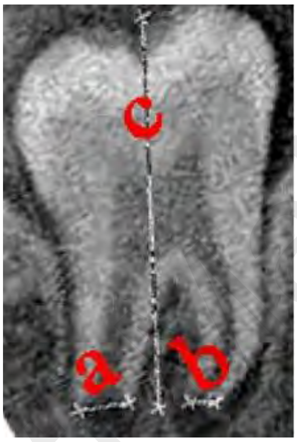

Figure 2.2: The linear measurements between the inner margins of the open apices of mandibular third molar

This method was further tested on other populations around the world and showed promising results. However, the accuracy varied among different populations and ethnic groups. The recently reported meta-analysis evaluated the effectiveness and accuracy of Cameriere's third molar maturity index ($I_{3M} < 0.08$) among different populations and 16 studies were analyzed (Santiago et al., 2018). The percentage of correctly classified studied population ranged from 72.4 to 96.0%. The overall results showed that the pooled sensitivity of the test was 86% (84% to 87%) and a pooled specificity was 93% (92% to 94%). Moreover, male showed more accurate results as compared to females.

2.4.5.1 Application of Cameriere's third molars maturity index (I_{3M} < 0.08) among different populations

Cameriere's third molar maturity index (I_{3M}) (Cameriere et al., 2008) has been tested on different populations and ethnic groups such Egyptian (Amal A. El-Bakary et al., 2019), Chinese (Chu et al., 2018), Brazilian (Nobrega et al., 2019), French (Ribier et al., 2020), Sardinian (Spinas et al., 2018), Polish (Rozylo-Kalinowska et al., 2018) and Dutch (Boyacioglu et al., 2018) to differentiate minors from adults. Its accuracy and reliability was investigated in terms of sensitivity (the proportion of individuals being 18 years of age or older whose test is positive) and specificity (the proportion of individuals younger than 18 whose test is negative) tests. Majority of the studies reported good applicability and reliability of (I_{3M}) with high sensitivity and specificity values to differentiate juveniles from adults. However, it must be noted that the sensitivity and specificity values varied among different populations. Lower sensitivity values were observed in comparison to specificity in majority of the reported studies. This means that third molar maturity index is more effective in determining the age of subjects younger than 18 years of age as compared to above 18 years. This is important in forensic and legal situations that a minor should not be wrongly classified as major, which may have more serious consequences.

Evidence from the literature indicates that validation studies on Cameriere's third molar maturity index resulted in varying specificity and sensitivity test values. This may indicate that third molars development may vary among different populations or ethnic groups. Validation studies on Turkish (Gulsahi et al., 2016) and Libyan (Dardouri et al., 2016) populations have reported the best specificity values of 100 % among females followed by South Indian (98.3%) (Balla et al., 2017) and Albanian study (96.6) (Cameriere, Santoro, et al., 2014). In males, 100% specificity results were reported among Libyan (Dardouri et al., 2016), Turkish (Gulsahi et al., 2016) and Saudi (S. AlQahtani et al., 2017) populations. The highest value of sensitive among females were reported in Libyan (Dardouri et al., 2016), Australian (Franklin et al., 2016) and Serbian (K. Zelic et al., 2016) studies with 90.6%, 90% and 86%, respectively. The highest sensitivity value of 96% was observed among males in Serbian (K. Zelic et al., 2016) and Peruvian (Quispe Lizarbe et al., 2017) studies. It is also evident in the literature that male showed better sensitivity test values as compared to females in French (Ribier et al., 2020; Tafrount et al., 2019), Sardinian (Spinas et al., 2018), Polish (Rozylo-Kalinowska et al., 2018), Serbian (K. Zelic et al., 2016), and Turkish (Gulsahi et al., 2016) populations. However, higher specificity values were observed among females as compared to males. Moreover, males showed faster development of third molars in comparison to females in Egyptian (Amal A. El-Bakary et al., 2019), Serbian (K. Zelic et al., 2016), Hispanic (Solari & Abramovitch, 2002), Saudi (S. AlQahtani et al., 2017), southern Chinese (Wang et al., 2019), Sardinian (Spinas et al., 2018) and Albanian (Cameriere, Santoro, et al., 2014) populations. This could be due to the late growth spurt among males as compared to females. The growth spurt among girls is between 10 to 14 years of age. However, in boys, on an average the growth spurt is about 2 years delayed in comparison to girls. Hormonal changes are accompanied by growth spurts among both genders that transform kids into physically mature teens as their bodies develop. It must be noted that despite variations, most of the studies did not report statistically significant difference in the development pattern of third molars among genders. Moreover, gender did not contribute to the fit of logistic regression models, which means the developed model for their respective population is gender independent (Cameriere et al., 2008; Chu et al., 2018; Wang et al., 2019; Tafrount et al., 2019; Boyacioglu et al., 2018; Kelmendi et al., 2018; Cavric et al., 2016).

Many populations specific cut off value or maturity index have been developed among different populations to differentiate minors from adults based on Cameriere’s 2D radiographic method (Chu et al., 2018; Rozylo-Kalinowska et al., 2018). In an attempt to develop Chinese population specific cut-off value, Cameriere's third molar maturity index (I_{3M}) was applied on 840 northern Chinese population ranging in age from 12 to 25 years (Chu et al., 2018). The results of the study showed that there is a delayed development of third molars among Chinese population as compared to Caucasian population (Cameriere et al., 2008). Cut off value or maturity index of 0.10 was derived for Chinese population as compared to the originally developed cut off value of 0.08 among Caucasian population. The results indicated that the Chinese population specific cut off value (0.10) was more reliable in comparison to I_{3M} (0.08) and showed high sensitivity (0.929 and 0.809) and specificity (0.940 and 0.973) in male and females. Moreover, gender had no significant effect on the age estimation model and gender independent model was developed for Chinese populations. These gender independent results were similar to the originally developed model by Cameriere among Caucasians (Cameriere et al., 2008).

Kalinowska et al., (2018) developed Polish population specific threshold value of 0.07 to differentiate minors from majors and compared the accuracy of Cameriere's third molar maturity index (I_{3M}) < 0.08 (Cameriere et al., 2008) with Polish population specific maturity index of 0.07 (Rozylo-Kalinowska et al., 2018). When Cameriere's third molar maturity index (I_{3M}) < 0.08 was applied, the results indicated that accurately classified individuals among males were 87.6%, while the sensitivity and specificity values were 86.2% and 91.2%. Among females, accurately classified individuals were 85.3% while the sensitivity and specificity values were 82.6% and 93%. Moreover, most wrongly classified individuals were among females. The results for the population specific cut of value of 0.07 showed that, accurately classified individuals were 86.5% in males, while sensitivity and specificity values were 83.2% and 95.3%. Among females, accurately classified individuals were 84.4%, while the sensitivity and specificity values were 80.7%

and 94.7%. The comparison results indicated that Cameriere's third molar maturity index (I_{3M}) < 0.08 showed better accuracy and sensitivity in comparison to Polish population specific cut of value of 0.07. However, cut of value of 0.07 resulted in better specificity of the test among Polish population. In legal justice system it's important not to wrongly ascertain an individual who is less than 18 years of age into a major category. However, the study did not test the newly developed cut-off value of 0.07 on a separate independent sample to validate their results.

AlQahtani et al., (2017) reported low sensitivity test values among Saudi population, when Cameriere's third molar maturity index was applied (S. AlQahtani et al., 2017). Low sensitivity values were reported in both genders (male: 52.3%; female: 51.3%) in comparison to the previously reported studies on other populations (**male**: Australian 90%, Turkish 94%, Black African 88%, Colombian 91%, Serbian 96%, Brazilian 78% and Albanian 94% ; **female**: Australian 90%, Turkish 85%, Black African 88%, Colombian 95%, Serbian 86%, Brazilian 78% and Albanian 75%) (Franklin et al., 2016; Gulsahi et al., 2016; Cavric et al., 2016; De Luca et al., 2016; K. Zelic et al., 2016; Cameriere, Santoro, et al., 2014; Deitos et al., 2015).

Despite the effectiveness of Cameriere's third molar maturity index among different populations. It must be noted that the technique is based on 2D panoramic images and the demerits of 2D radiographic images cannot be ruled out. There is a need to further refine age estimation method for juveniles and young adults. To date no study has investigated the correlation between chronological age and third molars apices development using advance imaging modalities like CBCT, MRI etc.

2.4.6 Relationship between chronological age and third molar development by employing CBCT

Previously reported radiographic dental age estimation studies have shown favorable correlation of third molars development with chronological age. But forensic odontological investigations have mainly employed 2D imaging/radiographic modalities (dental periapical, bitewing, and panoramic radiographs) to assess third molars development for age estimation (Demirjian et al., 1973; Mincer et al., 1993; Orhan et al., 2007; Bolanos et al., 2003). However, as stated earlier the development of 3D anatomical structures can be assessed more accurately using 3D imaging techniques. With the rapid development in cone-beam computed tomography (CBCT) technology, it has become one of the most reliable imaging techniques in forensic investigations (Scarfe & Farman, 2008). Due to the limitations of 2D radiographic modalities, Cantekin et al. (2013) employed CBCT images to assess third molars development and reported its effectiveness in assessing teeth maturation for age estimation based on the Demirjian et al., (1973) staging system among Turkish population (Cantekin et al., 2013). They reported a strong correlation between age and third molar development in males (R2 = 0.80) and females (R2 = 0.78). They concluded that CBCT images can be used effectively to assess third molars development.

In another study, Bassed et al. (2011) compared the usefulness of CBCT images to conventional 2D radiographs in assessing third molars developmental and found an excellent agreement of Demirjian scores between the two imaging modalities (Bassed et al., 2011). They suggested that CBCT is a useful imaging technique for differentiating juveniles from adults in Australian population.

2.5 Dental age estimation in adults

The teeth eruption pattern and development are specific with age and can be used to estimate dental age with reasonable accuracy. Permanent dentition development completes after the root completion of third molars at the age of around 21 to 23 years. However, dental age can also be predicted by evaluating different physiological age-related dental parameters such as apical migration of periodontal ligament, deposition of secondary dentin, attrition, cementum apposition, transparency of the root dentin and root resorption. Gustafson for the first time introduced the concept of estimating dental age by assessing different physiological age-related dental parameters (Gustafson, 1950). Six dental parameters were reported by Gustafson, which are as following:

1. Secondary dentine formation

2. Cementum apposition.

3. Root resorption.

4. Root transparency.

5. Apical migration of periodontal ligaments.

6. Attrition.

Assessing these structural changes with precision and accuracy was a challenge for the researchers at that time due to the less advancement of laboratory and radiographic modalities. The physiological age-related dental changes reported by Gustafson were further investigated by many researchers (Johanson, 1971; Dalitz, 1963; Bang & Ramm, 1970).

2.5.1 Criticism on Gustafson method of age estimation

Root resorption, cementum formation, and apical migration of periodontal ligaments were difficult to quantify due to the lack of any definite pattern at each stage in Gustafson point system. Maples (1978) reported that root resorption is difficult to score and it is the least reliable of all the physiological age-related changes reported by Gustafson, (1950) (Maples, 1978). Moreover, the study suggested that the combined measurements of transparent dentine and secondary dentine formation can give more reliable 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. However, this objection was addressed by Johanson (1971) and modified Gustafson's method by introducing multiple regression analysis with improved standard error value (5.16 years). He introduced seven stages to each variable based on the severity or extent of physiological changes (Johanson, 1971).

Physiological age-related dental parameters for age estimation were further investigated by many researchers. The new 5-point system was introduced by Dalitz (1963) that had overcome the limitations of Gustafson's system and improved the accuracy in estimating age (Dalitz, 1963). However, premolars and molars were not investigated in the 5-point system. In another research Bang and Ramm (1970) successfully investigated the changes in root dentine translucency with age at a microscopic level (Bang & Ramm, 1970). Root dentin translucency initiates in the apical portion of the root and increases in the coronal direction.

2.5.2 Secondary dentine as an important dental age estimation parameter in adults

Secondary dentine formation is one of the vital physiological age-related dental parameters to estimate age. When the root development completes, secondary dentine formation initiates and continues throughout life. This results in the shrinkage of pulp cavity. The decrease in the size of pulp cavity with age has been investigated by many researchers employing 2D and 3D radiographic modalities (Kvaal et al., 1995; Meinl et al., 2007; Star et al., 2011; Tardivo et al., 2014; De Luca et al., 2011; Sironi et al., 2018).

In recent years, CBCT imaging modalities have been successfully employed to assess volumetric changes of pulp cavity or secondary dentine formation among different populations for dental age estimation (Someda et al., 2009; V. Pinchi et al., 2015; Ge et al., 2016; Jagannathan et al., 2011). Although, CBCT studies have resulted in a strong correlation between chronological age and pulp cavity volume. However, the strength of correlation values varied among different populations and ethnic groups. The studies concluded that CBCT can be used with great accuracy and precision to measurement pulp/tooth volumetric changes for dental age estimation.

2.5.3 Age estimation from volumetric analysis of pulp/tooth ratio among Malaysian population using CBCT

In a recently reported CBCT study, two different volumetric methods were investigated to assess pulp/tooth volume ratio for age estimation among Malaysian population aged 16 to 65 years (Asif et al., 2018). 3D volumetric analysis of pulp cavity/tooth ratio was used in Method 1 and pulp chamber/crown ratio (up to cemento-enamel junction) was employed in Method 2. The results indicated strong correlation between age and pulp/tooth volume ratio for both methods. However, method 2 (R2 = 0.78) gave higher strength of correlation values as compared to method 1 (R2 = 0.64) (Table 2.1), This was the first attempt to study pulp/tooth volume ratio with age using CBCT data in a Malaysian population.

Table 2.1: Coefficient of correlation (r), determination (R2) and age estimation regression equations for the investigated teeth (PTV ratio= Pulp/Tooth volume ratio).

Methods of volumetric analysis	Coefficient of Correlation (R)	Coefficient of Determination (R2)	Std. Error of the estimate	Regression equation for dental age estimation 'y'
Method 1	0.799*	0.64*	8.646	Y=69.229-(953.04x PTV ratio)
Method 2	0.880*	0.78*	6.825	Y=66.180-(884.04x PTV ratio)

* Significant at the 0.01 probability level.

In another study, Asif et al., (2019) reported strong correlation between chronological age and pulp tooth volume ratio in maxillary canines and right central incisors (Table 2.2). The results indicated that the method was gender independent and no statistically significant difference was found in correlation values between maxillary left ($r = 0.73$) and right canines ($r = 0.74$) (M. K. Asif et al., 2019). Moreover, no significant difference was found in the strength of correlation values between Malay and Chinese ethnic groups. The study concluded that the maxillary right central incisors can be used with more precision and accuracy for age estimation using the pulp/tooth volume ratio among Malaysian adults as compared to maxillary canines.

Table 2.2: Coefficient of correlation (r), determination (R2), and regression equations for the investigated teeth (PTV ratio= Pulp/Tooth volume ratio) (P<0.01).

Type of Teeth (FDI notation)	Coefficient of Correlation (r)	Coefficient of Determination (R2)	Std. Error of the estimate	Regression equation for dental age estimation 'y'
11	0.83*	0.70*	7.603	Y=73.24-(1010.71 x PTV ratio)
13 & 23	0.73*	0.53*	9.672	Y=66.49-(736.64 x PTV ratio)

* Significant at the 0.01 probability level.

2.6 Increasing trend towards 3D dental age estimation techniques by employing CBCT

Majority of the dental age estimation techniques have been developed by employing 2D radiographic modalities mainly panoramic images (Demirjian et al., 1973; Cameriere, Ferrante, & Cingolani, 2006). It could be due to the unavailability and inaccessibility of 3D imaging modalities in the 20th and first decade of the 21st centuries. As stated previously, one of the most tested 2D radiographic dental age estimation methods around the world is the Demirjian's maturity scoring system (Demirjian et al., 1973; Orhan et al., 2007; Zandi et al., 2015; Willems et al., 2001). 2D radiographic studies have shown strong correlation between chronological age and developing permanent dentition. For years, these 2D techniques have shown their effectiveness in estimating chronological age in criminal investigations to implement the legal justice system around the world. However, there are limitations as 2D panoramic images have unequal magnifications and distortion of anatomical structures.

There is an increasing trend towards developing dental age estimation techniques by employing 3D imaging modalities such as CBCT (Cantekin et al., 2013; Bassed et al., 2011; Star et al., 2011; V. Pinchi et al., 2015). Tooth is a 3D structure and its maturation or development can be evaluated more accurately using 3D imaging modalities. The introduction of CBCT allows 3D visualization of developing tooth. Moreover, It causes no magnification error, which is normally associated with conventional 2D radiography (Vannier et al., 1997). CBCT technology is being broadly used in all fields of dentistry, including orthodontics, endodontics, oral surgery, periodontics and implant treatment planning. CBCT was commercially introduced for the first time in the European market in 1996. The technology of CBCT is constantly evolving and it is becoming one of the most reliable and less invasive imaging modalities in forensic sciences. Initially developed CBCT (NewTom QR-DVT 9000) design was more of a conventional CT, with patient positioned in a supine position. However, latest CBCT machines are much more compact and available in patients seating and standing assemblies. Unlike conventional CT which uses fan shaped x-ray beam, CBCT uses cone shaped x-ray beam.

There are various CBCT systems available in the market with varying properties such as range of field of view (FOV), voxel resolution and type of detectors. But each system has an x-ray source and a detector that rotates simultaneously around the patient head. Basis images are acquired during the rotation in intervals and is captured by the detector. A wide range of 150 to 600 2D images are collected in the detector within a few seconds (White & Pharoah, 2014; Scarfe & Farman, 2008). The time of image acquisition and degree of rotation determine the number of basis images. High quality images will be acquired in longer scan as they will have a greater number of basis images than fast scan.

The 3D reconstruction in a CBCT system can be cylindrical or spherical in appearance depending on the type of a detector used (Scarfe & Farman, 2008; Abramovitch & Rice, 2014). The two most used detectors in the CBCT systems are CCD/II and flat panel detectors. The main difference is the peripheral distortion experienced by the spherical reconstruction with a CCD/II detector. The flat panel detector does not result in this type of distortion. During the reconstruction, 2D basis images will be sent to the CBCT software to create a 3D volume reconstruction of the data volume and also the multiplanar (MPR) reformatted images in axial, coronal, and sagittal orientation (Gonzalez, 2014).

The advantages of employing CBCT in comparison to medical CT includes higher isotropic voxel resolution, lower cost, lower radiation exposure, rapid scan time and small field of view (FOV) (Scarfe & Farman, 2008). However, it must be noted that the quality of CBCT images and its ability to display anatomical and pathological features is dependent on different CBCT image acquiring factors such as type of CBCT system, the field of view (FOV), tube voltage and amperage, and also on spatial resolution defined by the voxel size (Kamburoglu et al., 2011).

Each CBCT system can offer images with different voxel sizes ranging from 0.076 to 0.4 mm. It must be noted that small voxel size will have high resolution images and increase capability to differentiate between minute structural details. Although, small voxel size will result in high spatial resolution but at the cost of high radiation dose to the patient. Another important aspect in selecting the appropriate voxel size is the partial volume effect (PVE), which is associated when a large voxel size is used. In PVE, the voxel lies at the junction of two anatomical structures with different densities. The average density of the two anatomical structures will be assigned instead of the actual value of each structure with different densities (Scarfe & Farman, 2008). In contrary, smaller voxel size will be less susceptible to PVE. However, caution must be taken as using small voxel size CBCT images may result in higher image noise (Al-Rawi et al., 2010). Moreover, contrary to the MDCT, CBCT offers isotropic voxel with uniform resolution and equal dimensions in all 3 planes. As a result, CBCT can be used with high accuracy in measuring anatomical structures in comparisons to MDCT.

In addition, CBCT data in the DICOM files (Digital Imaging and Communications in Medicine) can be exported to third party image enhancing software's like 3-Matics (Materialise NV, Belgium) and Mimics (Materialise NV, Belgium) for 3D volumetric analyses (Materialise, 2018).

2.7 Materialise Interactive Medical Image Control System (MIMICS) Software and 3-matics

MIMICSs (Materialise Interactive Medical Image Control System) is a medical 3D image-based engineering software that can be used for 3D reconstruction and modelling, manufactured by Materialize NV, a Belgian company specialized in additive manufacturing software and technology for dental, medical and additive manufacturing industries.

Mimics (Materialise Interactive Medical Image Control System) software is commercially available as part of the Materialise Mimics Innovation Suite, which also includes 3-matic software, a design and meshing software for anatomical data (Materialise, 2018). CBCT images can be further enhanced with Mimics software. Mimics with the assistance of 'Image segmentation module' calculate the volume of the 3D models from stacked image data provided by CBCT, Computed Tomography (CT), Micro CT, Magnetic Resonance Imaging (MRI) and Ultrasound. It calculates and creates images in the axial, coronal and sagittal planes. This enables a more comprehensive 3D feel of the 2D data. Multiple segmentation tools allow you to select a region of interest. Moreover, 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. Mimics software has been reported in various dental age estimation methods for the volumetric analysis of pulp cavity (Tardivo et al., 2014; Tardivo et al., 2011; Asif et al., 2018).

3-matic software is used to design patient-specific devices, 3D anatomical models for printing, or creating Finite Element Analysis (FEA) meshes based on patient anatomy. It is provided as part of the Materialise Mimics Innovation Suite by Materialise NV. It can be used to design personalized devices directly on the anatomical model using the extensive STL design toolbox. Moreover, virtual implantations can be performed to verify device design and fit.

2.8 Summary of the literature review

Majority of dental age estimation methods developed in the past have mainly employed 2D radiographic imaging modalities such as panoramic and intra oral radiographic images. 2D radiographic images may result in magnification, distortion and blurring of anatomical structures. Moreover, 2D radiographs does not represent the 3D anatomical structures. One of the 2D radiographic dental age estimation methods has been developed by Cameriere and his co-researchers in the year 2006 based on the linear measurements of open apices of 7 left mandibular teeth using panoramic image (Cameriere, Ferrante, & Cingolani, 2006). Similar method was developed to ascertain the age of adulthood among juveniles and young adults (Cameriere et al., 2008). This newly developed method was successfully applied on different populations. Despite of the effectiveness, the strength of correlation between chronological age and linear distance of the developing root apices varied among different populations. This could be due to the limitations associated with the 2D panoramic images. Recent studies have reported that the 3D imaging modalities such as CBCT and MRI can be used effectively for assessing developing teeth for age estimation (Cantekin et al., 2013). Therefore, there is a need to develop a more reliable and accurate dental age estimation method by assessing developing root apices using 3D imaging modalities.

CHAPTER 3: RESEARCH METHODOLOGY

3.1 Introduction

The current study aimed to develop two novel and validated dental age estimation models/equations for Malaysians using 3D surface area analyses of the developing permanent maxillary canines' apices among children and mandibular third molars apices among juveniles and young adults. Separate regression equations for age estimation were developed by investigating the relationship between chronological age and surface area of the developing root apices. Moreover, variations based on ethnicities (Malay/Chinese), gender and status of root development (closed/open apex) were also investigated in terms of fit to the age estimation models. The current study assessed maxillary canine root apex development among growing children aged 7 to 14 years and mandibular third molar apices among juveniles and young adults aged 13 to 24 years.

3.1 Ethical approval for the study

Ethical approval for the study was obtained from the University of Malaya, Medical Ethics Committee (Ref. No: DF OS1824/0085(P) (Appendix D). Prior written informed consents were obtained from all the participants included in the study. Participants were informed that their scanned CBCT data can be used for research and teaching purposes.

3.2 The materials of the study

3.2.1 Three-dimensional (3D) imaging technique and image enhancing software

The study employed cone beam computed tomography (CBCT) images. The CBCT images were further analysed with MIMICS (Materialise Interactive Medical Image Control System) and 3-Matics software's to measure surface area of the developing root apices. It must be noted that similar imaging technique and software's were used to achieve both the research objectives (1.3.1 & 1.3.2).

3.2.1.1 Cone-Beam Computed Tomography (CBCT)

CBCT images were retrieved from the stored database at the Oral and Maxillofacial Imaging Division, Faculty of Dentistry, University of Malaya using i-CAT Cone Beam 3D Dental Imaging System (version 3.1.62 supplied by Imaging Sciences International, Hatfield, USA). The CBCT images were selected based on the quality, image acquiring parameters and chronological age of the patients. All the selected CBCT images 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 selected data was then saved in the external hard disk. The CBCT images selected for the study dated back from January 2008 to April 2019.

The selected CBCT data was then transferred in the DICOM format to the MIMICS (Materialise NV, Belgium, version 21.0) and 3-matic software for creating 3D models and surface area analysis of developing root apices (Materialise, 2018).

3.2.1.2 Materialise Interactive Medical Image Control System (MIMICS) Software

Mimics (Materialise Interactive Medical Image Control System) software is commercially available as part of the Materialise Mimics Innovation Suite (Materialise, 2018). The current study used Mimics software (21.0 version) to create 3D models of the calcified developing root apices. The advanced and automated segmentation tools in the latest version (Mimics 21.0) allowed faster and easy reconstruction of 3D tooth models. However, it must be noted that in few investigated teeth the software was not able to demarcate small structural details, particularly in the radicular portion of the tooth. Therefore, manual intervention was required in the radicular portion with 'multiple slice editing' tool, which was offered by the software to cross check the segmentation process manually, slice by slice.

