AGE ESTIMATION BASED ON

TOOTH PULP VOLUME USING CBCT IMAGES

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AGE ESTIMATION BASED ON

TOOTH PULP VOLUME USING CBCT IMAGES

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LIST OF ABBREVIATIONS

2D	Two-dimensional
3D	Three-dimensional
ACR	American College of Radiology
BC	Before Christ
CBCT	Cone Beam Computed Tomography
micro-CT	micro-Computed Tomography
CVM	Cervical Vertebrae Maturation
DICOM	Digital Imaging and Communications in Medicine
FOV	Field of View
MRI	Magnetic Resonance Imaging
NEMA	National Electrical Manufacturers Association
NIFTI	Neuroimaging Informatics Technology Initiative
PET	Positron-Emission Tomography
ROI	Region of Interest
SEE	Standard Error of Estimate
SD	Standard Deviation
SPECT	Single-Photon Emission Computed Tomography
WHO	World Health Organization

ESTIMASI USIA BERDASARKAN VOLUME PULPA GIGI MENGGUNAKAN RADIOGRAF CBCT

ABSTRAK

Kajian terdahulu mengenai estimasi usia hanya dapat dilakukan pada usia 20 tahun kerana parameter pengukuran dilakukan dengan melihat pertumbuhan dan perkembangan gigi secara morfologi dari radiograf 2D. Pertumbuhan dan perkembangan ruang pulpa gigi akan berkurang ukurannya mengikut pertambahan usia dan perubahan ini dapat diperhatikan menggunakan radiograf 3D seperti CBCT. Volume ruang pulpa dapat menjadi parameter pengukuran dalam menentukan usia. Daripada pengetahuan penulis, belum pernah dilakukan kajian mengenai pengukuran volume ruang pulpa gigi untuk estimasi usia pada populasi subras Deutero-Melayu. Tujuan umum daripada kajian ini adalah untuk mengevaluasi, mengembangkan, dan mengoptimumkan radiologi dentomaksilofasial menggunakan radiograf CBCT untuk estimasi usia dari volume pulpa gigi akar tunggal dan berbilang akar mengikut perbezaan jenis kelamin pada populasi subras Deutero-Melayu. Kajian *cross-sectional* dilakukan untuk menganalisa volume pulpa gigi dari CBCT (Vatech, Korea) dan software ITK-SNAP 3.6.0, regresi non-linier/logaritmik untuk menganalisa hubungan antara perubahan penurunan volume pulpa pada gigi akar tunggal dan berbilang akar serta model matematik untuk menghasilkan formula estimasi usia. Kajian ini menggunakan data radiograf CBCT dari 16 jenis gigi di populasi subras Deutero-Melayu yang dipilih berdasarkan kriteria inklusi iaitu gigi tanpa karies, tanpa kalsifikasi pulpa, tanpa patologi periapikal, tanpa kelainan bentuk dan ukuran, tanpa restorasi gigi, tanpa artifak kerana bahan restorasi logam pada di gigi yang berdekatan,

kelihatan secara jelas daripada imbasan CBCT, pada pesakit dengan usia kronologis dari 10 hingga 61 tahun dan tanpa rawatan saluran akar. Total sampel yang digunakan berjumlah 5760 data CBCT. Kajian ini dilakukan di Unit Radiologi Hospital Pergigian Universitas Padjadjaran, Bandung Indonesia dan Pusat Pengajian Sains Pergigian Universiti Sains Malaysia, Kelantan. Untuk setiap hubungan antara pengurangan volume pulpa gigi dengan penambahan usia dapat dianalisa dari rumusan regresi; $y\ (age) = a \ln x\ (pulp\ volume) + b$. Setiap gigi memiliki rumusan yang berbeza. Kesimpulan hasil kajian ini adalah terdapat perbezaan yang signifikan antara volume pulpa gigi lelaki dan wanita, sehingga rumus juga diperoleh untuk memperkirakan usia antara perbezaan jenis kelamin. Pada gigi berakar tunggal, yang paling disarankan untuk estimasi usia adalah gigi seri lateral maksila, sedangkan yang paling tidak disarankan adalah gigi taring pada kelompok lelaki dan wanita. Pada gigi berbilang akar, geraham pertama rahang bawah adalah yang paling disarankan untuk estimasi usia pada lelaki dan wanita, sedangkan yang paling tidak disarankan adalah geraham ke-tiga rahang atas. Tren penurunan volume pulpa pada gigi berakar tunggal dan berbilang akar perlu dianalisa sebelum estimasi usia dilakukan untuk menilai apakah volume pulpa berada dalam kondisi normal atau tidak. Daripada pengetahuan penulis, ini adalah kajian pertama yang menentukan persamaan perkiraan usia dari volume pulpa berdasarkan populasi subras Deutero-Melayu yang menggunakan CBCT sebagai usaha mengembangkan dan mengoptimalkan radiograf 3D. Oleh karena itu, hasil novel dari kajian ini dapat diterapkan di masa depan sebagai rujukan untuk meramal usia manusia dengan lebih mengoptimumkan lagi data dentomaksilofasial radiologis dari CBCT untuk perkiraan usia dalam populasi subras Deutero-Melayu.