3.2.1.3 Materialise 3-matic Software

3-matic software is provided as part of the Materialise Mimics Innovation Suite by Materialise NV (Materialise, 2018). The current study employed 3-matic software (13.0 version) to measure the surface area of the developing root apices from the 3D models created in MIMICS software.

3.3 The subjects and methods of the first research objective of the study (To develop an age estimation model for Malaysian children by assessing maxillary canines apices)

3.3.1 Sample size calculation

Sample size of the first research objective of the study was calculated using G*Power software (version 3.1.9.2) employing F-test, Power of (1 – β err) 0.95 and α=0.05. The effect size (0.149) was determined by computing 0.13 coefficient of determination (R^2) value (Erdfelder et al., 1996). Effect size could not be determined based on the previous literature because no study has been reported in assessing the developing root apices using CBCT images for age estimation. Secondly, computing the R^2 values from previously reported age estimation 2D studies (Cameriere, Ferrante, & Cingolani, 2006) have resulted in very small sample size (14 to 25 sample size). Thus, in order to avoid any bias, moderate R^2 value (0.13) was used to determine the effect size. The calculated sample size was 130, however 191 study and 96 validation samples were selected for this comprehensive study (Table 3.1) (Figure 3.1).

Test Family: F-Tests

Statistical test: Linear multiple regression

Type of power analysis: A priori, Compute required sample size- given α, power and effect size

Input: Tail(s) = Two

Effect size determined based on R^2 value of 0.13 (f^2) = 0.149

α err prob = 0.05

Power (1-β err prob) = 0.95

Number of predictors: 4.

Output: Calculated total sample size = 130.

Note: However, 191 study and 96 validation samples were selected.

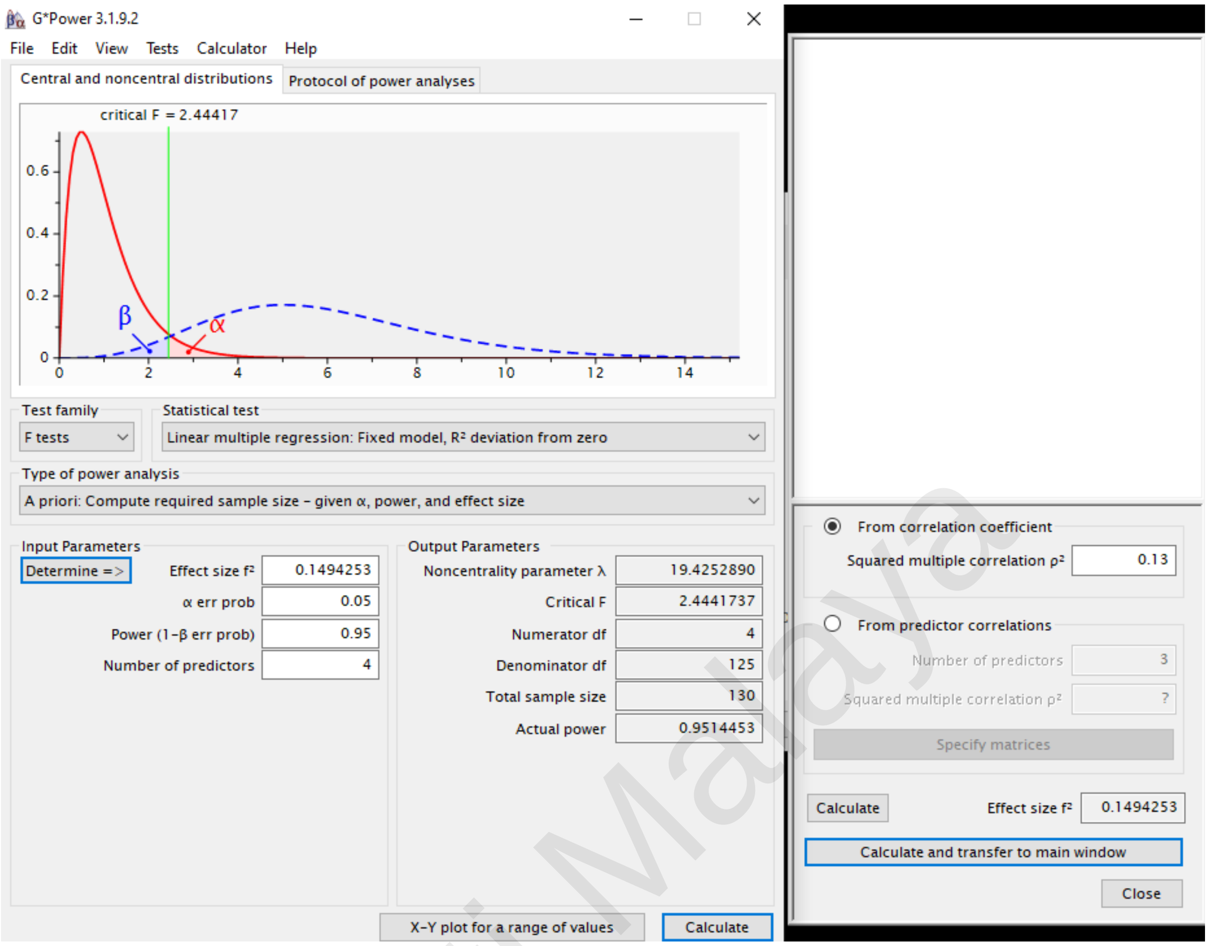

Figure 3.1: Sample size calculation for the first research objective of the study using G*Power software.

3.3.2 Sampling method

CBCT images for the retrospective study were obtained from the database of Oral and Maxillofacial Imaging Division, Faculty of Dentistry, University of Malaya based on the image acquiring parameters, quality and the registered age of the patients. CBCT images of patients ranging in age from 7 to 14 years were included in the study. The CBCT data mainly belonged to the patients living in the Selangor state and Kuala Lumpur city. Training sample of 191 intact permanent maxillary canines (without any caries or associated pathologies) were selected from 191 CBCT images belonging to 100 Malays (male = 51, female = 49) and 91 Chinese (male = 44, female = 47) (Table 3.1). Moreover, an independent validation sample of 96 permanent maxillary canines were selected from

96 CBCT images belonging to 48 Malays (male = 24, female = 24) and 48 Chinese (male = 24, female = 24) (Table 3.1). These samples were stratified into 8 age groups - each group with an interval range of 1 year to avoid any bias in the regression equation and to ensure a balanced data distribution (Figure 3.2). Each scan was selected following the strict inclusion and exclusion criteria. Only one tooth per CBCT image was selected to avoid any bias in the strength of correlation analysis.

Chronological age was calculated by subtracting the date of birth from the date of the CBCT scan and divided by 365.25 to convert it into decimal years. It must be noted that the permanent maxillary left canine was the priority while selecting the investigated tooth from a CBCT scan. However, maxillary right canine was selected in those cases where the left maxillary canine did not meet the selection criteria of the study. The sample for the first research objective of the study included 241 maxillary left canines and 46 maxillary right canines.

A total of 376 CBCT scans acquired during the period of 2009 to 2019, belonging to the Malay and Chinese population (7–14 years of age) were initially screened. 89 CBCT images were excluded from the study sample because they were not adhering to the inclusion criteria of the study.

Table 3.1: Distribution of training (n = 191) and validation (n = 96) samples based on gender and ethnicity.

Age groups	Age (years)	Training sample (n = 191)				Validation sample (n = 96)			
		n = Malays		n = Chinese		n = Malays		n = Chinese	
		n = Male	n = Female	n = Male	n = Female	n = Male	n = Female	n = Male	n = Female
G 1	7.00-7.99	15		12		6		6	
		7	8	6	6	3	3	3	3
G 2	8.00-8.99	15		12		6		6	
		8	7	6	6	3	3	3	3
G 3	9.00-9.99	12		12		6		6	
		7	5	6	6	3	3	3	3
G 4	10.00-10.99	10		11		6		6	
		5	5	6	5	3	3	3	3
G 5	11.00-11.99	12		10		6		6	
		6	6	5	5	3	3	3	3
G 6	12.00-12.99	12		11		6		6	
		6	6	5	6	3	3	3	3
G 7	13.00-13.99	14		12		6		6	
		7	7	5	7	3	3	3	3
G 8	14.00-14.99	10		11		6		6	
		5	5	5	6	3	3	3	3
Total		100		91		48		48	
		51	49	44	47	24	24	24	24

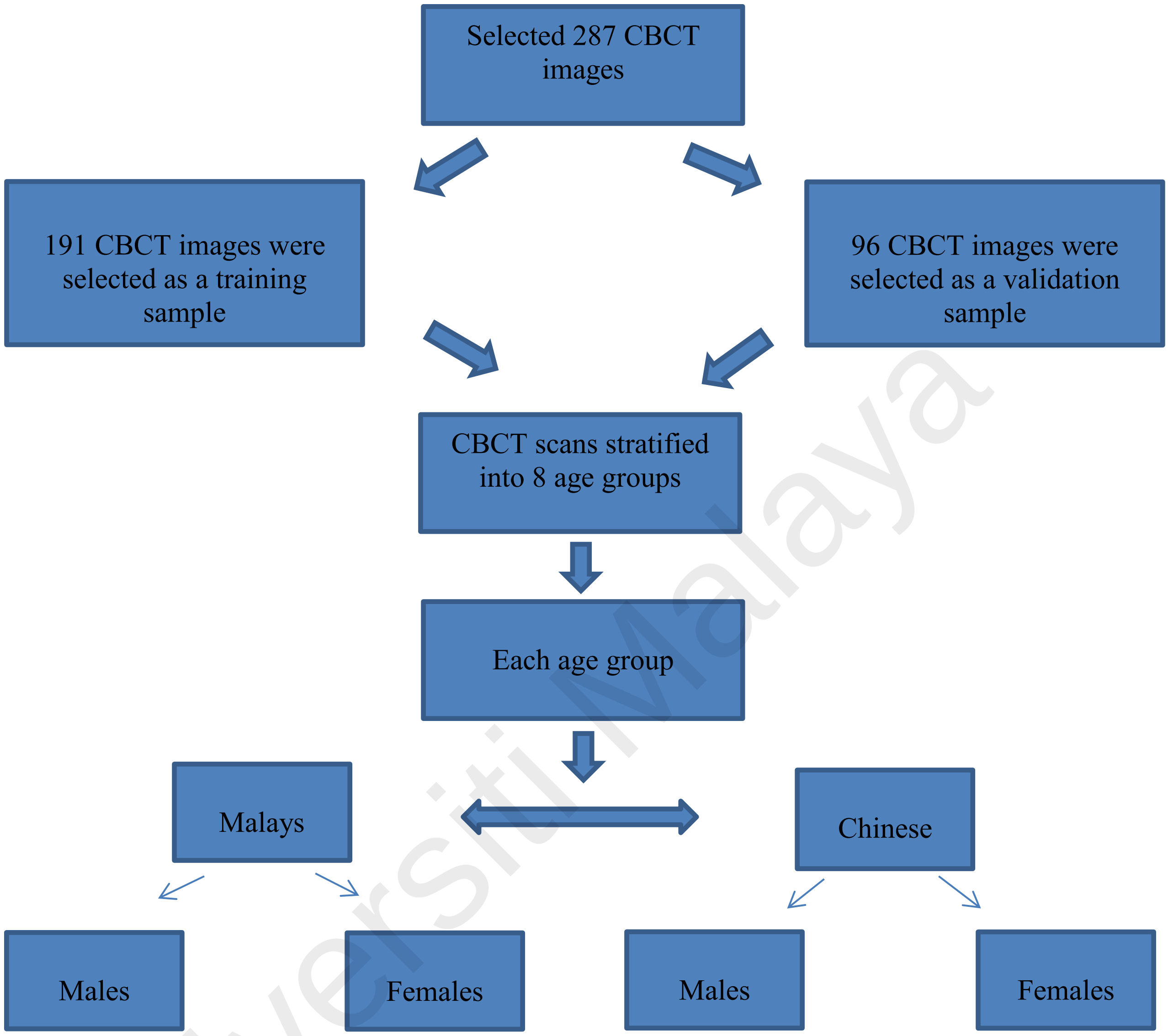

Figure 3.2: Process for sample distribution

3.3.3 Selection criteria of the sample

The training and validation sample of the study were selected from the database according to the inclusion and exclusion criteria as follows:

3.3.3.1 Inclusion criteria

1. Patients aged 7 to 14 years.
2. Mongoloid race (Malays and Chinese population).

3. Good quality CBCT images.
4. No caries or pathology associated with the selected permanent maxillary canines.
5. CBCT images acquired using i-CAT Cone Beam 3D Dental Imaging System, version 3.1.62.
6. CBCT images that were acquired using the following exposure parameters:
    - 0.30mm voxel size
    - 10 seconds scanning time
    - 120 kVp
    - 18 mA.

3.3.3.2 Exclusion criteria

1. Patients aged below 7 and above 14 years.
2. Non-Mongoloid population.
3. Caries or any pathology associated with the selected permanent maxillary canines.
4. CBCT images that were not acquired using scanning parameters set for the study.
5. Poor quality CBCT images.

3.3.4 Developing three-dimensional (3D) image models of maxillary canines using Mimics software

Mimics software (Materialise NV, Belgium, version 21.0) was used in the current study for creating and analyzing 3D image models of the developing maxillary canine's apices (Materialise, 2018). The selected 287 CBCT data (Training sample = 191, Validation sample = 96) were transferred to the MIMICS software in DICOM format. The images were oriented properly in axial, sagittal and coronal planes. Initially in the 'mask creation phase' of the software, pre-set greyscale threshold values (1200-3071) were selected for creating masks of developing maxillary canine (Figure 3.3). This pre-set greyscale threshold value was fixed for the calcified tooth structure by the software. The newly created mask was then cropped in all 3 planes to separate the tooth from the surrounding structures. Following 'mask creation phase', 'multiple slice editing segmentation' tool was used manually to allow accurate and precise selection of the developing maxillary canine (Figure 3.4). This step was important because in some of the instances, software was not able to demarcate the calcified radicular portion of the tooth from the surrounding bone. Then 'region growing phase' was used to ensure no connections of tooth with the surrounding bone (Figure 3.5). Three-dimensional image model of developing maxillary canine was then created automatically by the software (Figure 3.6). The overall procedure of converting DICOM files to the Mimics software, thresholding, multiple slice segmentation, region growing, and 3D reconstruction took approximately 2 hours and 15 minutes per tooth. It must be noted that half of the total time was utilized during 'multiple slice editing segmentation phase'.

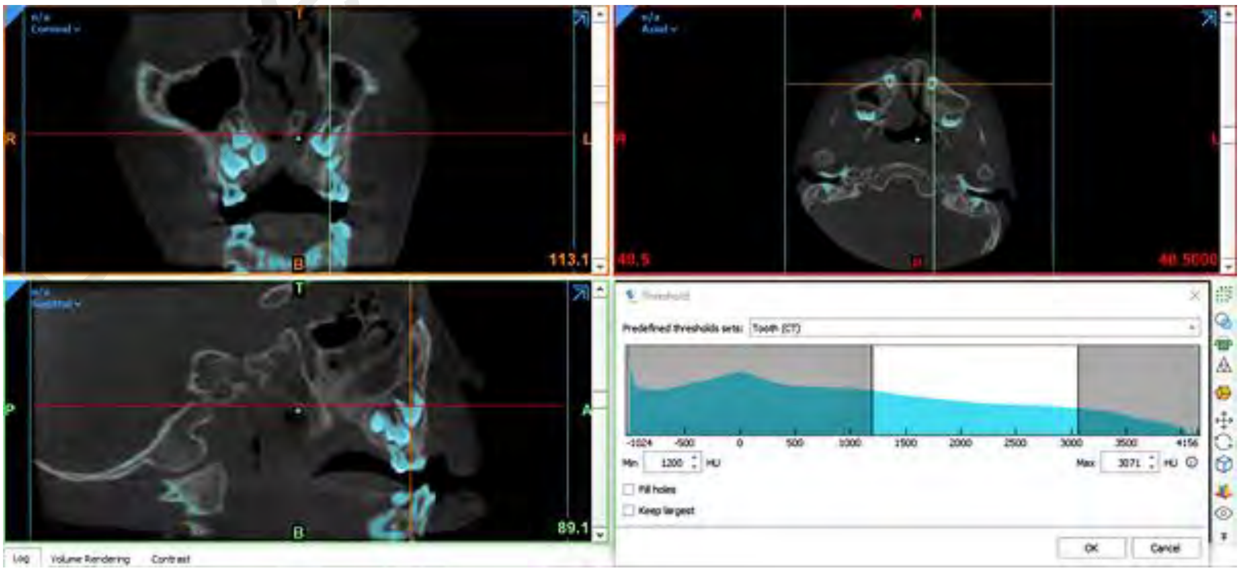

Figure 3.3: "Mask creation phase" in the Mimics software by selecting pre-set greyscale threshold values (1200-3071).

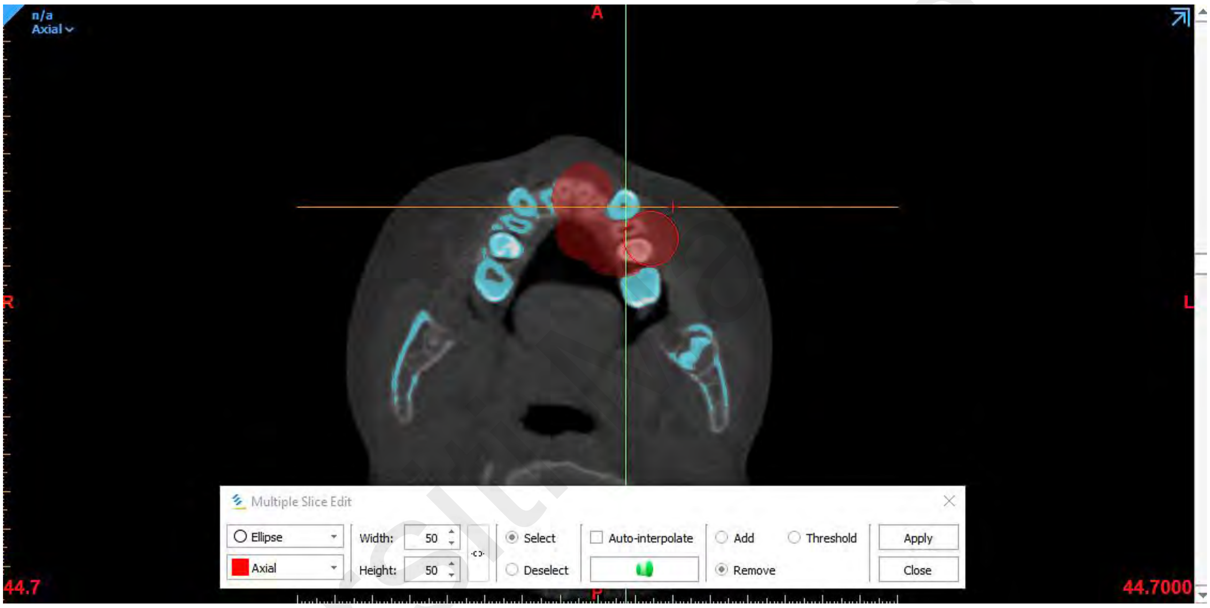

Figure 3.4: "Multiple slice editing segmentation phase" to manually cross check the exact selection of the developing maxillary canine on axial view.

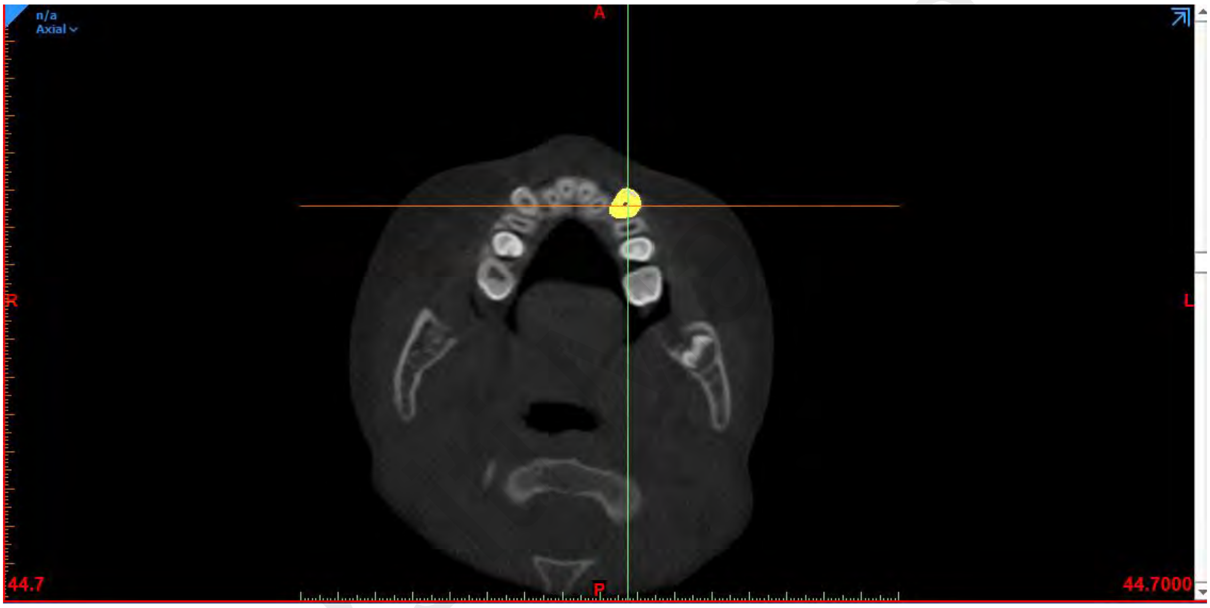

Figure 3.5: "Region growing phase" to ensure no connections of the calcified maxillary canine with the surrounding bone on axial view.

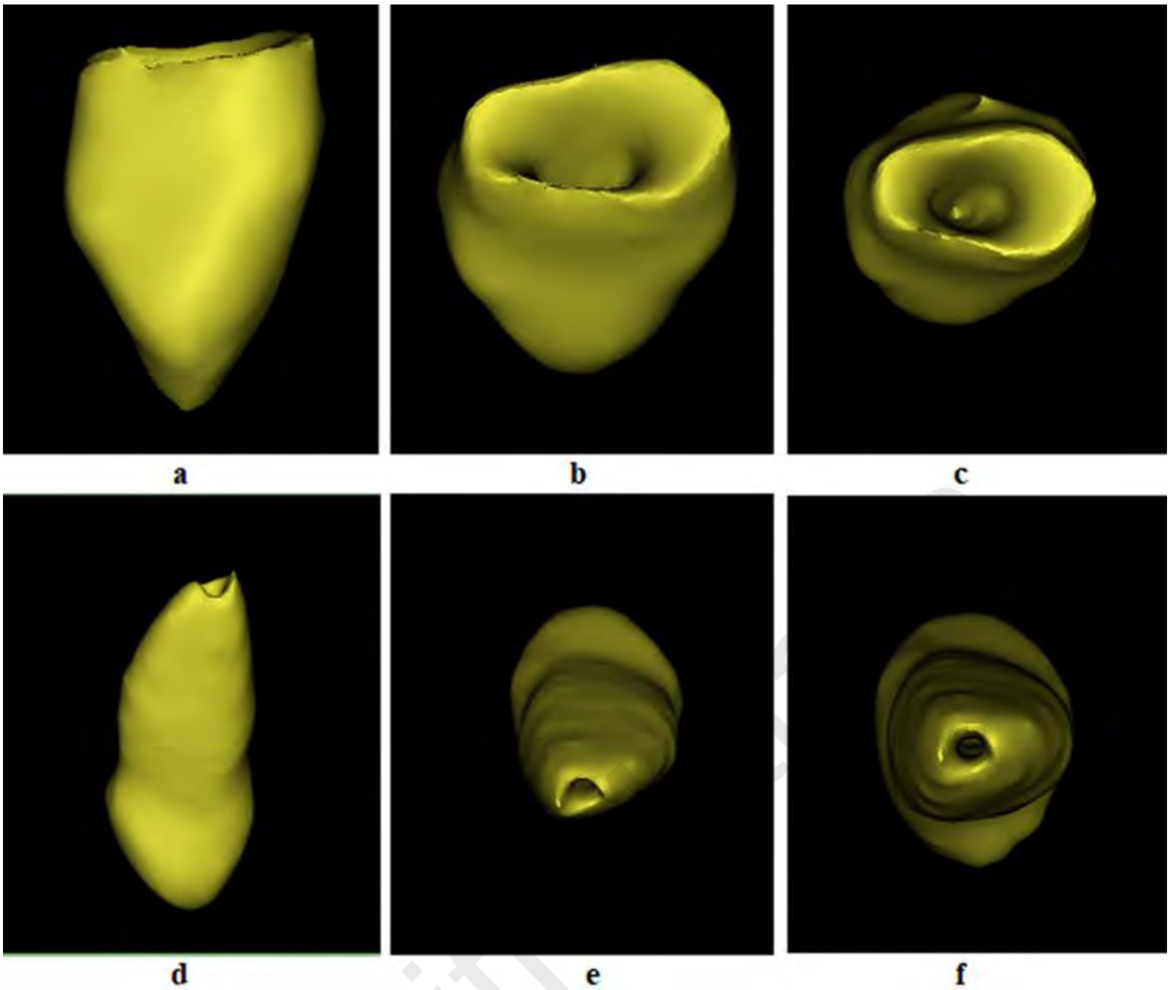

Figure 3.6: Three-dimensional (3D) image models of early (a, b & c) and advanced (d, e & f) stages of developing maxillary canines apices in different 3D orientations using Mimics software.

3.3.5 Surface area analysis of maxillary canines apices using 3-Matics software

3D models of developing maxillary canine apex created in the Mimics software was then transferred to the 3-Matics software (Materialise NV, Belgium, Version 13.0) for the surface area analysis of developing root apex (Materialise, 2018). A curve along the margins of the 3D developing maxillary canine apex was drawn by using 'curve creation' tool in the software (Figure 3.7). During this phase, operational tools of self-interaction, smoothen and close curves were selected to standardize the measurements. The software then automatically generated the surface area of canine apex in the 'surface reconstruction phase' of the software (Figure 3.8). Curve creation and surface reconstruction phase took approximately 20 minutes per tooth (Figure 3.9).

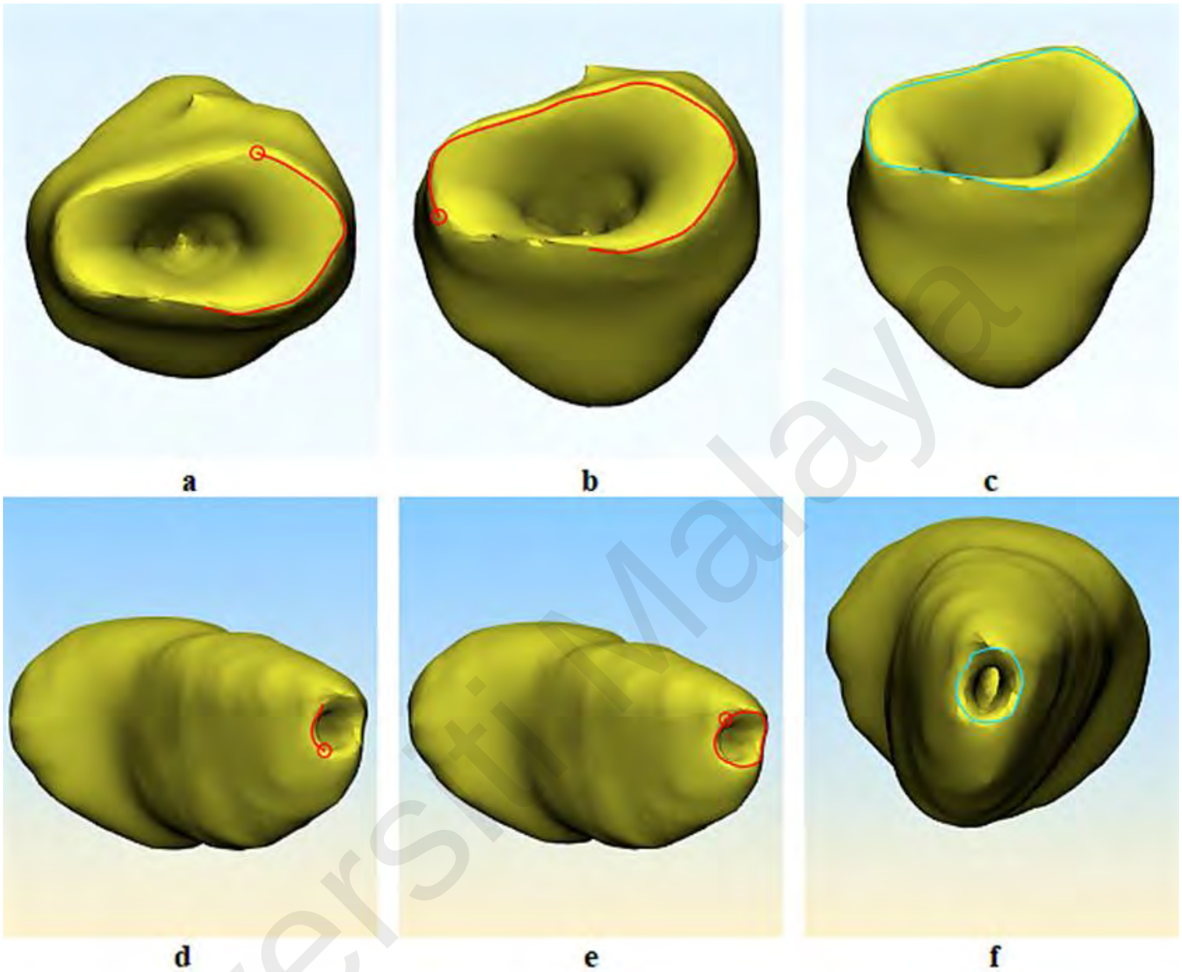

Figure 3.7: Curve creation along the margins of early (a, b & c) and advanced (d, e & f) stages of developing maxillary canines apices using 3-Matics software.

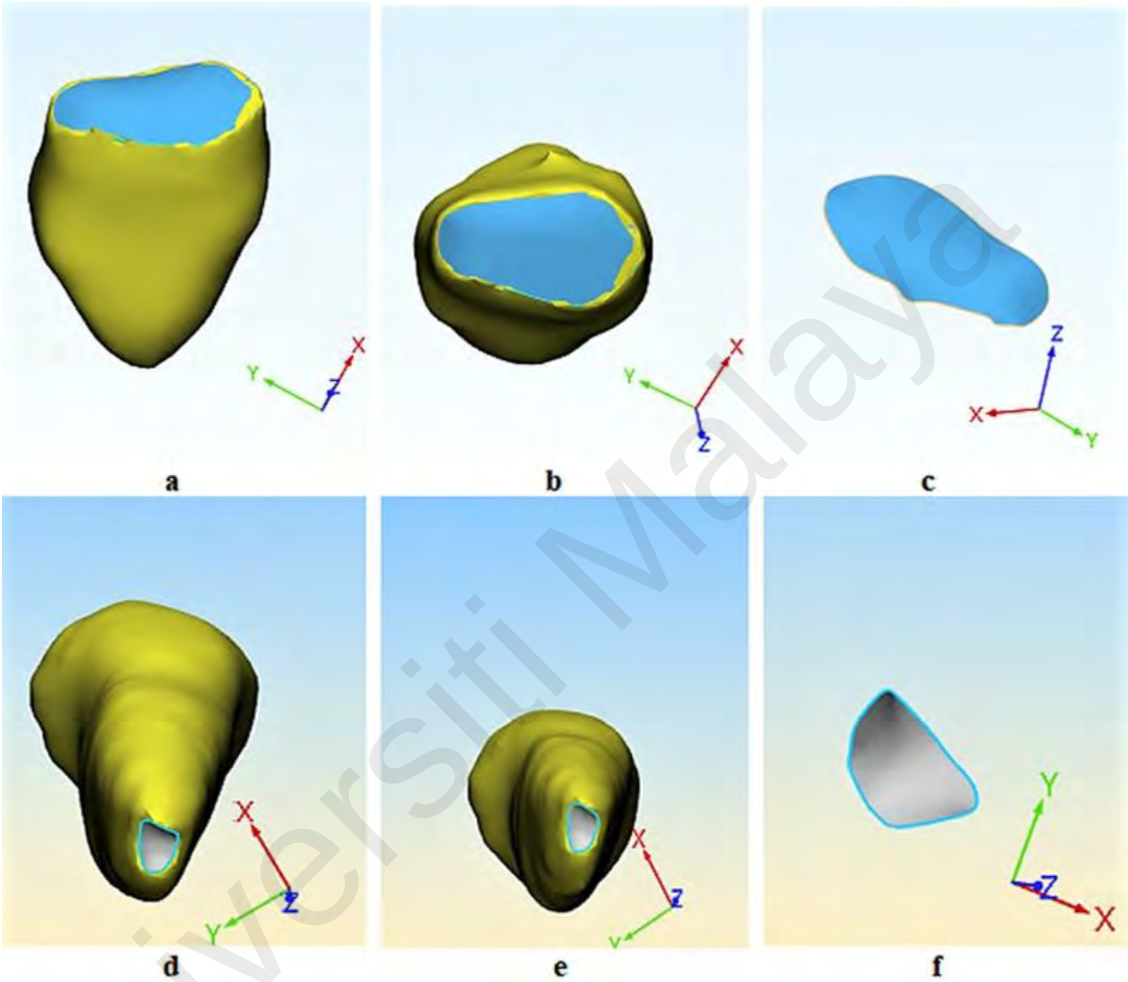

Figure 3.8: "Surface reconstruction phase" to create surface area of the newly created curve along the margins of early (a, b & c) and advanced (d, e & f) stages of canines apices using 3-Matics software.

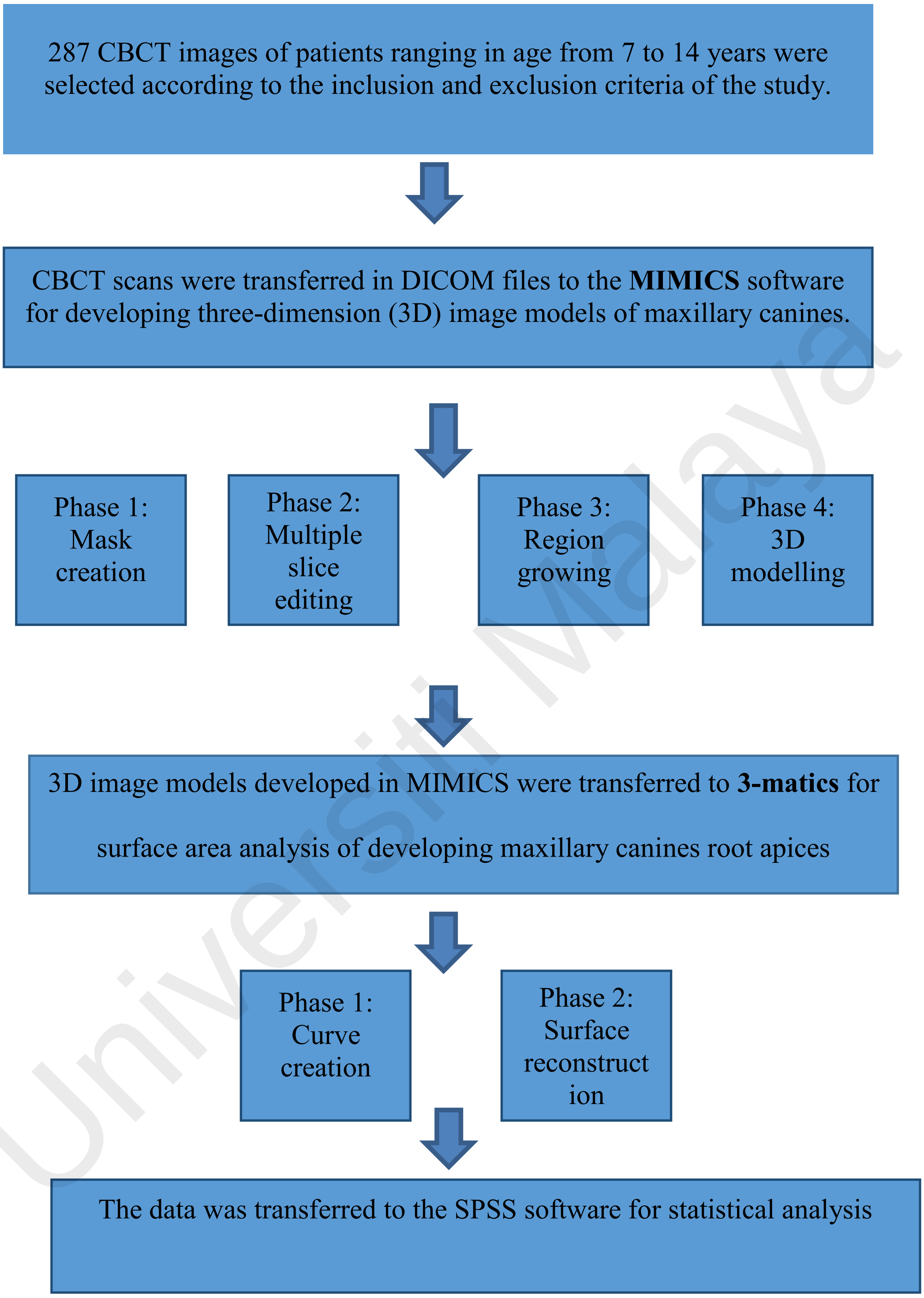

Figure 3.9: Flow chart of the methodology for the 3D image modeling and surface reconstruction of maxillary canines apices in MIMICS and 3-matics software.

3.3.6 Statistical Analyses

3.3.6.1 Dependent and Independent variables of the study

Chronological age of the Malaysian children included in the study was used as a dependent variable and surface area of the developing maxillary canines root apices, ethnicity, gender and status of root development (open/closed) were used as independent variables.

3.3.6.2 Intra and inter-examiner reliability

The intra and inter-examiner reliability of the measurements were assessed in the current study. Intraclass correlation coefficient (ICC) analysis was used to assess intra and inter-examiner reliability. Sixteen maxillary permanent canines were randomly selected from the study sample. 3D modeling and surface area analysis of the open apices were carried out twice by the examiner, with at least one week interval to assess intra-examiner reliability. Another examiner carried out the analysis separately on the same sample to assess inter-examiner reliability. Both the examiners were postgraduate dental students and had more than 4 years of experience using Mimics and 3-Matics software. The analysis was performed using SPSS version 22 (IBM, 2013).

3.3.6.3 Training sample

Pearson correlation coefficient analysis and Fishers Z test was used separately for each gender to investigate statistically significant difference in the strength of correlation coefficient (r) values between Malays and Chinese ethnic groups. Similar analyses were employed separately for each ethnic group to investigate any significant difference in the strength of correlation coefficient (r) values between males and females. In addition, group-wise (1-8) independent samples t-test was also employed to investigate the significant difference in the mean values of apex surface area measurements between

Malays and Chinese separately for each gender. Similarly, group-wise (1-8) independent samples t-test was also performed to investigate the significant difference in the mean values of apex surface area between male and female separately for each ethnic group. The development of root and subsequent frequency of the apical closure were also compared between gender in 12, 13 and 14 years age groups for each ethnic group to assess which gender is showing advance development of maxillary canines. Multiple linear regression analysis was used to derive an age estimation model using chronological age as a dependent variable and surface area of the canine apex, ethnicity, gender and status of the root development (open/closed apices) as predictor variables. Stepwise regression analysis was further employed to develop an age estimation model based on those predictor variables that contributed significantly to the model. Variables ethnicity, gender and status of the root development completion (open/closed apices) were dummy coded as 0 for Malay and 1 for Chinese ethnicities, 0 for males and 1 for females, 0 for open and 1 for closed apices. Assumption of normality of the residuals was tested using Kolmogorov-Smirnov test.

3.3.6.4 Validation sample

To test the reliability and accuracy of the newly developed age estimation model, the model was then applied on an independent validation sample (n = 96). The mean absolute error (MAE) values were assessed to determine the acceptability and applicability of the age estimation model on the Malaysian population in future. All statistical analyses were carried out using SPSS software (version 22) (IBM, 2013).

3.4 The subjects and methods of the second research objective of the study (To develop an age estimation model for Malaysian juveniles and adults by assessing mandibular third molars apices)

3.4.1 Sample size calculation

The second main objective of the study was to develop a novel and validated dental age estimation model/equation using 3D surface area analyses of mandibular third molars apices among Malaysian juveniles and young adults aged 13 to 24 years using CBCT data. Sample size to investigate the second objective of the study was also calculated using G*Power software (version 3.1.9.2) employing F-test, Power of (1 – β err) 0.95 and α=0.05 (Erdfelder et al., 1996). As stated earlier, to the best of our knowledge, no 3D CBCT study has been reported to measure the surface area of developing third molars apices for age estimation. This was the reason we computed lower coefficient of determination ($R^2 = 0.14$) value to determine the effect size (0.163). Previous 2D studies (Cameriere et al., 2008) in assessing the linear measurements of root apices have reported higher R^2 values (0.80 – 0.90). We did not use higher R^2 value to determine the effect size because that led to the small sample size (n = 14-26) calculation. Thus, large and adequate sample size was preferred to draw any conclusions from the current study. The calculated sample size was 119, however 128 study and 55 validation samples were selected for the comprehensive research (Table 3.2) (Figure 3.10).

Test Family: F-Tests

Statistical test: Linear multiple regression

Type of power analysis: A priori, Compute required sample size- given α, power and effect size

Input: Tail(s) = Two

Effect size determined based on R^2 value of 0.14 (f^2) = 0.163

α err prob = 0.05

Power (1-β err prob) = 0.95

Number of predictors: 4.

Output: Total sample size = 119.

Note: However, 128 study and 55 validation samples were selected.

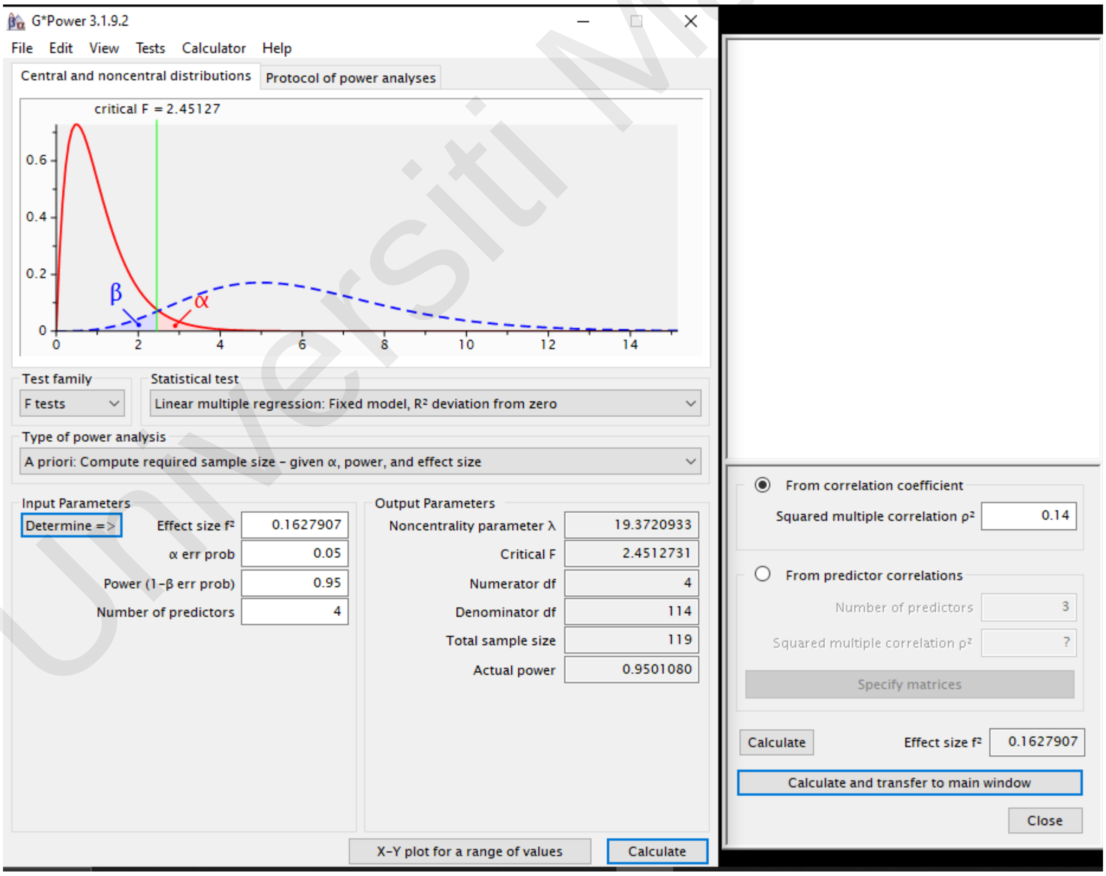

Figure 3.10: Sample size calculation for the second research objective of the study using G*Power software.

3.4.2. Sampling method

CBCT images of the patients ranging in age from 13 to 24 years were selected from the database of Oral and Maxillofacial Imaging Division, Faculty of Dentistry, University of Malaya based on the image acquiring parameters, quality and the registered age of the patients. The data mainly belonged to the patients living in the Selangor state and Kuala Lumpur city. Training sample of 128 intact mandibular third molars (without any caries or associated pathologies) were selected from 128 CBCT images belonging to 66 Malays (Male = 37, Female = 29) and 62 Chinese (Male = 26, Female = 36) (Table 3.2). Moreover, an independent validation sample of 55 permanent maxillary canines were selected from 55 CBCT images belonging to 27 Malays (Male = 14, Female = 13) and 28 Chinese (Male = 14, Female = 14) (Table 3.2). These samples were stratified into 6 age groups - each group with an interval range of 2 years to ensure balanced data distribution (Figure 3.11). Mandibular right third molar was the priority while selecting tooth from a CBCT image. However, in those cases where mandibular right third molar did not adhere to the study's selection criteria, then mandibular left third molar was selected. The sample for the second research objective of the study included 156 mandibular right third molars and 27 mandibular left third molars.

A total of 301 CBCT scans acquired during the period of 2009 to 2019, belonging to the Malay and Chinese population (13–24 years of age) were initially screened. 118 CBCT images were excluded from the study sample because they were not adhering to the inclusion criteria of the study. To ensure consistency in the methodology, the mandibular third molars with only 2 roots were included in the study. The surface areas of the mesial and distal roots apices were calculated and the sum of both the surface areas were recorded and analysed during statistical analysis. It must be noted that if one of the 2 apices of the investigated third molar was not closed then the tooth was still given a status of incomplete closure of the roots development. Only one tooth per subject was selected to exclude any bias in the regression analysis.

Table 3.2: Distribution of training (n = 128) and validation (n = 55) sample based on gender and ethnicity.

Age groups	Age (years)	Training sample (n = 128)				Validation sample (n = 55)			
		n = Malays		n = Chinese		n = Malays		n = Chinese	
		n = Male	n = Female	n = Male	n = Female	n = Male	n = Female	n = Male	n = Female
G 1	13.00 - 14.99	10		11		5		4	
		8	2	4	7	2	3	2	2
G 2	15.00 - 16.99	8		8		4		4	
		4	4	4	4	2	2	2	2
G 3	17.00 - 18.99	11		10		4		5	
		5	6	4	6	2	2	3	2
G 4	19.00 - 20.99	13		12		5		5	
		6	7	6	6	3	2	2	3
G 5	21.00 - 22.99	10		12		4		5	
		6	4	4	8	2	2	3	2
G 6	23.00 - 24.99	14		9		5		5	
		8	6	4	5	3	2	2	3
		66		62		27		28	
	Total	37	29	26	36	14	13	14	14

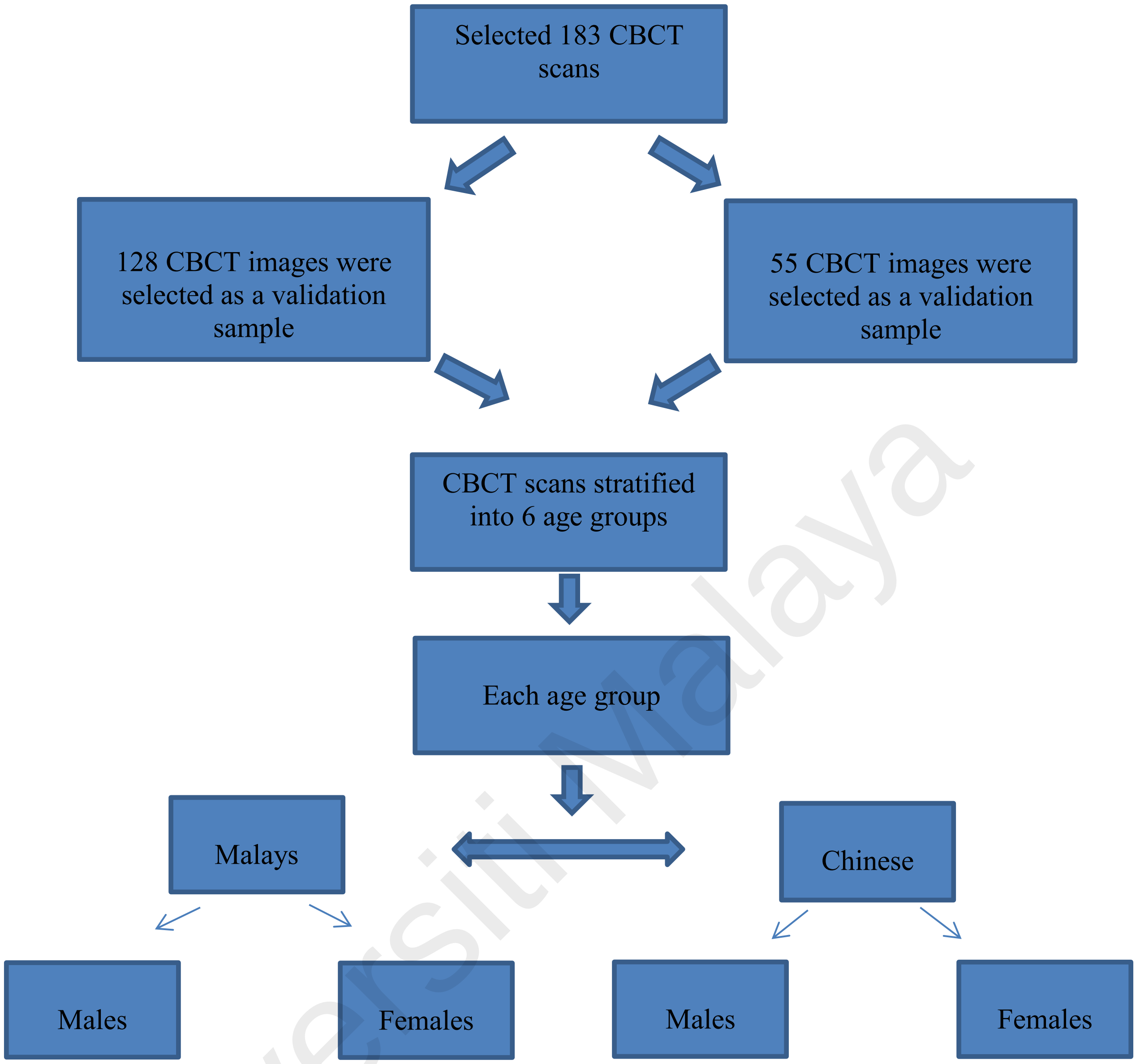

Figure 3.11: Process for sample distribution

3.4.3 Selection criteria of the sample

Following are the inclusion and exclusion criteria for the selection of training and validation samples of the study:

3.4.3.1 Inclusion criteria

1. Patients aged 13 to 24 years.
2. Mongoloid race (Malays and Chinese population).
3. Good quality CBCT images.

4. No caries or pathology associated with mandibular third molars.
5. If the root formation was initiated, then mandibular third molars with only 2 roots were included in the study.
6. CBCT images acquired using i-CAT Cone Beam 3D Dental Imaging System, version 3.1.62.
7. CBCT images that were acquired using the following exposure parameters :
    - 0.30mm voxel size
    - 10 seconds scanning time
    - 120 kVp
    - 18 mA.