AGE ESTIMATION BASED ON TOOTH PULP VOLUME USING CBCT IMAGES

ABSTRACT

Earlier studies on age estimation were unable to accurately estimate the age of over 20 years of age because they only examined tooth growth and development morphology from 2D radiographs as measurement parameters. In terms of growth and development, the tooth pulp volume will decrease in size over the years and this change can only be seen using 3D radiographs like CBCT. This study proposes that the tooth pulp volume can be used as a measurement parameter in determining age. As far as the author is aware, no study has been conducted regarding the measurement of tooth pulp volume for age estimation in the Deutero-Malay subrace. The general objectives of this study were to evaluate, develop, and optimize dentomaxillofacial radiology using CBCT images for age estimation via tooth pulp volume examination in single-rooted and multi-rooted teeth by gender in the Deutero-Malay subrace. A cross-sectional study was conducted to analyse tooth pulp volume using CBCT (Vatech, Korea) and ITK-SNAP 3.6.0 software. In addition, non-linear/logarithmic regression to examine the correlation between pulp volume decrease in single and multi-rooted teeth and mathematical models for producing the formula for age estimation. This study used CBCT data obtained from 16 types of teeth in selected Deutero-Malay subrace population. The inclusion criteria of the teeth were: without caries, pulpal calcification, and periapical pathology; the shape and size were normal without restorations; without artifacts from metal dental material; visible in detail from CBCT scans; from patients with the chronological age of 10 to 61 years old; and

without root canal treatment. In total, 5760 CBCT data were selected. The study was conducted at the Radiology Unit of the Dental Hospital at Universitas Padjadjaran, Bandung Indonesia and Pusat Pengajian Sains Pergigian Universiti Sains Malaysia, Kelantan. Correlation between tooth pulp volume decrease and the increase of could be observed from the regression equation: $y\,(age) = a \ln x\,(pulp\ volume) + b$. It was found that each tooth had a different equation. This difference in the equations of each tooth might be due to anatomic differences in the pulp volume. The conclusions of this study showed that there was a significant difference between the male and female tooth pulp volumes. Therefore, equations were obtained to estimate the age between the genders. In single-rooted teeth, in both male and female groups, maxillary lateral incisors were the most recommended, while canines were the least recommended. In multi-rooted teeth, in both male and female groups, mandibular first molar was the most recommended for age estimation, while the least recommended was the maxillary third molar. Examination of decreasing pulp volume trend in single and multi-rooted teeth needed to be done before age estimation, even though the pulp chamber showed no development of pathological disturbances. As far as the author is aware, this is the first study to determine the age estimation equation from the tooth pulp volume based on the Deutero-Malay subrace population using CBCT as an attempt to develop and optimize 3D radiography. Thus, the novel result of this study could be applied in the future as a reference for identifying the human age for further to optimize the dentomaxillofacial radiology data from CBCT for age estimation in the Deutero-Malay subrace population.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Age estimation is very useful in human identification because tooth is known as the strongest part of the human body. Furthermore, teeth can withstand physical and chemical stresses evident in cases of natural disasters like earthquakes and tsunami. Aside from DNA and fingerprint, teeth examination plays an imperative role in forensic odontology to recognize or identify unknown dead bodies. Hence, it is necessary for practitioners to agree on a precise and consistent method of tooth age estimation (Clark, 1994; Lain *et al*., 2003; Hinchliffe, 2011).

Age estimation can be performed by observing the change in teeth growth and development on radiographs (Mincer *et al*., 1993; Willems *et al*., 2001). The diverse physiological age-associated alterations in the tooth can complicate the age estimation process. Thus, this method can only be carried out after permanent teeth have completely emerged (Solheim, 1993; Yang *et al*., 2006).

Morphological degenerative changes by age factors are morphologically in the form of the reduced number of blood vessels and nerves and the form of pulp space shrinkage due to the gradual formation of secondary dentin which lasts a lifetime could affect the dental pulp volume (McKenna *et al*., 2002). The reduction in tooth pulp volume occurs owing to continual deposition of secondary dentine in the pulp cavity after tooth eruption which causes the size of the pulp cavity to gradually decrease with

age (Star *et al.*, 2011; Tardivo *et al*., 2011). In recent studies on age estimation, dentinal deposition has become the main focus. The estimation of age from radiographic imaging assessment of tooth pulp volume is distinctive because it is related to the characteristics of secondary dentine apposition. Previous studies have described that dentine deposition is responsible for the decrease in tooth pulp volume (Kvaal *et al*., 1995; Drusini *et al*., 1997; Kolltveit *et al*., 1998; Cameriere *et al*, 2004; Yang *et al*., 2006; Meinl *et al*., 2007; Someda *et al.*, 2009; Aboshi *et al*., 2010; Maret *et al*., 2011; Jagannathan *et al*., 2011; Ge *et al*., 2015 and 2016; Gulsahi *et al*., 2018; Asif *et al*., 2018). So far, there has been no study in Indonesia regarding age estimation using tooth pulp volume analysis.