3.4.3.2 Exclusion criteria

1. Patients aged below 13 and above 24 years.
2. Non-Mongoloid population.
3. Caries or any pathology associated with the selected mandibular third molars.
4. Mandibular third molars with fused or more than 2 roots were excluded.
5. CBCT images that were not acquired using scanning parameters set for the study.
6. Poor quality CBCT images.

3.4.4 Developing three-dimension (3D) image models of mandibular third molars using Mimics software

The selected 183 CBCT data (Training sample = 128, Validation sample = 55) were transferred to the MIMICS software (Materialise NV, Belgium, version 21.0) in DICOM format for creating 3D image models of the developing mandibular third molars. The images were oriented properly in axial, sagittal and coronal planes. Mask creation (Figure

3.12), multiple slice segmentation (Figure 3.13), region growing (Figure 3.14) and 3D reconstruction phases (Figure 3.15) that led to the creation of 3D image models of mandibular third molars apices were similar to the methodology explained in section 3.4.4 for creating 3D image models of maxillary canines. However, it must be noted that these steps were followed for both the mesial and distal roots of mandibular third molars apices in comparison to the 1 rooted maxillary canine.

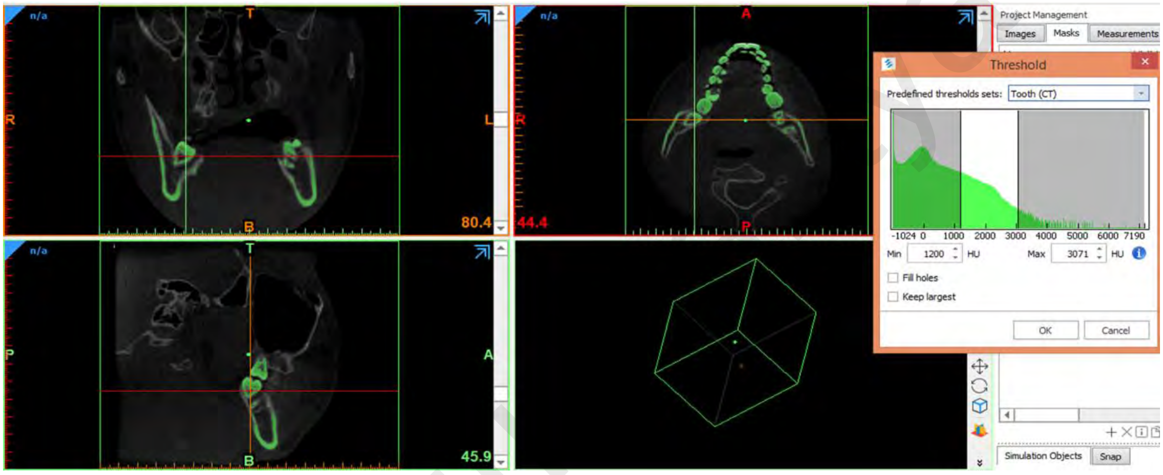

Figure 3.12: "Mask creation phase" by selecting predefined grayscale threshold values for mandibular right third molar in MIMICS software.

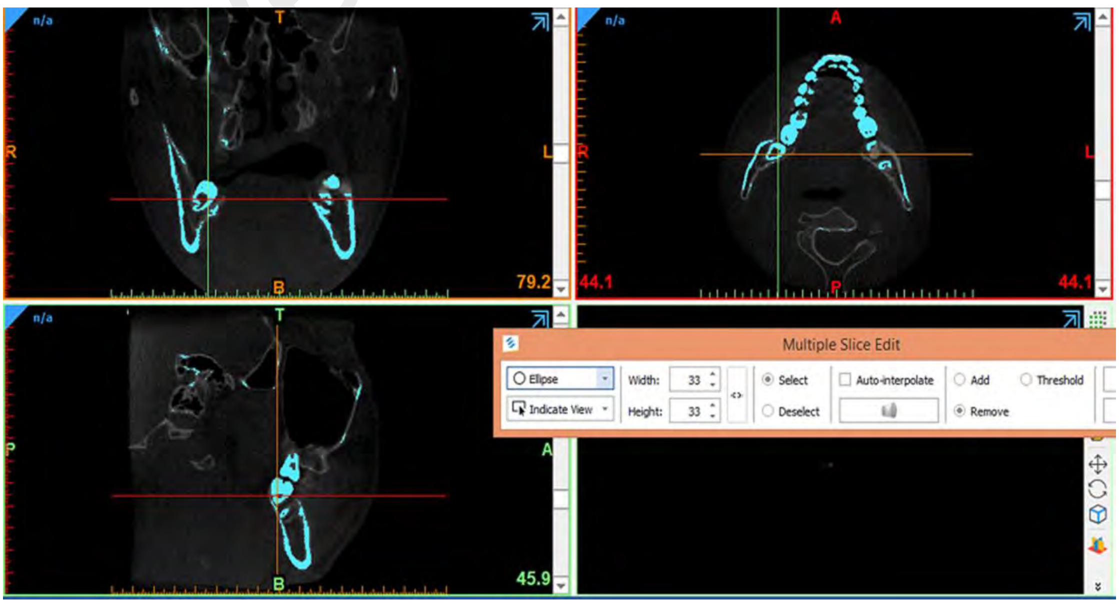

Figure 3.13: "Multiple slice editing segmentation phase "to determine accurate selection of the calcified tooth structure manually slice by slice.

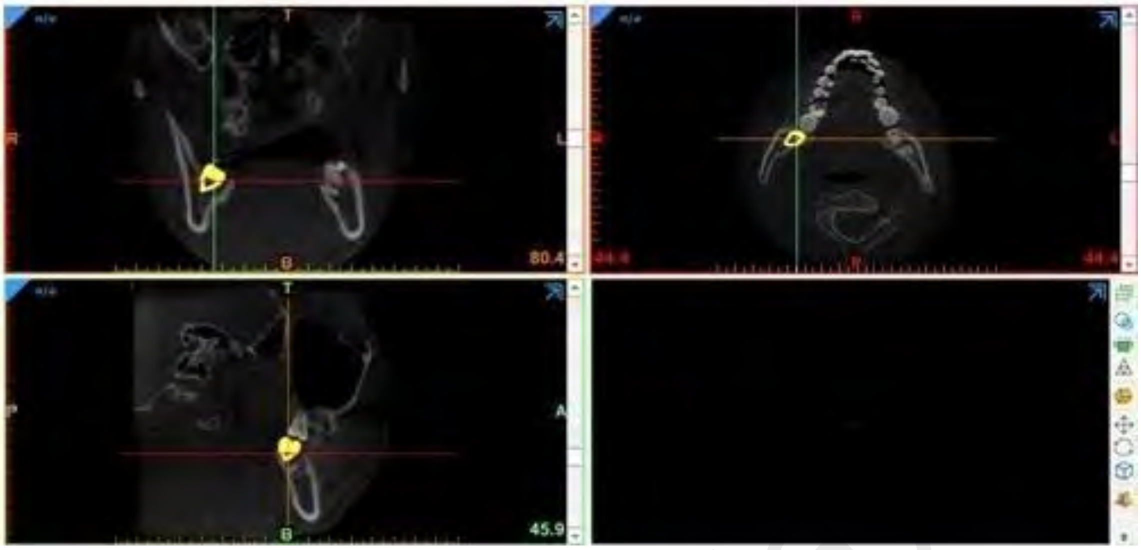

Figure 3.14: "Region growing phase" in MIMICS software on sagittal, coronal and axial views.

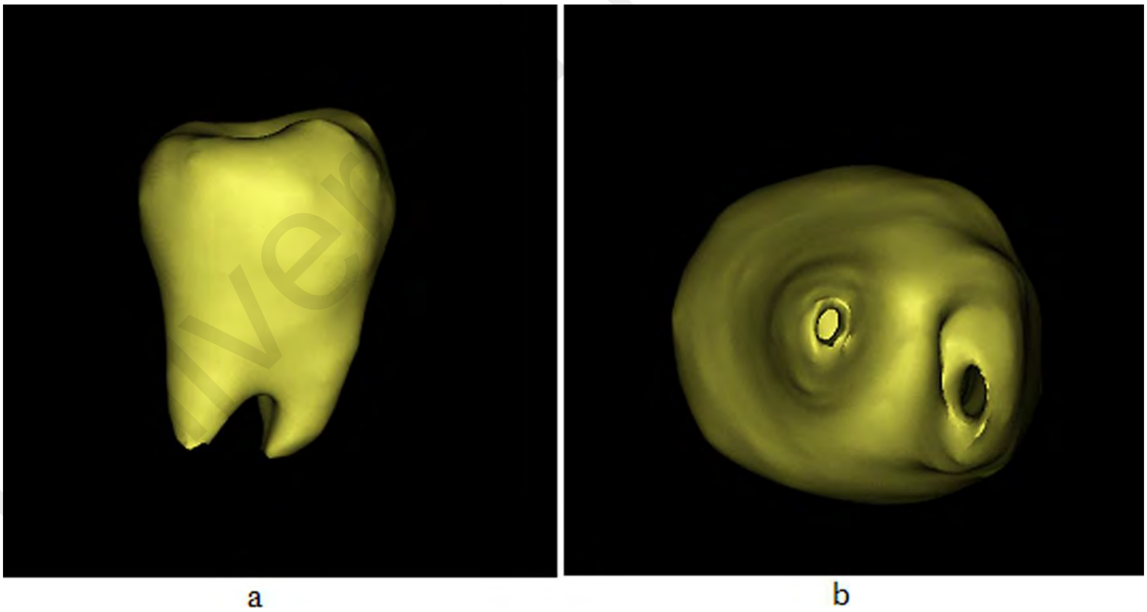

Figure 3.15: Three-dimensional (3D) model of developing right mandibular third molar (a) with open apices (b) in Mimics software.

3.4.5 Surface area analysis of mandibular third molars apices using 3-Matics software

3D image models of developing mandibular third molars apices created in the Mimics software were transferred to the 3-Matics software (Materialise NV, Belgium, Version 13.0) for the surface area analysis of developing root apices. A 'curve creation phase' (Figure 3.16) and 'surface reconstruction phase" (Figure 3.17) were employed to create curves along the margins and 3D surface reconstruction of third molars apices (Figure 3.18).

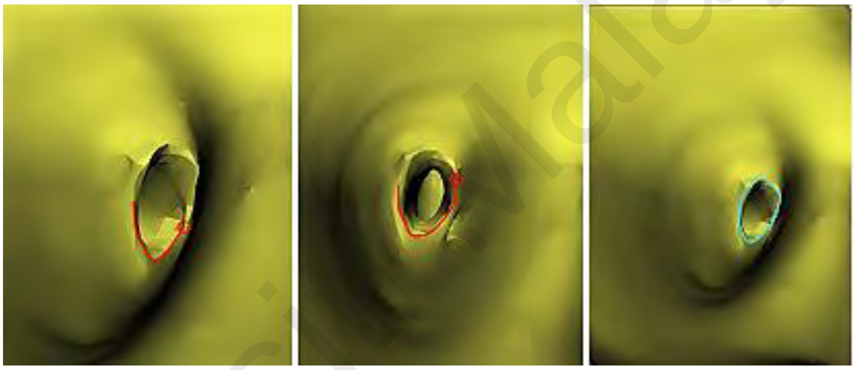

Figure 3.16: Mandibular third molar apices 'curve creation' at different orientation in 3-Matics Software.

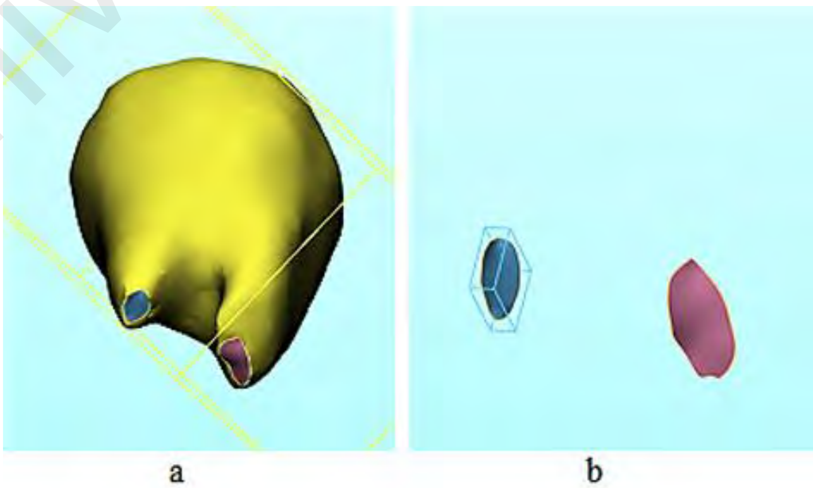

Figure 3.17: Image analysis of mandibular third molar apices showing surface reconstruction phase (a & b) in 3-Matics software.

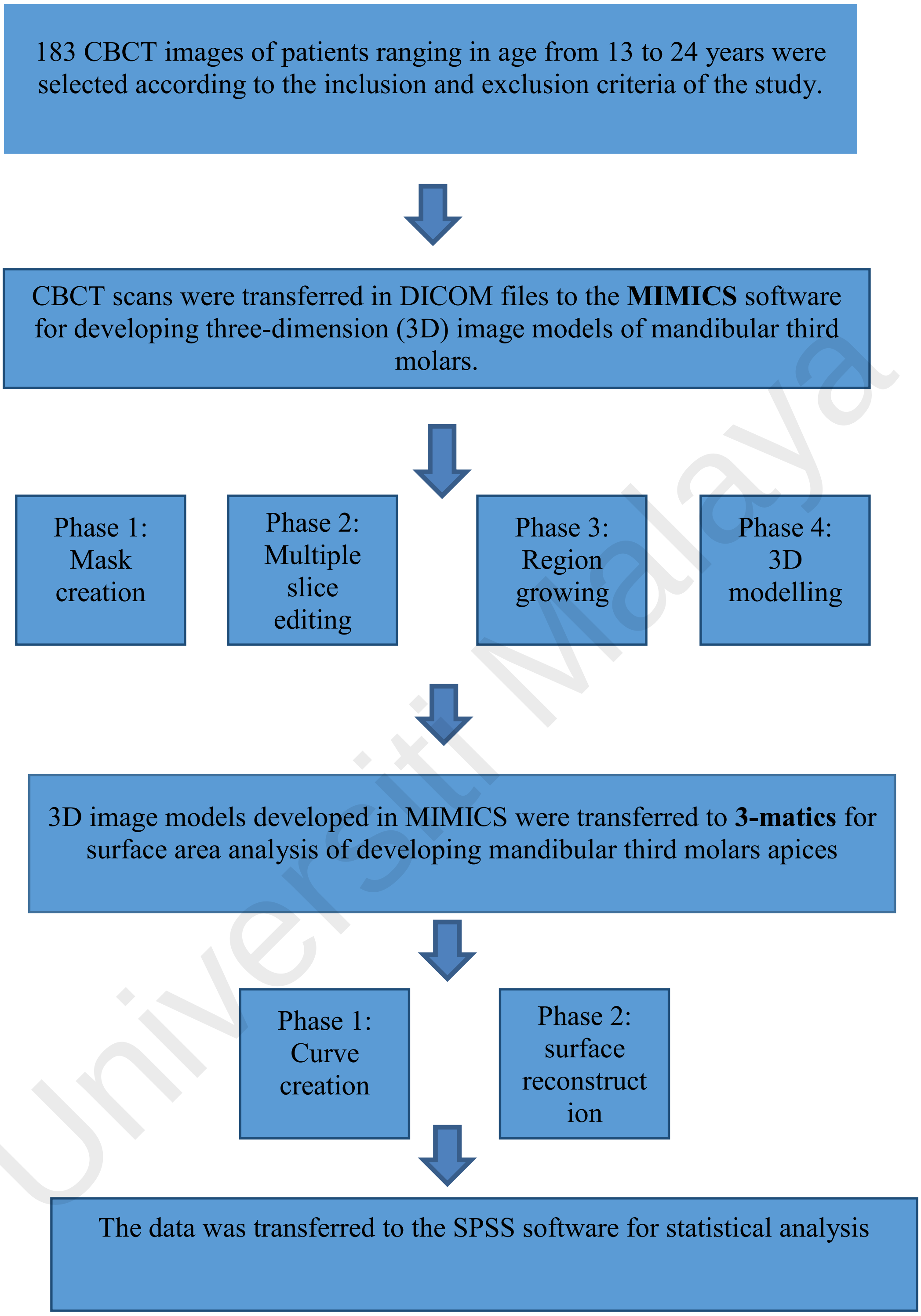

Figure 3.18: Flow chart of the methodology for the 3D image modeling and surface reconstruction of mandibular third molars apices in MIMICS and 3-matics software.

3.4.6 Statistical Analyses

3.4.6.1 Dependent and Independent variables of the study

Chronological age of the Malaysian juveniles and young adults included in the study was used as a dependent variable and surface area of the developing mandibular third molars apices, ethnicity, gender and status of root development (open/closed) were used as independent variables.

3.4.6.2 Intra and inter-examiner reliability

Intraclass correlation coefficient (ICC) analysis was used in the current study to assess intra and inter-examiner reliability. Fifteen mandibular third molars were randomly selected from the study sample. 3D modeling and surface area analysis of the open apices were carried out twice by the examiner, with at least one week interval to assess intra-examiner reliability. Another examiner carried out the analysis separately on the same sample to assess inter-examiner reliability. Both the examiners were postgraduate dental students and had more than 4 years of experience using Mimics and 3-Matics software. The analysis was performed using SPSS version 22 (IBM, 2013).

3.4.6.3 Training sample

Pearson correlation coefficient analysis and Fishers Z test was used separately for each gender to investigate statistically significant difference in the strength of correlation coefficient (r) values between Malays and Chinese ethnic groups. Similar analyses were employed separately for each ethnic group to investigate any significant difference in the strength of correlation coefficient (r) values between male and female. In addition, age group-wise (1-6) independent samples t-test was also employed to investigate the significant difference in the mean values of apex surface area measurements between

Malays and Chinese separately for each gender. Similarly, age group-wise (1-6) independent samples t-test was also performed to investigate the significant difference in the mean values of apex surface area between male and female separately for each ethnic group. The frequency of the apical closure was also compared between genders in 19-20, 21-22 and 23-24 years age groups for each ethnic group to assess which gender is showing advance development of mandibular third molars. Multiple linear regression analysis was used to derive age estimation model using chronological age as a dependent variable and surface area of the mandibular third molars apices, ethnicity, gender and status of the root development completion (open/closed apices) as predictor variables. Stepwise regression analysis was performed to develop age estimation model based on those predictor variables that contributed significantly to the age estimation formula/model. Variables ethnicity, gender and status of the root development completion (open/closed apices) were dummy coded as 0 for Malay and 1 for Chinese ethnicity, 0 for males and 1 for females, 0 for open and 1 for closed apices. Assumption of normality of the residuals was tested using Kolmogorov-Smirnov test. The significance threshold was set at 5%.

3.4.6.4 Validation sample

The derived age estimation formula was then applied on an independent separate validation sample (n=55) to calculate mean absolute error (MAE) values. All statistical analysis was carried out using SPSS software (version 22) (IBM, 2013).

CHAPTER 4: RESULTS AND DATA ANALYSIS

4.1 Results and data analysis

The current study aimed to develop a novel method of dental age estimation for Malaysian children, juveniles and young adults. The results of the study are presented based on the 2 main research objectives of the study.

4.1.1 Results of the first research objective of the study

The first research objective of the study was to develop an age estimation model for Malaysian children by assessing maxillary canines apices. Training sample of 191 intact permanent maxillary canines (without any caries or associated pathologies) were selected from 191 CBCT images belonging to 100 Malays (male = 51, female = 49) and 91 Chinese (male = 44, female = 47) (Table 4.1). Moreover, an independent validation sample of 96 permanent maxillary canines were selected from 96 CBCT images belonging to 48 Malays (male = 24, female = 24) and 48 Chinese (male = 24, female = 24) (Table 4.1).

Table 4.1: Distribution of training (n = 191) and validation (n = 96) samples based on gender and ethnicity.

Age groups	Age (years)	Training sample (n = 191)				Validation sample (n = 96)			
		n = Malays		n = Chinese		n = Malays		n = Chinese	
		n = Male	n = Female	n = Male	n = Female	n = Male	n = Female	n = Male	n = Female
G 1	7.00-7.99	15		12		6		6	
		7	8	6	6	3	3	3	3
G 2	8.00-8.99	15		12		6		6	
		8	7	6	6	3	3	3	3
G 3	9.00-9.99	12		12		6		6	
		7	5	6	6	3	3	3	3
G 4	10.00-10.99	10		11		6		6	
		5	5	6	5	3	3	3	3
G 5	11.00-11.99	12		10		6		6	
		6	6	5	5	3	3	3	3
G 6	12.00-12.99	12		11		6		6	
		6	6	5	6	3	3	3	3
G 7	13.00-13.99	14		12		6		6	
		7	7	5	7	3	3	3	3
G 8	14.00-14.99	10		11		6		6	
		5	5	5	6	3	3	3	3
Total		100		91		48		48	
		51	49	44	47	24	24	24	24

4.1.1.1 Intraexaminer and interexaminer reliability

The observed intraclass correlation coefficient (ICC) value of 0.931 for intra-examiner reliability indicated good accuracy in the repeated measurements. High level of agreement in the measurements between the two observers was also observed for inter-examiner reliability with 0.902 value.

4.1.1.2 Pearson correlation coefficient analysis based on gender and ethnicity

Pearson correlation coefficient analysis was used to investigate the strength of correlation (r) between chronological age and surface area of the developing maxillary canines apices in Malay and Chinese ethnic groups for both genders separately (Table 4.2 & 4.3). This analysis was performed in both training and validation samples. The strength of correlation values for Malay (r > 0.965) and Chinese (r > 0.965) were found to be very strong among both genders (Table 4.2 & 4.3).

Table 4.2: The strength of correlation (r) for Malays and Chinese ethnic groups based on each gender resulted from the training sample (n = 191)

Training sample (n= 191)				
Investigated teeth (FDI notation)	**Pearson correlation (r) values for male**		**P-value for male**	
	Malays	**Chinese**	**Malays**	**Chinese**
13 & 23	0.973	0.978	.00*	.00*
	Pearson correlation (r) values for female		**P-value for female**	
	Malays	**Chinese**	**Malays**	**Chinese**
	0.969	0.968	.00*	.00*

* Significant at the 0.05 probability level.

Table 4.3: The strength of correlation (r) for Malays and Chinese ethnic groups based on gender resulted from the validation sample (n = 96).

validation sample (n= 96)				
Investigated teeth (FDI notation)	**Pearson correlation (r) values for male**		**P-value for Male**	
	Malays	**Chinese**	**Malays**	**Chinese**
13 & 23	0.989	0.991	.00*	.00*
	Pearson correlation (r) values for female		**P-value for Female**	
	Malays	**Chinese**	**Malays**	**Chinese**
	0.979	0.980	.00*	.00*

* Significant at the 0.05 probability level.

4.1.1.3 Fisher Z-test analysis to investigate significant difference in the coefficient of correlation values among genders and ethnic groups

One of the objectives of the study was to investigate the difference in the strength of correlation (r) values among genders and ethnic groups. Fishers Z test analysis was used separately for each gender to investigate statistically significant difference in the strength of correlation coefficient values between Malays and Chinese ethnic groups (Table 4.4). Similar analyses were employed separately for each ethnic group to investigate any significant difference in the strength of correlation coefficient values between males and females (Table 4.5).

4.1.1.3.1 To investigate difference in the strength of correlation coefficient values between Malays and Chinese ethnic groups separately for each gender

Fishers Z test analysis indicated that there was no statistically significant difference in the strength of correlation coefficient (r) values ($p > 0.05$) between Malays and Chinese ethnic groups, when investigated for each gender. This pattern was consistent among both training (n = 191) and validation samples (n =96) (Table 4.4).

Table 4.4: Pearson correlation values and Fishers Z test analysis between Malays and Chinese for each gender (training sample: n = 191, validation sample: n = 96).

Training sample (n = 191)				
Investigated teeth (FDI notation)	**Pearson correlation (r) values for male**		**Fishers Z test**	
	Malays	**Chinese**	**Z-value**	**P-value**
13 & 23	0.973	0.978	0.49	0.624
	Pearson correlation (r) values for female		**Fishers Z test**	
	Malays	**Chinese**	**Z-value**	**P-value**
	0.969	0.968	0.08	0.936
Independent validation sample (n= 96)				
Investigated teeth (FDI notation)	**Pearson correlation (r) values for male**		**Fishers Z test**	
	Malays	**Chinese**	**Z-value**	**P-value**
13 & 23	0.989	0.991	0.33	0.741
	Pearson correlation (r) values for female		**Fishers Z test**	
	Malays	**Chinese**	**Z-value**	**P-value**
	0.979	0.980	0.08	0.936

* Significant at the 0.05 probability level.

4.1.1.3.2 To investigate difference in the strength of correlation coefficient values between male and female separately for each ethnic group

Fisher Z-test indicated no statistically significant difference in the strength of correlation coefficient (r) values ($p > 0.05$) between males and females, when investigated for each ethnic group (Table 4.5). Fisher Z-test also indicated no significant difference in the validation samples (n =96) (Table 4.5).

Table 4.5: Pearson correlation values and Fishers Z test between male and female for each ethnic group (training sample: n = 191, validation sample: n = 96).

Training sample (n= 191)				
Investigated teeth (FDI notation)	**Pearson correlation (r) values for Malays**		**Fishers Z test**	
	Male	**Female**	**Z-value**	**P-value**
13 & 23	0.973	0.969	0.34	0.733
	Pearson correlation (r) values for Chinese		**Fishers Z test**	
	Male	**Female**	**Z-value**	**P-value**
	0.978	0.968	0.87	0.384
Independent validation sample (n= 96)				
Investigated teeth (FDI notation)	**Pearson correlation (r) values for Malays**		**Fishers Z test**	
	Male	**Female**	**Z-value**	**P-value**
13 & 23	0.989	0.979	1.06	0.289
	Pearson correlation (r) values for Chinese		**Fishers Z test**	
	Male	**Female**	**Z-value**	**P-value**
	0.991	0.980	1.3	0.193

* Significant at the 0.05 probability level

4.1.1.4 Independent samples t-test analysis to investigate the significant difference in the mean values of maxillary canines apex surface area among genders and ethnic groups

In addition to Fisher Z-test, independent samples t-test was also performed on the training sample to assess the significant difference in the mean values of apex surface area measurements for each age group (1-8) among genders and ethnic groups.

4.1.1.4.1 Age group-wise (1-6) independent samples t-test to investigate difference in the mean values of apex surface area between Malays and Chinese separately for each gender

The results of the independent samples t-test analysis indicated that there was no significant difference in the mean values of canine apex surface area measurements ($p > 0.05$) between Malays and Chinese in the investigated age groups (1-8) (Table 4.6) (Figure 4.1). This pattern was consistent among both genders.

Table 4.6: Groupwise Independent samples t-test to investigate difference in the mean values of canine apex surface area measurements between Malays and Chinese for each gender (Training sample = 191).

	Male				Female			
	Paired Differences				Paired Differences			
Age groups	Mean Difference	Std. Error Difference	t	Sig. (2-tailed)	Mean Difference	Std. Error Difference	t	Sig. (2-tailed)
7 years	0.69	1.49	0.4	0.65	0.29	1.91	0.1	0.88
8 years	2.69	1.30	2.0	0.06	0.14	0.85	0.1	0.86
9 years	3.17	1.52	2.0	0.06	1.48	1.40	1.0	0.32
10 years	0.012	1.21	.01	0.99	0.39	2.26	0.1	0.86
11 years	1.46	2.24	0.6	0.53	1.68	1.16	1.4	0.18
12 years	1.33	1.52	0.8	0.41	0.79	1.18	0.7	0.52
13 years	1.29	1.01	1.2	0.22	0.46	0.49	1.0	0.32
14 years	00	00	00	00	00	00	00	00

* Significant at the 0.05 probability level

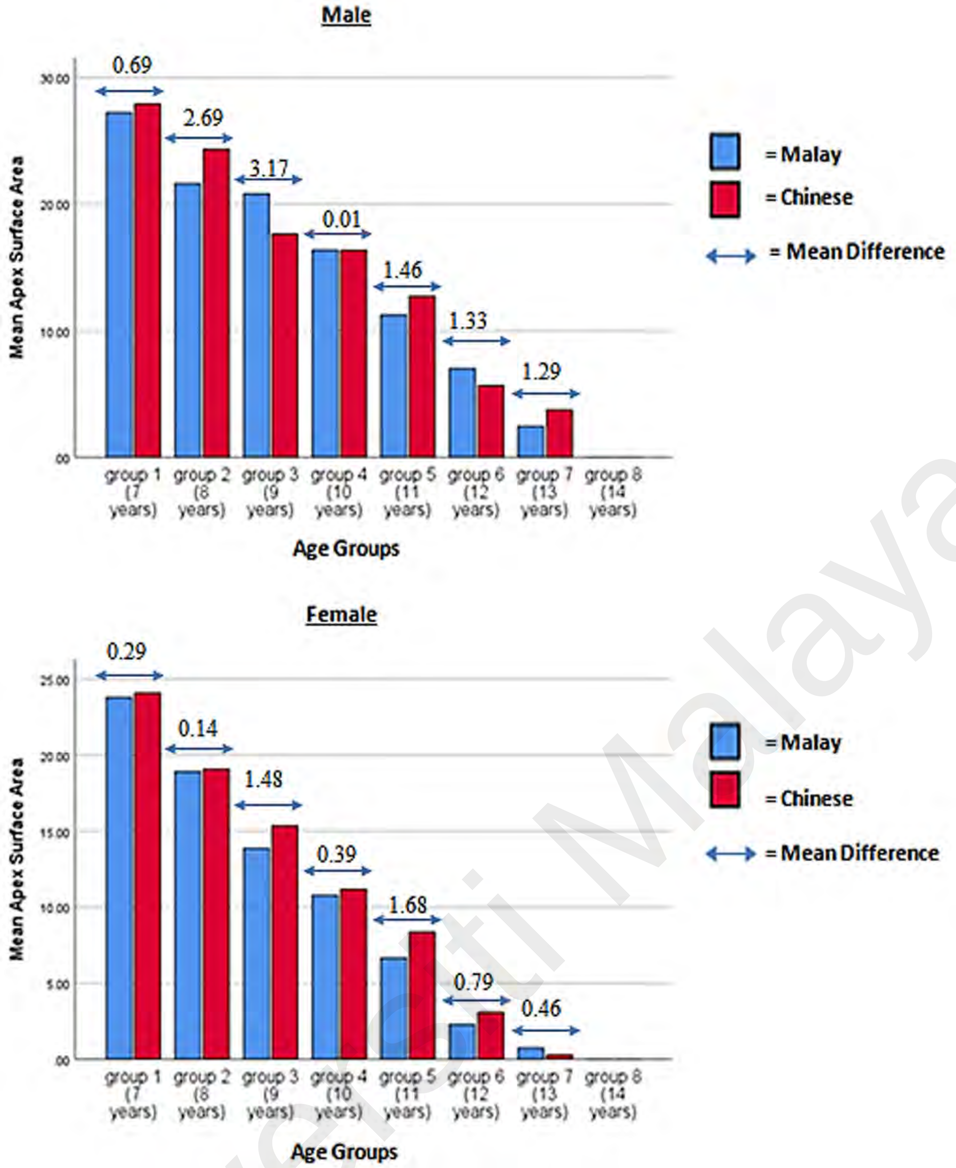

Figure 4.1: Bar charts illustrating gender-based differences in the mean values of canine apex surface area between Malays and Chinese among different age groups (* = Significant at the 0.05 probability level).

4.1.1.4.2 Age group-wise (1-6) independent samples t-test to investigate difference in the mean values of apex surface area measurements between males and females separately for each ethnic group

The results of independent samples t-test showed significant difference in the mean values of canine apex surface area measurements ($p < 0.05$) between males and females in majority of the investigated age groups particularly in the Malays ethnic group (Table

4.7) (Figure 4.2). The results also indicated the mean values of canine apices surface area measurements in 7, 9 and 10 years age groups among the Chinese ethnic group did not show significant differences between males and females. However, females showed advanced development of canines in comparison to males in all the investigated age groups among both ethnic groups (Figure 4.2).

Table 4.7: Groupwise Independent samples t-test to investigate difference in mean values of canine apices surface area measurements between male and female for each ethnic group (Training sample = 191).

	Malay				Chinese			
	Paired Differences				Paired Differences			
Age groups	**Mean Differ ence**	**Std. Error Difference**	**t**	**Sig. (2-tailed)**	**Mean Differ ence**	**Std. Error Difference**	**t**	**Sig. (2-tailed)**
7 years	3.42	1.58	2.1	0.05*	3.82	1.90	2.0	0.07
8 years	2.69	1.12	2.4	0.03*	5.23	1.08	4.8	0.00*
9 years	6.94	1.37	5.1	0.00*	2.28	1.59	1.4	0.18
10 years	5.59	1.20	4.6	0.00*	5.18	2.12	2.4	0.03*
11 years	4.57	2.05	2.2	0.05*	4.33	1.24	3.5	0.00*
12 years	4.72	1.31	3.6	0.00*	2.59	1.39	1.8	0.09
13 years	1.71	0.82	2.1	0.058	3.46	0.58	5.9	0.00*
14 years	00	00	00	00	00	00	00	00

* Significant at the 0.05 probability level

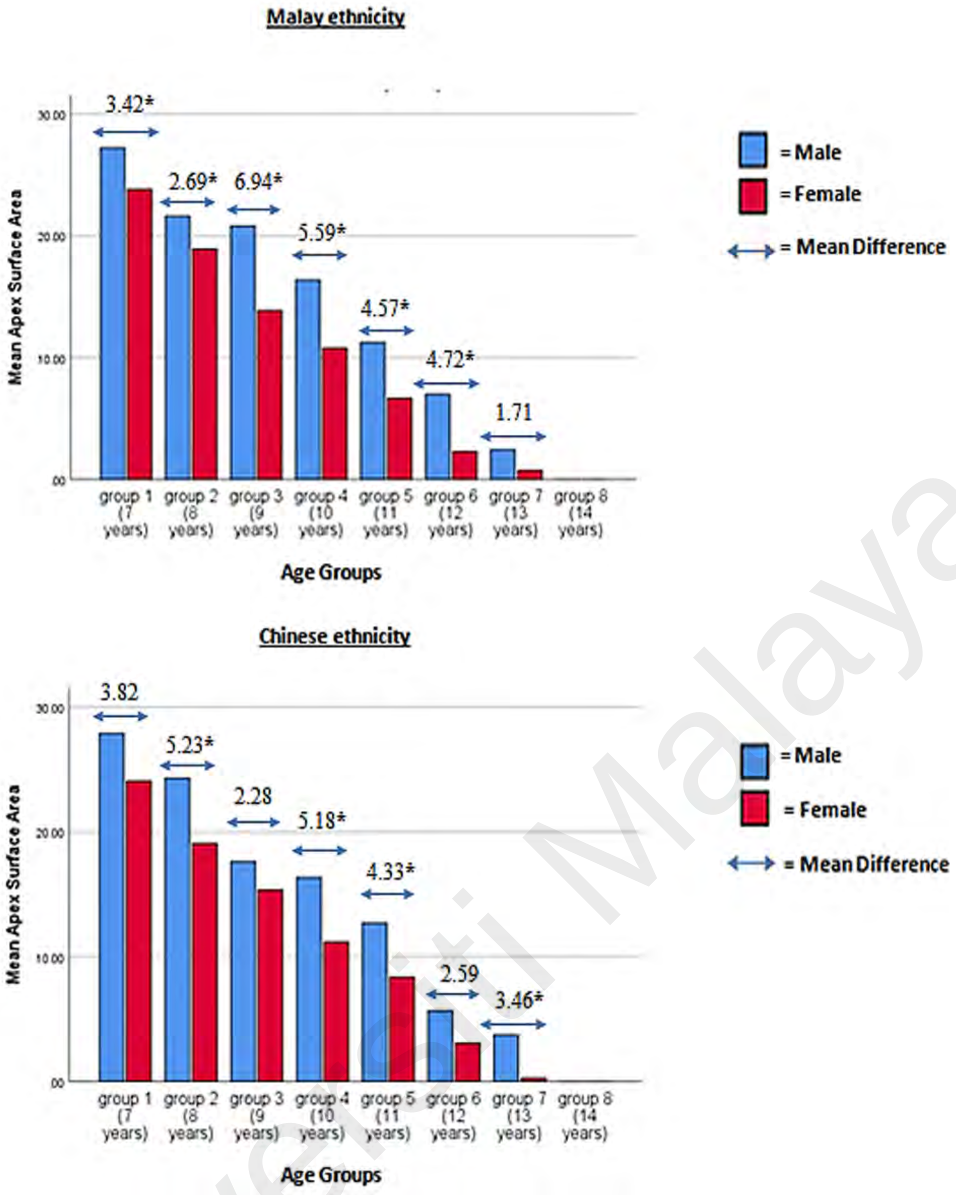

Figure 4.2: Bar charts illustrating ethnic based differences in the mean values of canine apex surface area between male and female among different age groups (* = Significant at the 0.05 probability level).

4.1.1.5 Frequency of maxillary canines apical closure in 12, 13 and 14 years age groups based on gender and ethnic groups

The frequency of maxillary canines apical closure were compared between genders for each ethnic group in 12, 13 and 14 years age groups (Table 4.8). The results indicated that female showed advanced development of maxillary canine in comparison to male (Table 4.8). Interestingly, the 13 year old females had their root development completed in more than 55% percent of the investigated teeth among Malays and more than 85% among Chinese ethnic groups. These results are also supported by the outcome of the independent samples t-test analysis in section 4.1.1.4.2, which indicated significant difference in the mean values of maxillary canines apices measurements between genders (Table 4.7). It was also observed that maxillary canines' apices development was completed in 14 years age group among both genders in each ethnic group.

Table 4.8: Frequency of maxillary canines apical closure in 12, 13 and 14 years age groups based on gender and ethnic groups.

	12 years age group				13 years age group				14 years age group			
	Male		Female		Male		Female		Male		Female	
	open apex	closed apex	open apex	closed apex	open apex	closed apex	open apex	closed apex	open apex	closed apex	open apex	closed apex
Malays	6	0	6	0	7	0	3	4	0	5	0	5
Chinese	5	0	6	0	5	0	1	6	0	5	0	6

4.1.1.6 Multiple linear regression analysis to derive an age estimation model for Malaysian children

One of the objectives of the study was to develop an age estimation model for Malaysian children. Multiple linear regression analysis was performed to derive an age estimation model using chronological age as a dependent variable and surface area of the maxillary canines apices, ethnicity, gender and status of the root development (open/closed apices) as predictor variables. A strong inverse correlation (r = 0.978, SE = 0.499) was observed between chronological age and the predictor variables (Table 4.9).

Table 4.9: Strength of correlation between chronological age and the predictor variables.

Type of Teeth (FDI notation)	Coefficient of Correlation (r)	Coefficient of Determination (R2)	Std. Error of the estimate	P - value
13 & 23	0.978	0.956	0.499	.000*

* Significant at the 0.05 probability level.

4.1.1.7 Stepwise regression analysis to identify those predictor variables that contributed significantly to the age estimation model

Age estimation model was developed by investigating those predictor variables that contributed significantly to the model using stepwise regression analysis (Table 4.10). The results indicated that all the predictor variables except ethnicity, contributed significantly to the age estimation model (Table 4.9). The R-Square (R2) value of 0.956 was observed, which indicated that 95.6 % of the variation in age can be explained by the surface area of the canine apex, gender and status of the root development (open/closed apices) (Table 4.9). The following age estimation model was derived:

Equation 4.1: Age = 13.897 - 0.228 (apex surface area) - 0.864 (gender) + 0.905 (open/closed apex).

Variables coding: Male = 0 / Female = 1, Open apex = 0 / closed apex = 1.

Table 4.10: Stepwise regression analysis to derive an age estimation regression model.

	Value	Std. Error	t- value	P - value	VIF
Intercept	13.897	0.091	153.092	.000*	
Apex surface area	- 0.228	0.005	- 48.160	.000*	1.517
Gender	-0.864	0.074	-11.659	.000*	1.050
Open/Closed apex	0.905	0.119	7.580	.000*	1.483

* Significant at the 0.05 probability level.

4.1.1.8 The reliability and accuracy of the derived age estimation model on an independent validation sample

The newly developed age estimation model was applied on an independent validation sample to test its reliability and applicability. Fishers Z test analysis indicated no statistically significant difference in the strength of correlation (r) values between Malays and Chinese ethnic groups, when investigated for each gender ($P > 0.05$) (Table 4.4). Similarly, no difference in the strength of correlation (r) values was found between male and female, when investigated for each ethnic group ($p > 0.05$) (Table 4.5). These results were in line with the outcome of the Fisher Z-test analysis resulted from the training sample (Table 4.4 & 4.5). The derived regression equation when applied on an independent validation sample (n = 96) resulted in the mean absolute error (MAE) value of 0.300 for the combined data of all the age-groups (Table 4.11). Based on the ethnicity and gender, Malay males and females resulted in the MAE values of 0.298 and 0.310 for the combined data of all the age-groups. Chinese males and females resulted in the MAE values of 0.301 and 0.302 respectively. Moreover, when investigated for each age group (1-8), lowest MAE value (0.15) was observed in 12 years age group, and highest (0.47) in 14 years age group. The resulted MAE values were in the acceptable range for all the investigated age groups (Table 4.11). The MAE values were based on the difference between the chronological and estimated age.

Table 4.11: Validation of the derived age estimation formula using mean absolute error (MAE) values (validation sample = 96).

Age Groups	Mean Absolute Error (MAE) values				
	Malay		Chinese		Combined data for the investigated ethnicity and gender
	Male	Female	Male	Female	
7 Years	0.49	0.23	0.36	0.26	**0.34**
8 Years	0.27	0.45	0.43	0.27	**0.35**
9 Years	0.33	0.23	0.12	0.13	**0.20**
10 Years	0.18	0.28	0.29	0.45	**0.30**
11 Years	0.5	0.13	0.43	0.25	**0.33**
12 Years	0.15	0.20	0.17	0.09	**0.15**
13 Years	0.21	0.35	0.23	0.35	**0.29**
14 Years	0.26	0.61	0.39	0.62	**0.47**
All age-groups together	**0.298**	**0.310**	**0.301**	**0.302**	**0.30**

4.1.1.9 Multicollinearity (variance inflation factor (VIF) =<5) among all predictor variables and Kolmogorov-Smirnov test to investigate normal distribution of the residuals

Multicollinearity expresses the situation where the independent variables are highly associated with each other. The variance inflation factor (VIF) values are below 5 in the current study, indicating that there is no problem of multicollinearity (Table 4.10). Durbin–Watson value in the current study was also close to 2 (1.612), which showed that there is no significant autocorrelation detected in the sample. Moreover, the Kolmogorov-Smirnov test of normality on the residuals resulted in a p-value of 0.096, which is more than 0.05 (Appendix E). Thus, the assumption of normality of the residual terms was also met. All these assumptions are important to report in the research article as these can have a significant impact on the final regression equation and analysis.

4.1.2 Results of the second main objective of the study

The second research objective of the study was to develop an age estimation model for Malaysian juveniles and young adults by assessing mandibular third molars apices. Training sample of 128 intact mandibular third molars (without any caries or associated pathologies) were selected from 128 CBCT images belonging to 66 Malays (Male = 37, Female = 29) and 62 Chinese (Male = 26, Female = 36) (Table 4.12). Moreover, an independent validation sample of 55 permanent maxillary canines were selected from 55 CBCT images belonging to 27 Malays (Male = 14, Female = 13) and 28 Chinese (Male = 14, Female = 14) (Table 4.12).

Table 4.12: Distribution of training (n = 128) and validation (n = 55) sample based on gender and ethnicity.

Age groups	Age (years)	Training sample (n = 128)				Validation sample (n = 55)			
		n = Malays		n = Chinese		n = Malays		n = Chinese	
		n = Male	n = Female	n = Male	n = Female	n = Male	n = Female	n = Male	n = Female
G 1	13.00 - 14.99	10		11		5		4	
		8	2	4	7	2	3	2	2
G 2	15.00 - 16.99	8		8		4		4	
		4	4	4	4	2	2	2	2
G 3	17.00 - 18.99	11		10		4		5	
		5	6	4	6	2	2	3	2
G 4	19.00 - 20.99	13		12		5		5	
		6	7	6	6	3	2	2	3
G 5	21.00 - 22.99	10		12		4		5	
		6	4	4	8	2	2	3	2
G 6	23.00 - 24.99	14		9		5		5	
		8	6	4	5	3	2	2	3
Total		66		62		27		28	
		37	29	26	36	14	13	14	14

4.1.2.1 Intraexaminer and interexaminer reliability

The intraclass correlation coefficient (ICC) value was 0.953 for intra-examiner reliability and 0.926 for inter-examiner reliability, which indicated high level of agreement between repeated measurements.

4.1.2.2 Pearson correlation coefficient analysis based on gender and ethnicity

Pearson correlation coefficient analysis was performed to assess the strength of correlation (r) between chronological age and surface area of the developing mandibular third molars apices in Malay and Chinese ethnic groups for both genders separately (Table 4.13 & 4.14). The results indicated very strong correlation for Malays ($r > 0.91$) and Chinese ($r > 0.94$) among both genders (Table 4.13 & 4.14).

Table 4.13: The strength of correlation for Malays and Chinese ethnic groups based on gender resulted from the training sample (n = 128)

Training sample (n= 128)				
Investigated teeth (FDI	**Pearson correlation (r) values for male**		**P-value for male**	
	Malays	**Chinese**	**Malays**	**Chinese**
38 & 48	0.915(37)	0.948(26)	.00*	.00*
	Pearson correlation (r) values for female		**P-value for female**	
	Malays	**Chinese**	**Malays**	**Chinese**
	0.896(29)	0.929(36)	.00*	.00*

* Significant at the 0.05 probability level.

Table 4.14: The strength of correlation for Malays and Chinese ethnic groups based on gender resulted from the validation sample (n = 55)

validation sample (n= 55)				
Investigated teeth (FDI notation)	**Pearson correlation (r) values for male**		**P-value for Male**	
	Malays	**Chinese**	**Malays**	**Chinese**
38 & 48	0.957(14)	0.872(14)	.00*	.00*
	Pearson correlation (r) values for female		**P-value for Female**	
	Malays	**Chinese**	**Malays**	**Chinese**
	0.919 (13)	0.871(14)	.00*	.00*

*** Significant at the 0.05 probability level.**

4.1.2.3 Fisher Z-test analysis to investigate significant difference in the coefficient of correlation values among genders and ethnic groups.

Fishers Z-test analysis was used separately for each gender to investigate statistically significant difference in the strength of correlation coefficient (r) values between Malays and Chinese ethnic groups (Table 4.15). Similar analysis was employed separately for each ethnic group to investigate any significant difference in the strength of correlation coefficient (r) values between males and females (Table 4.16). It must be noted that Fisher Z-test was applied on both training (n = 128) and validation (n = 55) samples.

4.1.2.3.1 To investigate difference in the strength of correlation coefficient values between Malays and Chinese ethnic groups separately for each gender

Results of the Fishers Z test analysis showed no statistically significant difference in the strength of correlation coefficient (r) values between Malays and Chinese ethnic groups, when investigated for each gender separately (Table 4.15). This pattern was consistent among both training (n = 128) and validation samples (n =55) (Table 4.15).

Table 4.15: Pearson correlation values and Fishers Z test analysis between Malays and Chinese for each gender (training sample: n = 128, validation sample: n = 55).

Training sample (n= 128)				
Investigated teeth (FDI notation)	**Pearson correlation (r) values for male**		**Fishers Z test**	
	Malays	**Chinese**	**Z-value**	**P-value**
38 & 48	0.915(37)	0.948(26)	-0.94	0.347
	Pearson correlation (r) values for female		**Fishers Z test**	
	Malays	**Chinese**	**Z-value**	**P-value**
	0.896(29)	0.929(36)	-0.76	0.447
Independent validation sample (n= 55)				
Investigated teeth (FDI notation)	**Pearson correlation (r) values for male**		**Fishers Z test**	
	Malays	**Chinese**	**Z-value**	**P-value**
38 & 48	0.957(14)	0.872(14)	1.33	0.183
	Pearson correlation (r) values for female		**Fishers Z test**	
	Malays	**Chinese**	**Z-value**	**P-value**
	0.919 (13)	0.871(14)	0.56	0.575

* Significant at the 0.05 probability level.