Dentomaxillofacial radiology can determine the human age and is one of the basic identification tools in forensic science, anthropology, archaeology, medicine and dentistry (Panchbhai, 2011; Bunyarit *et al*., 2019). In measuring secondary dentine deposition, many kinds of radiographic methods can be applied and the three-dimensional (3D) radiographic methods are generally more preferable than the two-dimensional (2D) ones (Someda *et al*., 2009; Aboshi *et al*., 2010; Agematsu *et al*., 2010; Star *et al*., 2011; Sakuma *et al*., 2013).

The first methodology that applied radiograph to estimate age was established by Kvaal *et al*. (1995). The study utilized periapical radiographs to measure the lengths of the tooth, pulp, and root. In 2005, panoramic radiographs became popular and widely utilized (Saxena, 2011; Thevissen *et al*., 2012; Erbudak *et al*., 2012; Limdiwala and Shah, 2013; Wiederhold and Riva, 2013). In recent years, various softwares have been introduced for dentomaxillofacial imaging studies with the advancement of

digital imaging modalities. The most commonly utilized softwares are Image J, Mimics, ITK SNAP, Osirix, and Amira (Karkhanis *et al*., 2014; Misirlioglu *et al*., 2014; Mittal *et al*., 2016; Marroquin *et al*., 2017).

Cone-Beam Computed Tomography (CBCT) imaging is an important tool in the field of forensic odontology due to its advanced computer technology incorporated with rapid scan time, low dose and image accuracy (Scarfe and Farman, 2008; Patcas *et al*., 2012). CBCT can give information about oral and maxillofacial structure in 3D. The resolution of CBCT images is dependent on the acquired voxel resolutions. CBCT's smaller size of field of view (FOV) and fewer frame images, compared to medical CT, are among the factors that can reduce the scanning time and in turn reduce the radiation exposure for patients (Alaei and Spezi, 2015).

Several studies have been conducted to estimate age based on tooth pulp volume, for instance by Sakuma *et al*. (2013) in the Japanese population, Jagannathan *et al*. (2011) in the Indian population, Queiroz *et al*. (2016) in the Brazilian population and Ge *et al*. (2016) in Chinese populations.

In Malaysia, a study was conducted on dental age estimation for Malaysian Chinese children and adolescents using Chaillet and Demirjian's methods (Bunyarit *et al*., 2019). In another study, Asif *et al*. (2019) examined the ratio of pulp volume/tooth volume using CBCT in Malaysian adults.

Similarly, in Indonesia, Amiroh *et al*. (2017) utilized a modified form of Demirjian's ten-stage method to compare two teeth regression equations. Hidayati *et al*. (2017)

conducted a study on age identification using a panoramic radiograph with the Thevissen method in Indonesian population.

The population in a country may consist of various heterogeneous origins. The total population of Malay race worldwide was 340 million individuals. In the context of Indonesia, Deutero-Malay subrace is the ancestors of the Austronesian people in the nation. They came to Indonesia during the second wave, after Proto-Malay United around 400-300 BC. This subrace then successfully integrated their culture with its predecessor's (Proto-Malay). Indonesian tribes descended from the original Deutero-Malay subrace are the Acehnese, Javanese, Sundanese, Minangkabau, Riau, Tamiang, Deli Malays, Jambi, Bengkulu, Palembang, Makassar, Bali, Sasak, and Bugis tribes (Anwar, 2011).

So far, studies in various parts of the world were conducted within the country-approach population. However, studies conducted on homogeneous populations in very limited subraces, especially for the Deutero-Malay subrace, are still lacking. In Indonesia, as far as the author is aware, there is no study of standard value for age estimation derived from tooth pulp volume in Deutero-Malay subrace.

Indonesia is very prone to natural disasters due to the collisions of three large continental plates, i.e Eurasian, Indo-Australian, and Pacific. Age identification is required as a legal tool to determine the age of humans, particularly in deciding whether the individual is legally a child or an adult (Putri *et al.*, 2013). In criminal cases, the victim's and perpetrators' ages also need to be identified. Hence, it is important to develop accurate age identification methods.