4.1.2.3.2 To investigate difference in the strength of correlation coefficient values between male and female separately for each ethnic group

Fisher Z-test indicated no statistically significant difference in the strength of correlation coefficient values between male and female, when investigated for each ethnic group in both training and validation samples (Table 4.16).

Table 4.16: Pearson correlation values and Fishers Z test between male and female for each ethnic group (training sample: n = 128, validation sample: n = 55).

Training sample (n= 128)				
Investigated teeth (FDI notation)	**Pearson correlation (r) values for Malays**		**Fishers Z test**	
	Male	**Female**	**Z-value**	**P-value**
38 & 48	0.915(37)	0.896(29)	0.41	0.681
	Pearson correlation (r) values for Chinese		**Fishers Z test**	
	Male	**Female**	**Z-value**	**P-value**
	0.948(26)	0.929(36)	0.59	0.555
Independent validation sample (n= 55)				
Investigated teeth (FDI notation)	**Pearson correlation (r) values for Malays**		**Fishers Z test**	
	Male	**Female**	**Z-value**	**P-value**
38 & 48	0.957(14)	0.919 (13)	0.75	0.453
	Pearson correlation (r) values for Chinese		**Fishers Z test**	
	Male	**Female**	**Z-value**	**P-value**
	0.872(14)	0.871(14)	0.01	0.992

* Significant at the 0.05 probability level

4.1.2.4 Independent samples t-test analysis to investigate significant difference in the mean values of mandibular third molars apices surface area among genders and ethnic groups.

In addition to Fisher Z-test, independent samples t-test was also applied on the training sample of mandibular third molars apices for each age group (1-6) to assess the significant difference in the mean values of apex surface area measurements among genders and ethnic groups.

4.1.2.4.1 Age group-wise (1-6) independent samples t-test to investigate difference in the mean values of mandibular third molars apices surface area measurements between Malays and Chinese separately for each gender

The results of the independent samples t-test analysis indicated that there was no significant difference in the mean values of mandibular third molars apices surface area between Malays and Chinese in majority of the investigated age groups (1-6) for both genders (Table 4.17) (Figure 4.3). However, among females, the age groups 13-14, 19-20 and 23-24 years showed significant difference in the mean values between Malays and Chinese ethnic groups.

Table 4.17: Groupwise Independent samples t-test to investigate difference in the mean values of mandibular third molars apices between Malays and Chinese separately for each gender (Training sample = 128).

	Male				**Female**			
Age groups (years)	**Paired Differences**				**Paired Differences**			
	Mean Difference	**Std. Error Difference**	**t**	**Sig. (2-tailed)**	**Mean Difference**	**Std. Error Difference**	**t**	**Sig. (2-tailed)**
13-14	-1.19	5.11	-2.3	0.82	23.14	5.14	4.5	0.00*
15-16	4.69	6.05	0.8	0.47	11.95	6.78	1.7	0.12
17-18	-3.24	9.27	-0.3	0.73	11.27	6.27	1.8	0.10
19-20	3.45	2.28	1.5	0.16	-3.80	1.43	-2.6	0.02*
21-22	-2.60	1.89	1.4	0.21	2.41	1.12	2.1	0.06
23-24	00	00	00	00	0.256	0.10	2.4	0.03*

* Significant at the 0.05 probability level

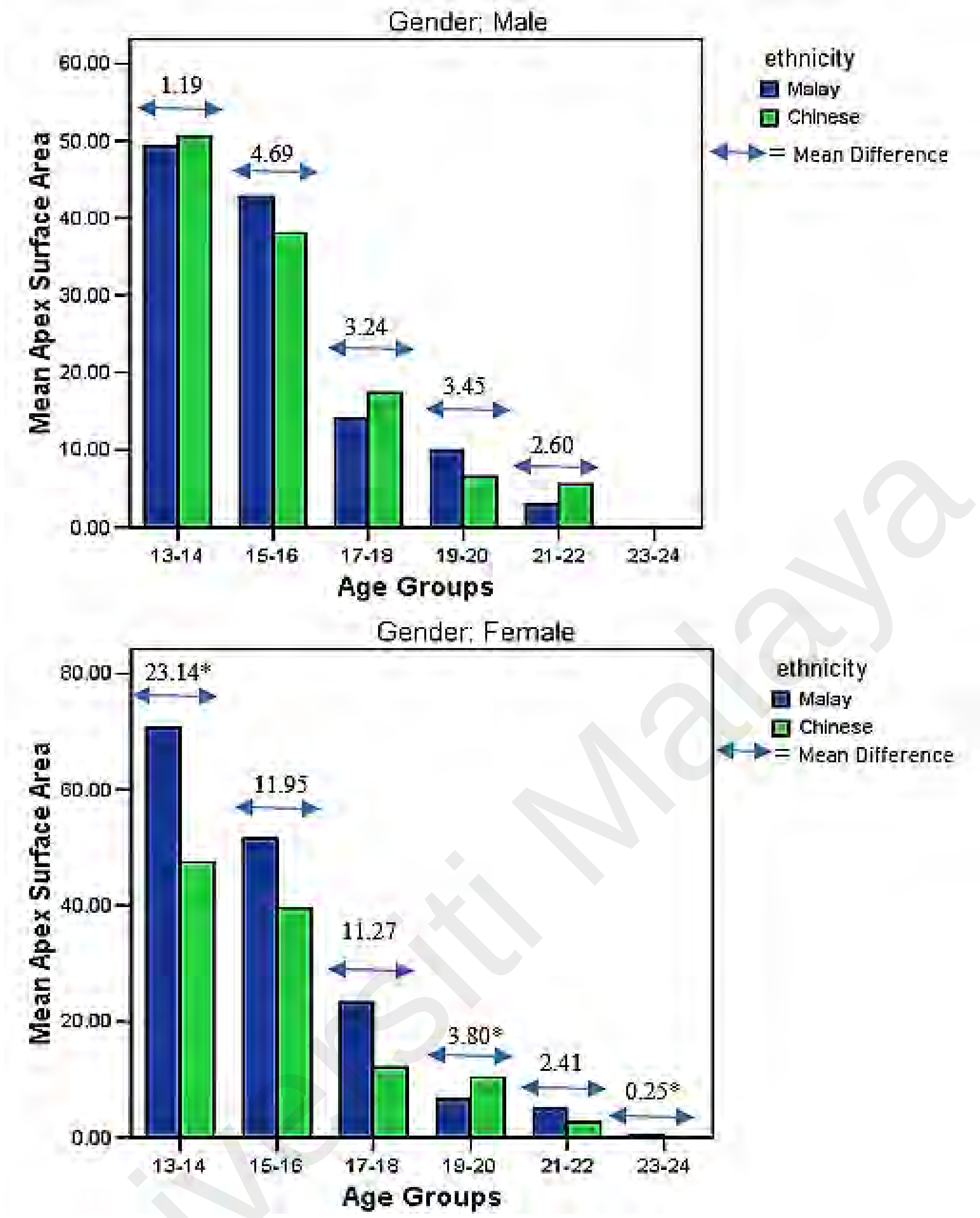

Figure 4.3: Bar charts illustrating gender based differences in the mean values of third molars apices surface area between Malays and Chinese among different age groups (* = Significant at the 0.05 probability level).

4.1.2.4.2 Age group-wise (1-6) independent samples t-test to investigate difference in the mean values of mandibular third molars apices surface area measurements between males and females separately for each ethnic group

The results of independent samples t-test showed no significant difference in the mean values of mandibular third molars apex surface area between male and female in majority of the investigated age groups (1-6) among both ethnic groups (Table 4.18) (Figure 4.4). However, among Malays, the age group 13-14 and 23-24 years showed significant difference in the mean values between males and females.

Table 4.18: Groupwise Independent samples t-test to investigate difference in the mean values of mandibular third molars apices between male and female separately for each ethnic group (Training sample = 128).

	Malays				**Chinese**			
Age groups (years)	**Paired Differences**				**Paired Differences**			
	Mean Difference	**Std. Error Difference**	**t**	**Sig. (2-tailed)**	**Mean Difference**	**Std. Error Difference**	**t**	**Sig. (2-tailed)**
13-14	21.3	7.42	-2.8	0.02*	3.06	3.50	0.9	0.40
15-16	-8.72	7.76	-1.1	0.30	-1.46	4.74	-0.3	0.77
17-18	-9.16	9.26	-0.9	0.35	5.35	4.64	1.1	0.28
19-20	3.39	1.77	1.9	0.08	3.86	1.97	1.9	0.07
21-22	-2.05	1.64	-1.2	0.25	2.96	1.38	2.1	0.06
23-24	-0.26	0.08	-3.1	0.00*	00	00	00	00

* Significant at the 0.05 probability level

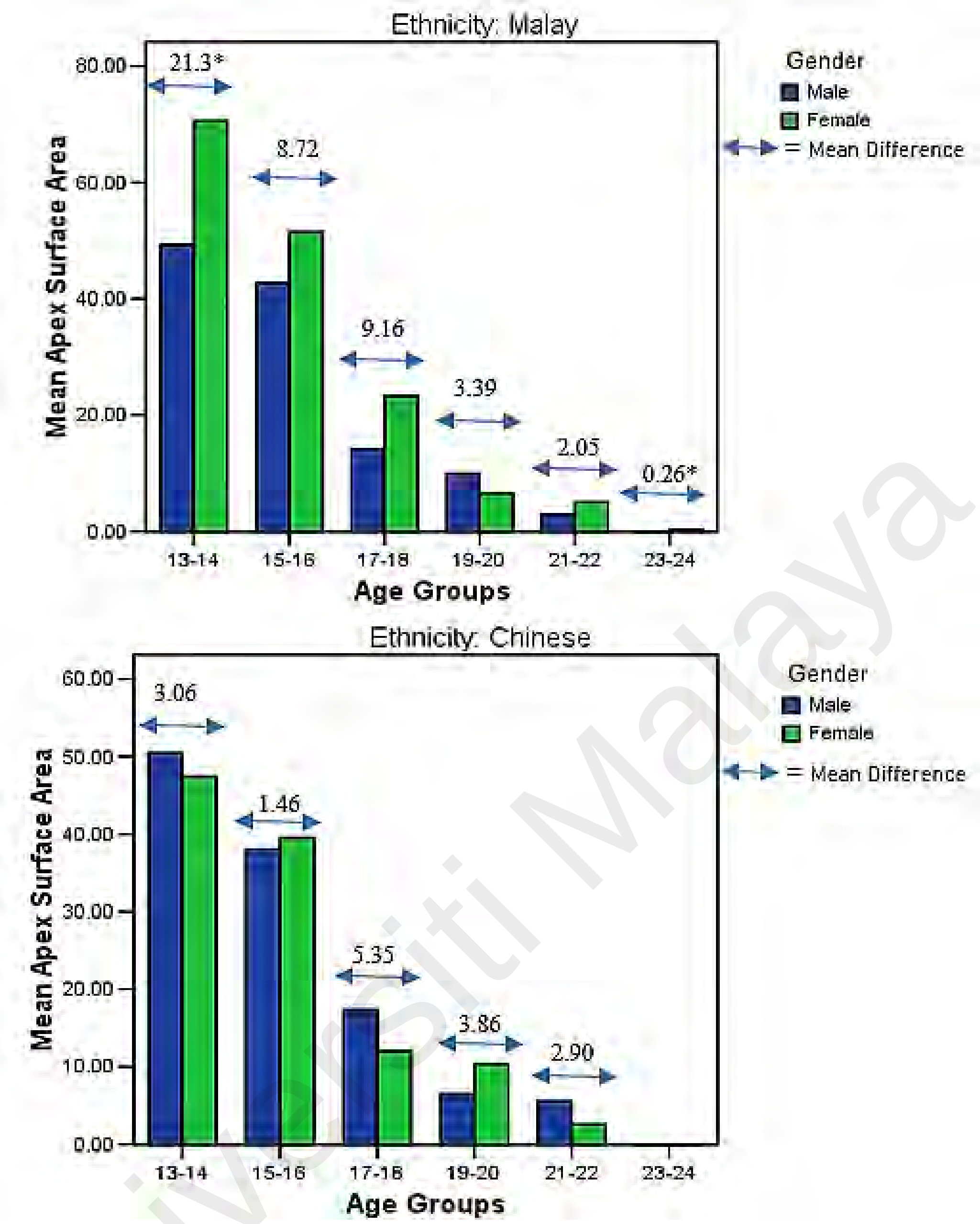

Figure 4.4: Bar charts illustrating ethnicity based differences in the mean values of third molars apices surface area between males and females among different age groups (* = Significant at the 0.05 probability level).

4.1.2.5 Frequency of third molars roots apical closure in 19-20, 21-22 and 23-24 years age groups based on gender and ethnic groups.

The mandibular third molars roots apical closure were compared between genders for each ethnic group in 19-20, 21-22 and 23-24 years age groups (Table 4.19). In 23-24 years age group, Malay males had their root development completed in all of the investigated third molars. However, more than 65% of third molars had open apices among Malay females in 23-24 years age group. This indicates that third molar development among Malay males is advanced in comparison to females. Interestingly, no difference was observed in the third molars root apical closure between Chinese male and females in the investigated age groups (Table 4.19).

Table 4.19: Frequency of apical closure in 19-20, 21-22 and 23-24 years age groups based on gender and ethnic groups.

	19-20 years age group				**21-22 years age group**				**23-24 years age group**			
	Male		**Female**		**Male**		**Female**		**Male**		**Female**	
	open apex	**closed apex**	**open apex**	**closed apex**	**open apex**	**closed apex**	**open apex**	**closed apex**	**open apex**	**closed apex**	**open apex**	**closed apex**
Malays	6	0	7	0	6	0	4	0	0	8	4	2
Chinese	6	0	6	0	4	0	8	0	0	4	0	5

4.1.2.6 Multiple linear regression analysis to derive an age estimation model for Malaysian juveniles and young adults

One of the aims of the study was to develop an age estimation model for Malaysian juveniles and young adults. Multiple linear regression analysis was used to derive an age estimation model using chronological age as a dependent variable and surface area of the mandibular third molars apices, ethnicity, gender and status of the root development completion (open/closed apices) as predictor variables. Multiple linear regression analysis showed strong inverse correlation (r = 0.95, SD = 1.144) between chronological age and the predictor variables (Table 4.20).

Table 4.20: Strength of correlation between chronological age and the predictor variables.

Type of Teeth (FDI notation)	Coefficient of Correlation (r)	Coefficient of Determinatio n (R2)	Std. Error of the estimate	P - value
38 & 48	0.95	0.896	1.144	.000*

* Significant at the 0.05 probability level.

4.1.2.7 Stepwise regression analysis to identify those predictor variables that contributed significantly to the age estimation model

Stepwise regression analysis was employed to develop an age estimation model based on those predictor variables that contributed significantly to the age estimation model. Stepwise regression analysis indicated that all the predictor variables contributed significantly to the age estimation model (Table 4.21). The R-Square (R2) value (0.896) indicated that 89.6% of the variation in age can be explained by surface area of the mandibular third molars apices, ethnicity, gender and status of the root development completion (open/closed apices). The following linear regression formula was derived:

Equation 4.2: Age = 21.396 - 0.137 (surface area) + 2.662 (open/closed apex) – 0.618 (ethnicity) + 0.415 (gender).

Variables coding: Open apex = 0 / closed apex = 1, Malay ethnicity = 0 / Chinese ethnicity = 1, Male = 0 / Female = 1.

Table 4.21: Stepwise regression analysis to derive an age estimation regression model.

	Value	Std. Error	t- value	P - value	VIF
Intercept	21.396	0.216	98.892	.000*	
Apex surface area	- 0.137	0.005	- 25.506	.000*	1.18
Open/Closed apex	2.662	0.310	8.574	.000*	1.19
Ethnicity	- 0.618	0.204	- 3.025	.003*	1.02
Gender	0.415	0.206	2.013	.046*	1.04

* Significant at the 0.05 probability level.

4.1.2.8 The reliability and accuracy of the derived age estimation model on an independent validation sample.

The newly developed age estimation model was applied on an independent validation sample of Malaysian juveniles and young adults to test its reliability and applicability. The results of the Fishers Z-test indicated no statistically significant difference in the strength of correlation (r) values between Malays and Chinese ethnic groups, when investigated for each gender (Table 4.15). Similarly, no difference in strength of correlation (r) values was found between male and female, when investigated for each ethnic group (Table 4.16). Similar results were observed in the training sample. Mean absolute error (MAE) value of 0.82 was observed, when the derived age estimation model was tested on an independent validation sample (n= 55) (Table 4.22). Based on the ethnicity and gender, Malay males and females resulted in the MAE values of 0.90 and 0.75 for the combined data of all the age-groups. Chinese males and females resulted in the MAE values of 0.73 and 0.87 respectively. When investigated for each age group (1-6), lowest MAE value (0.586) was observed for 23-24 years age group and 22-23 years age group resulted in highest MAE value (1.075).

Table 4.22: Mean absolute error (MAE) values resulted from validation of the derived regression equation (validation sample = 55)

Age Groups	Mean absolute error (MAE) values				
	Malay		Chinese		Combined data for the investigated ethnicity and gender
	Male	Female	Male	Female	
13-14 Years	1.22	0.75	0.23	1.04	**0.81**
15-16 Years	1.02	0.68	0.70	0.90	**0.82**
17-18 Years	0.63	1.19	0.82	0.87	**0.87**
19-20 Years	0.90	0.67	0.54	0.89	**0.75**
21-22 Years	1.04	0.81	1.36	0.96	**1.04**
23-24 Years	0.59	0.37	0.75	0.61	**0.58**
All age-groups together	**0.90**	**0.75**	**0.73**	**0.87**	**0.82**

4.1.2.9 Multicollinearity (variance inflation factor (VIF) =<5) among all predictor variables and Kolmogorov-Smirnov test to investigate normal distribution of the residuals

The variance inflation factor (VIF) values were below 5 in the current study, indicating that there is no problem of multicollinearity (Table 4.21). Durbin–Watson was also close to 2 (1.094), which showed that there is no significant autocorrelation detected in the sample. Moreover, the Kolmogorov-Smirnov test of normality on the residuals resulted in a p-value of 0.20, which is more than 0.05 (Appendix F). Thus, the assumption of normality of the residual terms was also met.

CHAPTER 5: DISCUSSION

5.1 Rational for choice of the study topic

The study was designed to develop new dental age estimation models for Malaysian children and juveniles and young adults. The current study investigated the relationship between chronological age and surface area of the developing permanent maxillary canines and mandibular third molars apices based on the 3D image analysis using CBCT data. Moreover, variations based on ethnicities, gender and status of root development were also investigated in terms of fit to the age estimation models. The study also investigated the statistically significant differences in the correlation coefficient (r) values among genders and ethnic groups (Malays & Chinese). In addition to the differences in the correlation coefficient (r) values, gender and ethnic based variations in the development of root apices were also investigated among different investigated age groups. The study was also designed to validate the newly developed age estimation models on an independent validation sample.

5.1.1 A novel three-dimensional (3D) image analysis technique for age estimation

The main reason for conducting the current research was based on the fact that to the best of our knowledge, no study has been reported to investigate the relationship between chronological age and changes in the surface area of developing permanent dentition apices using 3D image analysis by employing CBCT imaging modality. CBCT would assist in better analysis of developing root apices and due to the fact that the maxillary canines and mandibular third molars roots are generally conical and tapering towards the apices, the surface area measurements would have a negative correlation with age. Smaller the surface area, the age will be more advanced. Previous radiographic age estimation studies have mainly reported the relationship between chronological age and developing root apices using panoramic images (Cameriere, Ferrante, & Cingolani, 2006;

Cameriere et al., 2008; Cameriere, Ferrante, Scarpino, et al., 2006). These 2D radiographic studies have shown promising results, however it must be noted that 2D radiographs doesn't represent the 3D anatomical structures and may involve bias in the measurements. Moreover, as stated earlier, assessing panoramic images for measuring linear distance of open apices can be difficult due to the lower image clarity and resolution, higher magnification, distortion of structures and without any sectional information (Peker et al., 2008; Ladeira et al., 2012). These limitations can be nullified by employing CBCT scanning systems to measure the surface area of developing root apices. The findings of the current study will be beneficial for the future research in an effort to develop a more reliable, valid and simple method of dental age estimation among different ethnic groups.

5.1.2 Population specific age estimation method

2D radiographic dental age estimation studies have reported varying results among different ethnic groups by employing developmental and physiological dental age related parameters (Willems et al., 2001; Mani et al., 2008; Nik-Hussein et al., 2011). Dental age estimation method developed by Cameriere et al. (2006) based on the linear measurements of developing permanent dentition roots apices has resulted in different strength of correlation (r) values and regression equations among different populations around the world (Cameriere, Ferrante, & Cingolani, 2006; Cameriere et al., 2008) (Cameriere et al., 2007; Cugati et al., 2015; Rai et al., 2010; Guo et al., 2015; Angelakopoulos et al., 2019; Solari & Abramovitch, 2002; S. AlQahtani et al., 2017). Majority of these validation studies have suggested population specific age estimation model for their respective populations. Therefore, the current study aimed to develop Malaysian population specific age estimation models. Malaysia is a multi-ethnic society in which three major ethnic groups namely Malays, Chinese and Indians dominate. Malays and Chinese ethnic groups were selected for the study to investigate age related changes in the surface area of developing permanent dentition (maxillary canine and mandibular third molar) because they belong to the same Mongoloid race. Individuals belonging to Mongoloid race share similar genetic make-up and have similar features such as skin folds covering the corners of the eyes, and shovel shaped teeth. A shovel-shaped incisor is formed because of a raised cingulum on the lingual surface of incisors. It is found in approximately 90% of Mongoloids. Some other distinctive dental features are the tubercle on the buccal cusp or the central groove of premolars which may be seen among Mongoloids. Moreover, the distal cusp (5^{th}) in mandibular molars is usually more lingually placed than Caucasoid. Due to the highly mineralised tooth composition and racial specific characteristics, teeth can be used effectively for racial differentiations.

Despite of similarities in the dental features among Malays and Chinese ethnic groups, ethnic based variances were investigated between them in the current study. Moreover, gender variances were also investigated in the current study to observe the effect of growth spurt on the early or advanced tooth maturation. This was important as few studies have reported sexual and ethnic dimorphism in the morphometric dental measurements between Malays and Malaysian Chinese ethnic groups (Khamis et al., 2014).

5.1.3 Selection of the type of investigated teeth

The current study evaluated the relationship between chronological age and changes in the surface area of developing permanent maxillary canines and mandibular third molars apices. One of the reasons for selecting these two different types of teeth was due to their different development and eruption stages with aging, which was used to develop two new age estimation models. One of the models was developed for Malaysian children aged 7 to 14 years by employing permanent maxillary canines and another model for Malaysian juveniles and young adults aged 13 to 24 years using mandibular third molars.

Therefore, the newly developed method of dental age estimation in the current study can be applied on children, juveniles and young adults.

It must be noted that CBCT images for each investigated tooth were thoroughly examined to exclude teeth with any associated pathology. The selection of permanent maxillary canine was based on the fact that it is the most stable and longest tooth in the oral cavity. The roots of the maxillary canines are the strongest among all human teeth. The average length of maxillary canine is approximately 17mm. Moreover, due to its longevity, the changes in the pattern of root development may be observed more precisely during 3D image analyses. Canine root appears slender, tapered, and conical in form with blunt or sharp apex. Developmental depressions are found on the mesial and distal root surfaces. The root of maxillary canine is broader labiolingually than mesiodistally, thereby giving a triangular shape. Labial and lingual root surfaces are convex in shape. The root development completes at the age of around 13 to 15 years. In addition, previous studies have reported the prevalence of maxillary canine agenesis between 0.07 and 0.3%, which is considered extremely low as compared to other permanent dentition (Polder et al., 2004; Dolder, 1937; Rozsa et al., 2009). Thus, canines can be used effectively for dental age estimation.

Mandibular third molars were selected in the current study to develop age estimation model for Malaysian juveniles and young adults because they are the only permanent teeth which are developing in adults. Crown completion of mandibular third molars is around 13 to 15 years and the root development completes at 20 to 23 years (Jung & Cho, 2014; Orhan et al., 2007; Zandi et al., 2015). Despite of variations in the development and eruption pattern, this is the only available tooth amongst the permanent dentition which continues to develop even after 16 years of age. This is critical because this may prove to be useful and effective in criminal investigations and implementing the legal justice system. The mandibular third molars were selected in the current study instead of maxillary third molars to develop dental age estimation model because of the increased bone mineral density of the mandibular jaw in comparisons to maxilla. This was considered because mandibular jawbone can resist physical and mechanical force more effectively during natural or manmade disasters. So, the chances of losing mandibular third molars due to any physical insult is less, which can play a significant role in forensic identification and age estimation during disaster victim management. The current study investigated mandibular third molars with only 2 roots. This was considered due to the fact that although the root canal morphology and anatomy of mandibular third molars are highly variable, most of the studies have reported that prevalence of two roots is higher in comparison to one and three roots (Sidow et al., 2000; Kuzekanani et al., 2012). The surface areas of the mesial and distal roots apices were calculated and the sum of both the surface areas were recorded and analysed in the current study.

5.1.4 Sudden increase in the frequency of mass disasters

There is a growing need to develop a simple and reliable dental age estimation method due to the abrupt rise in the frequency of mass disasters. These events are sudden, unexpected and involves large number of loss of life. Mass disasters can be natural such as earthquakes, tsunamis, volcanic eruptions and landslides. It can also be due to human errors like road traffic accidents, aviation disasters or accidents through illegal sea route migration. It is difficult to undergo disaster victim identification with the help of biometric fingerprinting and DNA profiling techniques on such a catastrophic large scale. Moreover, these advance identification techniques require accredited expensive laboratories. The local government resources in underdeveloped or developing countries are mostly limited to undergo advance identification procedures specially in mass disasters where most of the infrastructure is destroyed. Thus, forensic odontologists play an important role in the identification and age estimation of deceased in mass disasters. They provide more likely less invasive, inexpensive and quickest way of identification in such events. Moreover, teeth can be extracted, examined and scanned with imaging modalities for greater understanding of their stages of development.

5.1.6 Cone beam computed tomography and image acquiring parameters

The current study employed CBCT images to develop new method of dental age estimation among children, juveniles and young adults. CBCT is currently being used in different specialties of dentistry such as third molar surgical extractions, pathological lesion analyses, implant placements, orthodontics, periodontal treatment (Alamri et al., 2012) and endodontics (van Vlijmen et al., 2009; Haney et al., 2010; Kamburoglu et al., 2009; Worthington et al., 2010; Ahmad et al., 2012). Moreover, it provides isotropic voxel resolution, rapid scan time, multiplanar reformatting (MPR) and small field of view (FOV) (Benavides et al., 2012) (Scarfe & Farman, 2008). As stated earlier, the main reason for the selection of CBCT as a imaging modality for the current study was due to less exposure time to radiation and its cost effectiveness in comparison to other 3D imaging modalities (Kumar et al., 2015; Dawood et al., 2009; Ziegler et al., 2002; Scarfe et al., 2006). Few studies have even reported more accuracy and higher resolution of CBCT as compared to CT, despite of less exposure to radiation (Cotton et al., 2007).

i-CAT Cone Beam 3D Dental Imaging System (version 3.1.62 supplied by Imaging Sciences International, Hatfield, USA) was used in the current study to acquire CBCT data. All the scanned CBCT images were acquired using voxel size of 0.30 mm and a scanning time of 20 seconds. The selected scans had exposure parameters of 120 kV, 18 mA. It must be noted that the reason for including CBCT images with same exposure parameters was due to the effect of different scanning parameters on the quality of CBCT images. Moreover, the current study selected CBCT images with 0.30 mm voxel size because CBCT images acquired with different voxel sizes have shown different volumetric results for the same anatomical structures (Whyms et al., 2013). Maret et al.

(2012) reported that the volumetric measurements from the CBCT images acquired with voxel size of 0.30 mm were significantly different from the CBCT images acquired using 0.076 mm voxel size (Maret et al., 2012). This could be attributed to Partial volume effect (PVE) associated with larger voxel size. Moreover, as mentioned earlier larger voxel size CBCT images can be difficult to segment minute structures having similar densities such as teeth very close to each other. This could be the reason that minor apical constriction in the completely formed maxillary canines and third molars were not visualised or detected on 3D constructed image models in MIMICS and 3-matics software. The average diameter of apical foramen for anterior teeth is approximately between 0.28 mm to 0.35 mm, and for mandibular third molars approximately between 0.23 to 0.34 mm (Abdullah et al., 2013; Chapman, 1969; Marroquín et al., 2004). The diameter of apical foramina of the investigated teeth is almost similar or lesser than the voxel size employed to acquire the CBCT images in the current study. Thus, apical foramen were not detected in the completely formed roots.

5.2 Effectiveness of Mimics and 3-matics software in 3D image analysis

MIMICS software (Materialise NV, Belgium, version 21.0) was used in the current study to develop 3D image models of developing maxillary canines and mandibular third molars apices. This software has been used in dental age estimation studies for reconstructing 3D image models of various dental tissues and has proved to be effective in volumetric assessment of dental structures (Tardivo et al., 2014; Tardivo et al., 2011; M. K. Asif et al., 2019). It allows to do the volumetric analysis in all 3 planes (axial, coronal and sagittal). It also allows to precisely measure distance, diameter, density and angles either on 2D images or directly on the 3D image model of developing tooth apices. Another advantage of the software is that it 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 (Materialise, 2018). 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.

'Multiple slice editing segmentation' provided by the software has proved to be useful in the current study in identifying those calcified structures which were not selected automatically by the software. It also allowed the investigator to add or remove mask, where the software was not able to automatically demarcate minute structural details. Another advantage of the software was that it provided pre-set grayscale threshold values (1200-3071) for the calcified tooth structures, which helped in standardising the thresholding or segmentation for all the investigated teeth. Thresholding method turns a grey scale image into a binary image by using a selected threshold value (Batenburg & Sijbers, 2009). This is the simplest form of image segmentation. However, it must be noted that despite selecting predefined threshold values, the developing calcified tooth was manually checked during 'multiple slice editing segmentation phase' in MIMICS software because it was noticed that in some cases the software was not able to identify minute structural details, especially in advanced developed roots whose apices were near to closure. This could be due to the fact that the investigated teeth were at developing stages and the calcification or mineralisation of the developing root apices were not completed to be recognised by the software.

3-matics software (Materialise NV, Belgium, Version 13.0) has been used in the current study for the surface area analysis of developing root apices. The advantage of this software in the current study was that it allowed to mark and create curve along the margins of the developing root apices on the 3D image models (Figure 3.7 & 3.16). The software was effective as it provided self-interaction tool during the creation of curve along the developing root apices, which was consistently followed in all the investigated teeth. Another feature offered by the software was the 'smoothen curve' option. This was important because majority of the investigated teeth were developing. Due to the variability in the calcification or maturation along the margins of the root, some of the margins were not clear enough to be marked so this feature assisted the examiner to mark the margins with more precision. It must be noted that the smoothen curve tool was used in all the investigated teeth to standardise the measurements.

5.3 Two-dimensional verses three-dimensional measurements of developing root apices

Dental age estimation methods developed in the 20th and first decade of the 21st century have mainly employed 2D radiographic modalities mainly panoramic images, such as a method of assessing linear distance of the open apices in developing teeth among growing children for age estimation (Cameriere, Ferrante, & Cingolani, 2006). Cameriere et al. (2008) also employed the same 2D radiographic method using third molars to distinguish juveniles from adults (Cameriere et al., 2008). Despite of varying results among different populations, the newly developed method showed strong association of age with the changes in the linear distance of the roots open apices. However, with recent advancements in 3D imaging modalities such as MRI, CT and CBCT, it is possible to nullify the limitations associated with panoramic images in assessing developing root apices such as unequal magnification and distortion of the images. The results of the current study showed strong correlation between chronological age and changes in the surface area of maxillary canine and mandibular third molars apices. Other researchers also supported the effectiveness of CBCT images in assessing third molars development for age estimation (Cantekin et al., 2013). In a recently reported systematic review on the accuracy and reliability of CBCT verses panoramic images in dental age estimation methods, they concluded that CBCT is more reliable in assessing teeth maturation in comparison to panoramic images (Dalessandri et al., 2020). It must be noted that Cameriere et al. (2006) divided the linear measurements of root apices by the tooth length to nullify the differences in magnification and angulations among panoramic radiographs (Cameriere, Ferrante, & Cingolani, 2006; Cameriere et al., 2008). This was effective but it must be noted that this additional step involved more human manual intervention during measurements. In contrast, the current CBCT study has developed age estimation method by measuring the surface area of root apices alone without considering tooth length due to the high accuracy of CBCT in acquiring anatomical structures without any distortion or magnification. This may result in a more reliable and accurate measurements, as it limits manual human intervention. Moreover, as stated earlier 2D radiograph doesn't represent the 3D anatomical structures. Thus, assessment of 3D developing roots was not possible in previously reported 2D age estimation studies using panoramic images. This could have resulted in inaccurate measurements and a biased correlation between chronological age and the developing root apices. In addition, the mineralisation or development of root apices may not be uniform in all 3 planes. So, the method employed in the current study enabled the examiner to assess and mark the margins of developing root apices in any plane along with simultaneous viewing of axial, coronal and sagittal sections of the investigated tooth.

5.4 Age estimation model for Malaysian children by assessing developing maxillary canines' apices

One of the research objectives of the current study was to develop dental age estimation regression equation for Malaysian children aged 7 to 14 years. To derive an age estimation model, the current study investigated the correlation between chronological age and surface area of the developing root apices. Due to the high evidence of ethnic and gender variances in the previously reported 2D dental age estimation studies, the current study also investigated these predictor variables in terms of fit to the age estimation model. Furthermore, ethnic and gender based differences in the developmental patterns of the investigated teeth were also investigated.

5.4.1 Correlation between chronological age and surface area of the developing maxillary canines apices

A strong correlation (r = 0.978) was found between the chronological age and surface area of developing permanent maxillary canines' apices among Malaysian children (Malays & Chinese) aged 7 to 14 years (Table 5.1). The results in the current study showed better coefficient of correlation value (r = 0.978) as compared to the originally developed 2D Cameriere's method (r = 0.914) (Cameriere, Ferrante, & Cingolani, 2006) (Table 5.1). This could be due to the effectiveness and accuracy of CBCT image analysis in measuring developing root apices in comparison to the panoramic images employed in Cameriere's technique. However, it must be noted that the current study only assessed permanent maxillary canines in comparison to the seven left mandibular permanent teeth in Cameriere's originally developed 2D method (Cameriere, Ferrante, & Cingolani, 2006).

Table 5.1: Comparison with originally developed Cameriere 2D age estimation method

	Age estimation studies	Radiographic method employed	Coefficient of correlation values (r)
1	Cameriere et al., 2006 [56]	Cameriere 2D technique	0.914
2	Present study	3D CBCT technique	0.978

5.4.1.1 Comparison with two-dimensional radiographic Malaysian studies

Validation studies of Cameriere's 2D radiographic dental age estimation method have also been reported on Malaysian population (Alghali et al., 2016; Kumaresan et al., 2016; Cugati et al., 2015). These validation studies have resulted in a strong correlation between chronological age and 2D radiographic linear measurements of root apices using panoramic images. Even one of the Malaysian study reported that the Cameriere's method is more accurate and reliable in comparison to Demirjian's, Willems, Nolla's and Haavikko's among Malaysian children (Kumaresan et al., 2016). The current CBCT study has shown higher coefficient of correlation value (r = 0.978) in comparison to the Malaysian 2D radiographic Cameriere's validation studies reported by Cugati et al. (2015) (r = 0.933) and Alghali et al. (2016) (r = 0.892) (Table 5.2). As stated earlier, this could be attributed to the fact that the surface area measurements of developing permanent maxillary canine's apices by 3D analysis might be more accurate and precise as compared to the 2D analysis using panoramic images.

Table 5.2: Comparison with Malaysian 2D radiographic studies.

No.	Age estimation studies	Radiographic method employed	Coefficient of correlation values (r)
1	Alghali et al., 2016	Cameriere 2D technique	0.892
2	Cugati et al., 2015	Cameriere 2D technique	0.933
3	Present study	3D CBCT technique	0.978

5.4.1.2 Comparison of the strength of correlation (r) with 2D radiographic studies on other populations

The originally developed Cameriere's method (Cameriere, Ferrante, & Cingolani, 2006) was also tested on European children belonging to various parts of Europe (Croatia, UK, Germany, Italy, Kosovo, Spain, Slovenia) (Cameriere et al., 2007; Cameriere, Ferrante, Scarpino, et al., 2006). The results indicated a strong correlation between chronological age and linear measurements of developing root apices using panoramic images. However, no significant difference was reported in the strength of correlation values among various European populations. The results of the current study indicated higher strength of correlation coefficient value (r = 0.978) between chronological age and changes in the surface area of developing maxillary canines apices as compared to the European 2D radiographic study (r =0.927) (Cameriere et al., 2007).

The 2D radiographic age estimation technique developed by Cameriere's et al. (2006) was employed by other researchers to develop their own population specific age estimation models. The strength of correlation (r) values between chronological age and linear measurements of root apices were strong among various populations such as South African, Indian, Brazilian, Chinese, Saudi and German children. However, again it must be noted that the strength of correlation (r = 0.9780) found in the current study was higher in comparison to all these population specific studies (Angelakopoulos et al., 2019; Sharma et al., 2016; Rai et al., 2010; Mazzilli et al., 2018; Guo et al., 2015; AlShahrani et al., 2019; T. Halilah et al., 2018). It must be noted that although strong correlations (r) were observed, but the values among all these reported studies varied indicating that population or ethnic specific age estimation formula's should be developed. It was observed that the Chinese (r = 0.955) (Guo et al., 2015) and Brazilian (r = 0.954) (Mazzilli et al., 2018) population specific 2D radiographic studies showed highest strength of correlations followed by Indian (r =0.947) (Rai et al., 2010), German (r = 0.917) (T. Halilah et al., 2018), South African (r = 0.871) (Angelakopoulos et al., 2019) and Saudi (r = 0.795) (AlShahrani et al., 2019) populations (Table 5.3).

Table 5.3: Comparison with 2D radiographic studies reported on other populations.

	Age estimation studies	Investigated population	Radiographic method employed	Coefficient of correlation values (r)
1	AlShahrani et al., 2019	Saudi	Cameriere 2D technique	0.795
2	Angelakopoulos et al., 2019	South African	Cameriere 2D technique	0.871
3	Mazzilli et al., 2018	Brazilian	Cameriere 2D technique	0.954
4	Halilah et al., 2018	German	Cameriere 2D technique	0.917
5	Guo et al., 2014	Chinese	Cameriere 2D technique	0.955
6	Rai et al., 2010	Indian	Cameriere 2D technique	0.947
7	Cameriere et al., 2007	European	Cameriere 2D technique	0.927
8	Present study	Malaysian	3D CBCT technique	0.978

5.4.2 Ethnicity based comparison in the development of permanent maxillary canines apices with aging

The investigated Malaysian sample in the current study belonged to the same Mongoloid race but different ethnicities, namely Malay and Chinese. Previous studies have shown morphological variations in the phenotypic expression of Malays and Chinese ethnic groups. One of the objectives of the study was to investigate inter-ethnic differences in maxillary canine root maturation by testing the statistically significant difference in the correlation coefficient (r) values. Fishers Z test analysis in the current study indicated no statistically significant difference in the strength of correlation coefficient (r) values between Malays and Chinese ethnic groups, when investigated for each gender (Table 4.3). This pattern was consistent among both training (n = 191) and validation samples (n =96). Fisher Z test analysis was used in the current study because previously reported 2D radiographic studies in assessing linear measurements of root apices have only investigated the effect of ethnicity in terms of fit to the derived age estimation models.

In addition to the specific objective of investigating the strength of correlation (r) values between Malays and Chinese ethnic groups, the current study also tested the statistical difference in the mean values of canines apices surface area measurements between Malays and Chinese ethnic groups (Table 4.5). The results were similar to the Fisher Z-test analysis because no significant difference was observed in the mean values of canines apices surface area measurements between Malays and Chinese in the investigated age groups (1-8) (Table 4.5) (Figure 4.1). However, age group-wise (1-8) comparison should be investigated on a larger sample in future.

It must be noted that ethnic variations in terms of fit to the age estimation model have been investigated by different researchers using 2D Cameriere's formula (Rai et al., 2010; Mazzilli et al., 2018; Cugati et al., 2015). However, the research is limited in terms of investigating dental development variations among different ethnic populations including Malays and Chinese. This aspect should be further investigated as a study has reported significant differences in the pattern of root development among people belonging to different parts of the country in India (Rai et al., 2010). However, the results of the current study supported the fact that both Malays and Chinese belonging to the same Mongoloid ancestry showed no significant difference in the development of permanent maxillary canine root apices.

5.4.3 Gender based comparison in the development of permanent maxillary canines' apices with aging

Gender or sexual dimorphism in dental traits have been reported in many morphometric studies (Prabhu & Acharya, 2009; Schwartz & Dean, 2005; Ling & Wong, 2007). Teeth development and eruption pattern may vary due to different environmental, nutritional, ethnic and hormonal factors during sexual maturation. Fisher Z-test analysis in the current study indicated no significant difference in the correlation coefficient (r) values between genders in both ethnic groups (Table 4.4). 2D radiographic studies using Cameriere's technique for age estimation among children have not tested the significant difference in the strength of correlation (r) values among genders. Nonetheless, gender based differences between estimated age and chronological age has been reported in their respective populations by other researchers (Balla et al., 2016; Ozveren et al., 2019; Apaydin & Yasar, 2018; Fernandes et al., 2011; Alsudairi & AlQahtani, 2019; T. Halilah et al., 2018). In addition, to investigate the effect of gender predictor variable in terms of fit to the newly derived age estimation model in the current study, it was also vital to investigate the correlation coefficient (r) values between genders to better understand gender based variances.

Although no statistically significant difference was observed in the correlation coefficient (r) values between chronological age and surface area of the developing maxillary canines apex among genders but interestingly significant differences in the mean values of canines apices surface area were observed in most of the investigated age groups - particularly in the Malay ethnic group (Figure 4.2) (Table 4.6). However, the mean values of canine apices surface area measurements in 7, 9 and 10 years age groups did not show significant differences between males and females among the Chinese ethnic groups. It was observed that females showed advance or early development of maxillary canines in comparison to males in all the investigated age groups in both Malays and Chinese ethnic groups (Table 4.6) (Figure 4.2). Gender based findings were critical and may be attributed to the early growth spurt due to pubertal changes among females as compared to males. This perception was supported by the results of early apical closure of maxillary canines among females in comparison to males (Table 4.7). The 13 year old females had their root development completed in more than 55% percent of the investigated teeth among the Malays and more than 85% among the Chinese ethnic group (Table 4.7). However, among males none of the investigated teeth had their root development completed in 13 years age group. It was also observed that maxillary canines' apices development was completed in 14 years age group among both genders in each ethnic group. It must also be noted that the tooth maturation is not only affected by growth spurt or hormonal changes but also depends on nutritional and other environmental factors. Therefore, this pattern of advanced maxillary canine development among females in comparison to males may not be similar among different populations or ethnic groups due to other factors such as malnourishment of young girls among developing or underdeveloped countries (Rai et al., 2010).

5.4.4 Dental age estimation regression model for Malaysian children

One of the objectives of the current study was to develop a new and reliable dental age estimation regression equation for Malaysian children. Multiple linear regression analysis in the current study showed a strong correlation between chronological age and the predictor variables (surface area of the maxillary canine apices, ethnicity, gender and status of the root development) (Table 4.8). Moreover, the stepwise regression analysis was performed to identify those predictor variables that contributed significantly to the age estimation model. The results indicated that all the predictor variables except ethnicity, contributed significantly to the age estimation model. The R-Square (R2) value of 0.956 was observed, which indicated that 95.6 % of the variation in age can be explained by the surface area of the developing maxillary canines' apex, gender and status of the root development (open/closed apices) (Table 8). The following age estimation model was developed:

Equation 5.1: Age = 13.897 - 0.228 (apex surface area) - 0.864 (gender) + 0.905 (open/closed apex).

Variables coding: Male = 0 / Female = 1, Open apex = 0 / closed apex = 1.

The strong correlation between chronological age and surface area of maxillary canines apices indicated that the newly developed 3D technique can be employed in assessing developing roots apices for age estimation. As stated earlier, it must be noted that the current study investigated only permanent maxillary canines in comparison to mandibular seven left permanent teeth in Cameriere's 2D radiographic technique (Cameriere, Ferrante, & Cingolani, 2006). Previously reported 2D Cameriere technique on Indian (Rai et al., 2010; Sharma et al., 2016), German (T. Halilah et al., 2018), Saudi Arabian (AlShahrani et al., 2019) and Chinese (Guo et al., 2015) children have reported variations in terms of significant contribution of the type of teeth (morphological predictor) to the age estimation model. The reason for investigating only permanent maxillary canines in the current study was because it is one of the most stable, strongest and has the longest root in the human dentition. As stated earlier, any changes in the surface area of the developing maxillary canines apices can be detected more effectively in comparison to other permanent teeth. Another reason for developing single tooth age estimation model was due to the fact that previously developed multiple teeth age estimation models had limitations that if one of the teeth is missing than age estimation is not possible (Demirjian et al., 1973). However, future studies should investigate the effect of different type of teeth on the accuracy of the age estimation model using newly developed 3D image analysis technique. This will help in determining which of the single or multirooted tooth can be used with more accuracy during criminal investigations and disaster victim identification.

Ethnicity as a predictor variable in the current study did not contribute significantly to the age estimation model. Moreover, Fisher Z-test analysis and independent sample t-test indicated that there was no significant difference among ethnic groups in terms of correlation coefficient (r) values and mean surface area measurements of maxillary canine's apices (Table 4.3 & 4.5). Despite of phenotypic variations reported in the Malay and Chinese ethnicities, even the Malaysian specific age estimation formula derived by Cugati et al. (2015) based on Cameriere's method for Malaysians (Malays, Chinese and Indians) showed no significant effect of the ethnicity variable on the age estimation model (Cugati et al., 2015). These results were similar to the current study. This could be attributed to their similar origin of the Mongoloid race. Malaysia has a multi-ethnic population comprising Malays, Chinese, Indians and other ethnicities. However, limited research has been reported on the variance in teeth developmental pattern with age based on ethnicity. The investigation of ethnic based variations in terms of fit to the age estimation model was vital in the current study because population specific age estimation models are more reliable and accurate. Similar results were reported by Cameriere et al. (2007), when they investigated the effect of children belonging to different parts of Europe on the age estimation model by assessing 2D linear measurement of developing roots using panoramic images. The results also suggested that children belonging to different European countries, but same Caucasian race do not have a significant effect on the age estimation model. However, Rai et al. (2010) reported contrary results that children belonging to different parts of India have a significant effect on the age estimation model.

It must be noted that validation studies of Cameriere's age estimation formula on the Indian (Pratyusha et al., 2017), Brazilian (Mazzilli et al., 2018), Chinese (Guo et al., 2015), Saudi Arabian (AlShahrani et al., 2019) and German (T. Halilah et al., 2018) populations indicated that population specific formulas should be developed. However, a study has also reported higher accuracy of Cameriere's European formula when applied among Mexican population (De Luca et al., 2012). But the authors did mention that this could be due to abrupt increase in the migration of people of European origin in Mexico City.

Gender contributed significantly to the derived age estimation model in the current study as reported in originally developed 2D radiographic Cameriere's technique. Significant contribution of gender predictor variable to the age estimation regression equation in the current study was similar to the validation studies of Cameriere's technique among Malaysian population (Cugati et al., 2015; Alghali et al., 2016). Cugati et al. (2015) indicated that gender contributed significantly to the Malaysian specific formula. Similar results were reported by Alghali et al. (2016) in another Malaysian specific Cameriere's validation study. However, it must be noted that in both of the Malaysian specific Cameriere's validation studies, gender distribution was not equal. Cameriere's European population specific formula also indicated significant contribution of gender to the fit of the age estimation model among children (Cameriere et al., 2007). Similarly, gender dimorphism in term fit to the age estimation model based on Cameriere's 2D technique was also reported among the Saudi Arabian (AlShahrani et al., 2019), German (T. Halilah et al., 2018), Mexican (De Luca et al., 2012), South African (Angelakopoulos et al., 2019), Chinese (Guo et al., 2015), Brazilian (Mazzilli et al., 2018) and Malaysian (Cugati et al., 2015) populations. However, an Indian specific Cameriere's model did not show any significant contribution of gender to the fit of the age estimation model (Rai et al., 2010).

5.4.5 Validation of the newly derived age estimation model for Malaysian children on an independent validation sample.

One of the objectives of the study was to develop an accurate and validated dental age estimation model for Malaysian children. The age estimation model was developed on 191 training CBCT sample belonging to Malaysian children (Malays and Chinese) ranging in age from 7 to 14 years. The 3D technique developed in the current study to assess developing root apices has not been previously reported in the literature. Therefore, it was important to validate the developed model on a separate 96 CBCT samples. The mean absolute error (MAE) values were assessed to determine the reliability and applicability of the age estimation model on the Malaysian population in future (Table 4.10). The derived regression equation resulted in the MAE value of 0.30 years for the combined data of all the age-groups. Based on the ethnicity and gender, Malay males and females resulted in the MAE values of 0.298 and 0.310 for the combined data of all the age-groups. Chinese males and females resulted in the MAE values of 0.301 and 0.302 respectively. These results indicated that the derived age estimation formula is reliable and can be used as a valid method of age estimation in Malaysian children. Moreover, there were no major differences in the MAE values between genders within each ethnic group. Moreover, no obvious differences were observed in the MAE values between ethnic groups (Table 4.10).

when investigated for each age group (1-8), the resulted MAE values were in the acceptable range for their respective age groups (Table 4.10). The MAE values were based on the absolute difference between chronological and estimated age using the derived age estimation model.

Previously reported validation studies of Cameriere's 2D radiographic dental age estimation technique on Malaysian population has resulted in the mean difference between chronological and estimated age by 0.648 (Alghali et al., 2016) and 0.41 (Kumaresan et al., 2016) years (Table 5.4). This indicated that the current 3D CBCT analysis has resulted in better estimation of age in comparison to Cameriere's 2D radiographic technique among Malaysian children. Cameriere's validation studies on South African (0.718-0.769 years), Saudi Arabian (0.89 years), Brazilian (1.24 years), Indian (0.58 years), Turkish (0.35 years) populations have also resulted in higher differences between chronological and estimated age in comparisons to the results of the current study (0.30) (Angelakopoulos et al., 2019; Gulsahi et al., 2015; Balla et al., 2016; Mazzilli et al., 2018; Alsudairi & AlQahtani, 2019) (Table 5.4). However, it must be noted that German population specific age estimation Cameriere's formula has resulted in high accuracy (0.04-0.08 years) (T. Halilah et al., 2018). Interestingly, few of the validation studies of originally developed Cameriere's European formula have also resulted in a very high accuracy in estimating age among Mexican (0.00-0.10 years) (De Luca et al., 2012) and Bosnian-Herzegovian (0.02-0.09 years) (Galic et al., 2011) populations.

It must be noted that despite few of the Cameriere's validation studies reported more accuracy in estimating chronological age (Talal Halilah et al., 2018; Galic et al., 2011; De Luca et al., 2012), the strength of correlation (r) values in the originally developed Cameriere's European model and German population specific model were lower in comparison to the current CBCT study (r = 0.978) with higher standard of error estimate values resulted from multiple linear regression analysis (Table 5.3). This shows that the validation results of the current study are more reliable and statistically valid. However, future studies should investigate both 2D Cameriere's formula and the newly developed 3D CBCT age estimation formula on the same population sample to compare the accuracy and precision of both methods.

Table 5.4: Comparison of the mean absolute error (MAE) values with the 2D Cameriere validation studies

	Age estimation studies	Investigated population	Radiographic method employed	Difference between CA-EA (years)
1	Alghali et al., 2016	Malaysian	Cameriere 2D technique	0.648
2	Cugati et al., 2015	Malaysian	Cameriere 2D technique	0.41
3	AlShahrani et al., 2019	Saudi	Cameriere 2D technique	0.89
4	Angelakopoulos et al., 2019	South African	Cameriere 2D technique	0.72-0.77
5	Mazzilli et al., 2018	Brazilian	Cameriere 2D technique	1.24
6	Halilah et al., 2018	German	Cameriere 2D technique	0.04-0.08
7	De Luca et al., 2012	Mexican	Cameriere 2D technique	0.00-0.10
8	Gulsahi et al., 2015	Turkish	Cameriere 2D technique	0.35
9	Balla et al., 2016	Indian	Cameriere 2D technique	0.58
10	**Present study**	**Malaysian**	**3D CBCT technique**	**0.30**

5.5 Age estimation model for Malaysian juveniles and adults by assessing developing mandibular third molars apices

Another aim of the study was to develop dental age estimation regression equation for Malaysian juveniles and adults aged 13 to 24 years. The correlation between chronological age and surface area of the developing mandibular third molars root apices was investigated to derive an age estimation regression model. Gender and ethnicity (Malays & Chinese) based variances in the strength of correlation (r) values and in terms of fit to the age estimation model were also investigated.

5.5.1 Correlation between chronological age and surface area of developing mandibular third molars apices.

The current study is a pioneering work to investigate the correlation between chronological age and surface areas of developing mandibular third molars apices using 3D CBCT image analysis. Evidently 3D image analysis of the surface area of developing apices can provide a more accurate and reliable measurements to derive an age estimation regression formula in comparison to 2D subjective assessment of tooth maturation studies. The results of the current 3D study showed a strong correlation (r = 0.95) between chronological age and surface area of the developing mandibular third molars apices (Table 4.18). These results were in line with the 2D radiographic study originally reported by Cameriere et al. (2008) on third molars using panoramic images among Caucasian population. However, the main objective of their study was to ascertain the age of adulthood or to differentiate minors from adults by developing a threshold value or maturity index (I_{3M}) (Cameriere et al., 2008). The current study aimed to develop an age estimation regression model that can be used among Malaysian (Malays and Chinese) juveniles and young adults ranging in age from 13 to 24 years. The reason for this approach was to develop a reliable model that can be used on a larger age range (13-24 years) instead of focusing only to ascertain the age of adulthood. Moreover, the newly developed age estimation model can also be used to ascertain the age of adulthood (18 years). Recently, we developed a threshold value of 9.25 mm^2 to differentiate juveniles from adults among Malay population using the same 3D CBCT technique that we developed in the current study (M K Asif et al., 2019) (Appendix C). The results of the study indicated that if the mandibular third molars apices surface area is below 9.25 mm^2, then it is more likely that the person is 18 years of age or above, irrespective of gender. However, the threshold value has not been validated yet on a separate CBCT data. It must be noted that in some countries the age of adulthood is not 18 years of age such as in

Saudi Arabia, Indonesia, United Kingdom and Kuwait. Therefore, it is better to develop an age estimation technique or regression formula that is applicable on a larger age range.

Each country has their own laws to implement legal justice system based on their religion, cultural values and socio-economic demographics. Malaysia's 60 percent population is below 30 years of age and child offenses has increased in frequency and severity over the last few decades. The principal act governing the handling of children in conflict with the law is the Child Act 2001, which became the part of the Malaysian justice system in August 2002 ("Laws of Malaysia. The commissioner of law devision. (2006). Act 611. Child Act 2001. Incorporating all amendments upto 1st January 2006"). The extra protection provided by the act includes privacy of the offender child or juvenile, the trial shall be in closed court (in camera). Only certain specified persons are allowed to attend the trial. It also contains provisions to protect the child from associating with adult offenders in prison or elsewhere. Moreover, the sentences or punishments to children are different than those offenders above 18 years of age. This newly developed model can therefore be used effectively to assist in criminal investigations when juvenile versus adult disputes occur. Additionally, girls below 16 years of age are not allowed to have sexual intercourse, with or without their consent. It is considered rape in the Malaysian law and can be sentenced to 30 years imprisonment.

Despite of few differences in the aims of the Cameriere's 2D radiographic (Cameriere et al., 2008) and the newly developed current 3D technique for age estimation, the results of both studies indicated that the developmental changes in the mandibular third molars root apices can be used effectively for dental age estimation. Cameriere's 2D radiographic technique was effectively applied on Egyptian (Amal A. El-Bakary et al., 2019), Chinese (Chu et al., 2018), Brazilian (Deitos et al., 2015; Nobrega et al., 2019), French (Ribier et al., 2020; Tafrount et al., 2019), Sardinian (Spinas et al., 2018), Polish (Rozylo-Kalinowska et al., 2018), Kosowar (Kelmendi et al., 2018), Dutch (Boyacioglu et al., 2018), Saudi (S. AlQahtani et al., 2017), Serbian (K. Zelic et al., 2016), Croatian (Galic et al., 2015) and Albanian (Cameriere, Santoro, et al., 2014) populations. Most of these studies showed good applicability of Cameriere's 2D radiographic technique with variances in the sensitivity (the proportion of individuals being 18 years of age or older whose test is positive) and specificity (the proportion of individuals younger than 18 whose test is negative) test values. It is important to note that different populations had varying sensitivity and specificity test values.

No validation study of Cameriere's third molar maturity index has been reported on Malaysian population. However, Johan et al. (2012) reported 0.843 correlation value (r) between chronological age and third molars development using Demirjian's stages (A-H) of tooth development among Northeast Malaysian population ranging in age from 14 to 25 years. The correlation value ($r = 0.843$) in their study was lower than what observed in the current study ($r = 0.95$).

5.5.2 Ethnicity based comparison in the development of mandibular third molars apices with aging

The present study investigated difference in the strength of correlation coefficient (r) values between Malays and Chinese ethnic groups by employing Fisher Z test analysis (Table 4.13). The results of Fishers Z test showed no statistically significant difference in the strength of correlation coefficient (r) values between Malays and Chinese ethnic groups, when investigated for each gender separately (Table 4.13). This pattern was consistent among both training ($n = 128$) and validation samples ($n = 55$). To the best of our knowledge, no previous 2D radiographic dental age estimation study has investigated and compared the strength of correlation values between Malays and Chinese ethnic groups. This aspect was important to investigate because 2D radiographic studies on third molars have shown variances in the developmental pattern among different ethnicities

(Liversidge, 2008; Solari & Abramovitch, 2002; Kasper et al., 2009; Uys et al., 2018; Blankenship et al., 2007). A study even reported 8 to 18 months of advance development of third molars among Hispanic population in comparison to American Caucasians (Kasper et al., 2009). However, no research has been reported on Malaysian population in assessing variance in third molars maturation based on different ethnic groups.

In addition to the Fisher Z test, independent samples t-test analysis was also used to assess the significant difference in the mean values of third molars apices surface area measurements between Malays and Chinese ethnic groups (Table 4.15). The results were in line with the Fisher Z test analysis, as no significant difference in the mean values between Malays and Chinese ethnic groups was observed in majority of the investigated age groups (1-6) when investigated for each gender separately (Table 4.15) (Figure 4.3). However, among females, the age groups 13-14, 19-20 and 23-24 years showed significant difference.

These results were similar to what we observed for permanent maxillary canines in the current study as no statistically significant difference in the strength of correlation (r) values and mean values in the surface area measurements were observed between Malays and Chinese ethnic groups. As stated earlier this could be due to their affiliation to the same Mongoloid race.

Investigating Malays and Chinese ethnic variance in terms of the strength of correlation coefficient (r) values, surface area measurements of root apices and in terms of fit to the age estimation model in the current study was important because variances in the accuracy of Cameriere's third molar maturation index (I_{3M}) have been reported by many researchers among different populations. A validation study of Cameriere's third molar maturation index (I_{3M}) on Chinese population indicated delayed development of roots in comparison to Caucasian population (Chu et al., 2018). Similarly, Cameriere's third molar maturity index showed less sensitivity (51.7%) among Saudi population (S. AlQahtani et al., 2017). The recently reported meta-analysis evaluated the effectiveness and accuracy of Cameriere's third molar maturation index (I_{3M}) < 0.08 among different populations and 16 studies were analyzed (Santiago et al., 2018). The percentage of correctly classified studied population ranged from 72.4 to 96.0%, which shows wide range of variability among different populations around the world.

5.5.3 Gender based comparison in the development of mandibular third molars apices with aging

The current study also investigated statistically significant difference in the strength of correlation coefficient (r) values between chronological age and surface area of the developing mandibular third molars apices among males and females using Fisher Z test analysis. The result indicated no statistically significant difference in the strength of correlation coefficient (r) values between males and females, when investigated for each ethnic group in both training and validation samples (Table 4.14). No previous 2D radiographic dental age estimation study has compared the strength of correlation between chronological age and developmental pattern of third molars among genders. Most of the 2D radiographic studies have only investigated gender variance in terms of fit to the age estimation models.

The results of the independent samples t-test analysis, which was employed to assess the significant difference in the mean values of mandibular third molars apices surface area between male and female showed no significant difference in majority of the age groups (1-6) (Table 4.16) (Figure 4.4). However, among Malays, the age group 13-14 and 23-24 years showed significant difference in the mean values. It must be stated here that despite of showing some variances in the developmental pattern of third molars, the previously reported 2D radiographic studies indicated no statistically significant difference between genders among Caucasians (Cameriere et al., 2008), Chinese (Chu et al., 2018), Brazilian (Nobrega et al., 2019), French (Tafrount et al., 2019), Libyan (Dardouri et al., 2016) and Dutch (Boyacioglu et al., 2018) populations.

The mandibular third molars roots apical closure were also compared between genders for each ethnic group in 19-20, 21-22 and 23-24 years age groups (Table 4.17). In 23-24 years age group, Malay males had their root development completed in all of the investigated third molars. However, more than 65% of third molars had open apices among Malay females in 23-24 years age group. This indicates that third molar development among Malay males is advanced in comparison to females. This could be due to the fact that by the time third molars develop, the growth spurt among males is at its peak in comparison to females. Interestingly, no difference was observed in the mandibular third molars root apical closure between Chinese male and females in the investigated age groups (Table 4.17). The results of the independent samples t-test also supported these results as the mean values of Malay males were significantly different from Malay females in an age group of 23-24 years age group (Table 4.16). 2D radiographic studies have also reported slightly faster development of third molars among male as compared to female in Egyptian (Amal A. El-Bakary et al., 2019), Serbian (K. Zelic et al., 2016), Hispanic (Solari & Abramovitch, 2002), Saudi (S. AlQahtani et al., 2017), southern Chinese (Wang et al., 2019), Sardinian (Spinas et al., 2018) and Albanian (Cameriere, Santoro, et al., 2014) populations. Similar results were also reported in a Malaysian population (Johan et al., 2012).

Although, gender based results in terms of the strength of correlation (r) values and mean surface area measurements did not show significant difference, it is preferable that this aspect should be investigated on a larger sample in each age group (1-6) to better understand the differences among genders. It must be noted that investigating age group-wise comparison between genders in terms of the apices surface area measurement was the secondary objective of the current study as the primary objective was to investigate the difference in the strength of correlation (r) values.

5.5.5 Dental age estimation regression model for Malaysian juveniles and young adults

Another aim of the study was to develop a novel and validated dental age estimation regression formula for Malaysian juveniles and young adults. Multiple linear regression analysis showed strong correlation (r = 0.95, SD = 1.144) between chronological age and the predictor variables (surface area of the mandibular third molars apices, ethnicity, gender and status of the root development) (Table 4.18). The R-Square (R2) value (0.896) indicated that 89.6% of the variation in age can be explained by surface area of the mandibular third molars apices, ethnicity, gender and status of the root development (open/closed apices). Stepwise regression analysis indicated that all the predictor variables contributed significantly to the age estimation model (Table 4.19), resulting in the following linear regression formula:

Equation 5.2: Age = 21.396 - 0.137 (surface area) + 2.662 (open/closed apex) – 0.618 (ethnicity) + 0.415 (gender).

Variables coding: Open apex = 0 / closed apex = 1, Malay ethnicity = 0 / Chinese ethnicity = 1, Male = 0 / Female = 1.

Despite of showing no significant differences in the strength of correlation coefficient (r) values and mean values of mandibular third molars apices surface area measurements between Malays and Chinese ethnic groups, stepwise regression analysis was performed to remove any bias in assessing the effect of ethnic variations on the dental age estimation model (Table 4.13 & 4.15). The results indicated that ethnicity contributed significantly to the age estimation model (Table 4.19).

Gender predictor variable contributed significantly to the age estimation model in the current study. Gender dependent result was in contrary to the 2D radiographic study originally developed by Cameriere et al. (2008) in assessing developing third molars apices among Caucasians juveniles and young adults. Other researchers also reported gender independent results among Chinese (Chu et al., 2018), Brazilian (Nobrega et al., 2019), French (Tafrount et al., 2019), Libyan (Dardouri et al., 2016), Kosovar (Kelmendi et al., 2018), black American (Cavric et al., 2016) and Dutch (Boyacioglu et al., 2018) populations. These conflicting results could be attributed to the fact that all these studies have employed 2D radiographic imaging modalities for assessing developing root apices. It must be reiterated here that limitations such as unequal magnification and distortion of the anatomical structures are associated with panoramic images. The current study is the first study to investigate the developing root apices using 3D image analyses for age estimation which is considered more reliable and accurate in comparison to 2D imaging modalities. However, future studies need to further investigate this aspect by employing 3D imaging modalities.

5.5.5 Validation of the newly derived age estimation model for Malaysian juveniles and young adults on an independent validation sample

It is vital in a modern scientific world to test the applicability of any newly developed regression equation on a separate validation sample. The current study developed an age estimation model by analyzing 128 CBCT training sample belonging to Malaysian juveniles and young adults aged 13-24 years. A separate 55 validation CBCT sample was selected to test the reliability and accuracy of the derived model. Mean absolute error (MAE) values were calculated by subtracting estimated age from the chronological age. The results indicated the MAE value of 0.82 years for the combined data of all the age-groups (Table 4.20). Based on the ethnicity and gender, Malay females showed better MAE value (0.75) in comparison to males (0.90). However, contrasting results were observed among Chinese ethnic group, where males showed better MAE value (0.73) in comparison to females (0.87). Moreover, MAE values for each age group ranged from 0.586 to 1.075, which is considered an acceptable range in forensic sciences. The validation results indicated that this newly developed model for age estimation can be used effectively in the future among Malaysian (Malays and Chinese) juveniles and young adults.

Third molars are the only developing teeth in sub adults and young adults. Thus, this newly developed 3D image analysis of developing root apices can provide a useful method to estimate age among Malaysian juveniles and young adults, and thereby rendering a significant assistance in the criminal investigations and administration of law, and also furtherance of the justice system.

To date no study has been reported on the validation of Cameriere's radiographic age estimation method by employing third molars among Malaysian population. However, dental age estimation using Demirjian's third molars staging radiographic method has been tested among Northeast Malaysian population (Johan et al., 2012). The study reported mean prediction error in the observed and chronological age by 1.14 among males and 0.94 among females.

5.6 Limitations of the study

i. The current study employed CBCT images with 0.30 mm voxel size which may have caused the partial volume effect (PVE) (Vannier et al., 1997). To nullify this unfavorable effect, 'multiple slice editing phase' was used to manually cross check that the software had accurately selected the calcified developing root apices. This was important because in a few instances the software was not able to accurately demarcate the developing root from the surrounding bone. Maret et al. (2012) also reported that segmentation of a tooth is difficult using CBCT images acquired with larger voxel size. It must be noted that CBCT images acquired with smaller voxel size may give sharp images, but caution must be expressed here as it may be at the cost of higher radiation exposure and increase image noise (Al-Rawi et al., 2010).

ii. The study was designed to select the CBCT images from the database stored at the Oral and Maxillofacial Imaging Division, Faculty of Dentistry University of Malaya. The exclusion of any pathology or previous traumatic history associated with the investigated teeth was merely based on the assessment of CBCT images and there was no direct contact of the researcher with the patients.

iii. Ethnicity (Malays and Chinese) based sample distribution and allocation of the CBCT images was based on the registered names of the patients at the time of scanning. Thus, this could have resulted in a biased sample distribution in few cases due to the increased trend of inter-marriages amongst various ethnicities in Malaysia.

CHAPTER 6: CONCLUSION

6.1 Introduction

The study was designed to develop a new dental age estimation method for Malaysians using CBCT scanned data. Age estimation models were developed for Malaysian children aged 7 to 14 years and Malaysian juveniles and young adults aged 13 to 24 years by investigating the relationship between chronological age and surface area of permanent maxillary canines and mandibular third molars apices. Moreover, variances based on ethnicity, gender and status of the root development (open/closed) were also investigated. Following are the research outcomes, clinical applications and recommendations for future studies.

6.2 Research outcomes

6.2.1 Age estimation model for Malaysian children by assessing developing maxillary canines apices

(1) A strong correlation (r = 0.978) was observed between chronological age and surface area of the developing maxillary canines apices. Thus, CBCT imaging modality can be used effectively among Malaysian children for assessing the surface area of developing permanent maxillary canines apices for dental age estimation.

(2) The results indicated no significant difference in the strength of correlation coefficient (r) values between Malays and Chinese ethnic groups. Moreover, based on each age-group (1-8) no significant difference was observed in the mean values of maxillary canines apices surface area measurements between ethnic groups. This supports the fact that both Malays and Chinese belonged to the same Mongoloid ancestry and did not show significant variations in the maxillary canine's apices development.

(3) The results showed no significant difference in the strength of correlation coefficient (r) values between males and females. However, when investigated for each age-group (1-8) a significant difference was observed in maxillary canines apices surface area measurements between genders in both ethnic groups. Females showed advanced development of maxillary canines apices as compared to males among Malaysian Children.

(4) A new dental age estimation model was developed by investigating the relationship between chronological age and the predictor variables (surface area of the canine's apex, ethnicity, gender and status of the root development). All the predictor variables except ethnicity, contributed significantly to the newly developed age estimation model. The R-square (R2) value indicated that 95.6 % of the variation in age can be explained by the surface area of the canines apices, gender and status of the root development (open/closed apices). Thus, ethnicity independent new dental age estimation regression model was developed for Malaysian children.

(5) When the newly developed age estimation model was applied on a separate Malaysian validation sample, the results indicated that derived age estimation model is reliable and can be applied for age estimation among Malaysian children in future.

6.2.2 Age estimation model for Malaysian juveniles and young adults by assessing developing mandibular third molars apices

(1) A strong `correlation ($r = 0.95$) was observed between chronological age and surface area of the developing mandibular third molars apices. 3D CBCT image analyses of mandibular third molars apices can be used effectively for dental age estimation among Malaysian juveniles and young adults.

(2) Malays and Chinese ethnic variations based on the strength of correlation values were not statistically significant in the current study. Moreover, no significant difference in the

surface area measurements of mandibular third molars apices was observed between Malays and Chinese in majority of the investigated age groups (1-6). However, among females, the age groups 13-14, 19-20 and 23-24 years showed significant difference in the mean values between Malays and Chinese ethnic groups. Thus, it can be concluded that despite few variations, the pattern of development of mandibular third molars apices are similar among Malays and Chinese juveniles and young adults.

(3) The results indicated that no significant difference was observed in the correlation coefficient (r) values between genders. Moreover, no significant difference was observed in terms of surface area measurements of mandibular third molars apices between males and females in majority of the investigated age groups (1-6) in both ethnic groups. However, Malay males showed faster development of mandibular third molars in 13-14 and 23-24 years age groups compared to females. The roots apical closure among Malay males was faster in comparison to females.

(4) A new dental age estimation regression model was developed for Malaysian juveniles and young adults. 89.6% of the variation in age was explained by the surface area of developing mandibular third molars apices, ethnicity, gender and status of the root development (open/closed apices). All the predictor variables contributed significantly to the newly developed age estimation model.

(5) Validation results of the Mean absolute error (MAE) values indicated that derived age estimation model is reliable and can be applied on Malaysian juveniles and young adults in future.

6.3 Clinical applications

(1) The newly developed Malaysian population specific dental age estimation models can be used with more accuracy among Malaysian children, juveniles and young adults. It is more logical to derive a regression equation for age estimation by assessing teeth

maturation using 3D imaging modality (CBCT) instead of using 2D radiographs (panoramic images) which have been employed in the past. The need to develop a simple, accurate and reliable age estimation method is on the rise due to the abrupt increase in illegal immigrants, armed conflicts and poverty leading to human trafficking. The current study would pave a new approach for the worldwide researchers in an attempt to develop a more reliable and accurate 3D CBCT technique for dental age estimation.

(2) The newly developed age estimation models can assist in criminal investigations and implementing the legal justice system in Malaysia. Third molars are the only developing teeth in sub adults and young adults, so the newly developed model can also be used to differentiate juveniles from adults.

(3) The 3D image analysis technique developed in the current study can be used effectively for age estimation and identification of victims in mass disasters such as tsunami, earthquake and aviation disasters. The CBCT image analysis employed in the current study is less invasive and the newly developed age estimation model can be more effective and applicable in the developing and underdeveloped countries, where DNA profiling and other advance biological profiling methods are not available.

(4) CBCT images were used in the current study to nullify the limitations of 2D radiographic linear measurements in assessing developing root apices. CBCT is cost effective as compared to other 3D advanced imaging modalities. Small field of view (FOV), higher voxel resolution, rapid scan time and beam limitations are the evolving features of CBCT technology which makes it suitable for clinical and research studies. Moreover, with the introduction of collimator cassettes and field size-defining blades in the CBCT systems has further limited the extent of the X-ray beam. Therefore, employing 3D imaging modality such as CBCT to assess root apices development can further improve the accuracy and reliability of dental age estimation.

6.4 Recommendations for future research

(1) The newly developed 3D dental age estimation technique and models should be tested on other populations or ethnic groups to further assess its accuracy and reliability. If required, then population specific age estimation models should be developed for their respective populations. This aspect needs to be investigated because previous studies reported ethnic based variations in different dental age estimation methods.

(2) The current study used permanent maxillary canines and mandibular third molars to develop dental age estimation models for Malaysian children, juveniles and young adults. Future studies should investigate the relationship of chronological age and surface area of the developing teeth by employing different types of single and multi-rooted teeth. Another type of teeth may possibly result in a more accurate and reliable age estimation model (or equation). Moreover, multiple teeth assessment may improve the accuracy of the age estimation.

(3) CBCT images with voxel resolution of 0.30 mm were selected in the current study which could have resulted in PVE. This could be the reason why MIMICS software was not able to detect minute structural details in few investigated teeth. Therefore, 'multiple slice editing phase' was employed to overcome the PVE of large voxel size and allows accurate selection of the calcified tooth structures. Therefore, future studies should investigate the effect of CBCT scans performed with different voxel sizes on the 3D surface area analysis of developing root apices. Future studies should also investigate the influence of various other scanning parameters of different CBCT systems on the strength of correlation (r) values and accuracy in age estimation models. Moreover, the overall procedure of converting DICOM files to the Mimics and 3-matics software, thresholding, multiple slice segmentation, region growing, curve creation and 3D reconstruction took approximately 2.5 to 3 hours per tooth. This duration of image processing and analyses

can be improved with further advancements in the CBCT technology and 3D image analyses software.

(4) Future studies should compare the accuracy of the newly developed 3D image analysis method for assessing developing root apices between CBCT and CT imaging modalities. Developing age estimation model using CT scans may prove to be effective in disaster victim identifications as mortuaries are increasingly equipped with medical-imaging X-ray CT.

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