Therefore, the purposes of this study were to evaluate, develop, and optimize dentomaxillofacial radiology using CBCT images for estimation of age estimation via tooth pulp volume examination by gender in single-rooted and multi-rooted teeth in Deutero-Malay subrace.

1.2 Gap statement and justification of the study

Previous studies on age estimation were limited by their use of 2D radiographs, such as intraoral and panoramic, which made it difficult to accurately estimate the age of over 20 years. In addition, these studies have not utilized open-source software and their samples in the world were heterogeneous. Hence, the present study aimed to evaluate, develop, and optimize the age estimation by gender from tooth pulp volume in single-rooted and multi-rooted teeth for a homogenous population, i.e. the Deutero-Malay subrace in Indonesia, using 3D radiograph CBCT images with ITK-SNAP open-source software.

1.3 Objectives of the study

1.3.1 General objectives

The general objectives of this study were to evaluate, develop, and optimize dentomaxillofacial radiology using CBCT images for age estimation by gender using the examination of tooth pulp volume in single-rooted and multi-rooted teeth in Deutero-Malay subrace.

1.3.2 Specific objectives

The specific objectives of this study were:

1. To determine the pulp volume in single-rooted and multi-rooted teeth using ITK SNAP software from CBCT data by gender.
2. To develop the regression equation for age estimation from the pulp volume of single-rooted teeth by gender.
3. To develop the regression equation for age estimation from the pulp volume of multi-rooted teeth by gender.
4. To evaluate the relationship between the pulp volume in single-rooted teeth with chronological age by gender.
5. To evaluate the relationship between the pulp volume in multi-rooted teeth with chronological age by gender.
6. To assess the trend of pulp volume decrease in single-rooted teeth by gender.
7. To assess the trend of pulp volume decrease in multi-rooted teeth by gender.

1.4 Benefits of the study

Theoretically, this study is significant in its determination of the standard reference data for age estimation based on pulp volume in single and multi-rooted teeth images in the Deutero-Malay subrace. Furthermore, the study portrayed the trend in pulp volume decrease in single and multi-rooted teeth as a basis for age estimation. The practical benefit of this study is to estimate the age population in the Deutero-Malay subrace for dentomaxillofacial radiologists, forensic odontologists, and dentists.

1.5 Study hypothesis

This study hypothesized that the data obtained from CBCT could be used for age estimation and that there was a correlation between age and tooth pulp volume.

CHAPTER 2

LITERATURE REVIEW

2.1 Methods of age estimation

Normal individual anatomical and physiological development is very closely related to age. Several methods for determining age have been developed, e.g. using chronological age, biological age, bone age, dental age, and psychological age. Chronological age is the simplest method for determining the age, in which age is calculated based on the time the individual was born as documented on birth certificates, government data, and hospital records (Vodanović, 2011; Al-Qahtani, Hector, and Liversidge, 2014).

Dental age estimation is one of several physiological development measurements. It is based on permanent tooth growth (Rai *et al.*, 2014). Teeth are vital in determining chronological age because their development lacks variability. The reasons teeth are the focus of age estimation are that they start to develop in the early embryonic period (Al-Qahtani, Hector, and Liversidge., 2014), that they are the hardest part of the body structure, and they can withstand any conditions. Moreover, the age estimates, as well as the data provided by the chronology of tooth development, are more reliable than that provided by bone development (Patel *et al.,* 2015). Because of all these characteristics, human teeth are frequently used, with the support of an orderly anatomical and radiological examination to estimate age. Nutritional and hormonal status in a human does not have much effect on tooth maturity. The process of tooth

maturity is more stable to analyse than the eruption process, which is influenced by space and systemic factors (McKenna *et al*. 2002).

There are various techniques for dental-based age estimation, depending on the age range of the individual. Age estimates carried out until the age of 24 is based on the eruption sequence and the stages of tooth development. After an individual reaches the phase of adult tooth growth and development, it will be difficult to estimate the age because so far, there has been no reliable method to do so. For the determination of age in adults, two criteria can be used, namely the assessment of tooth pulp volume and the development of the third molar (Panchbhai, 2011). However, after the eruption of the third molar is completed, age determination in adults is still difficult to identify (Biuki *et al*., 2017).

Age estimation may help in various investigations, whether it is for legal purposes or medical purposes, by narrowing the field. For instance, it can help in the identification of a body. Age estimation also helps in determining the age of perpetrators of crimes, which will determine the penalties for the committed crime. It is especially important in cases in which the perpetrator is underage (Rai *et al.*, 2016). Dental age estimation is a very important practice for forensic science and clinical interests. In forensic science, identification of the age of alive or dead individuals are useful for various cases, including in criminal law and marriage law. Unclear or dubious data regarding one's birth causes an issue in age determination, and dental age estimation can help in such cases. It also helps in physiological age determination. Located between the parts of the body, the teeth are most resistant to decay. This makes teeth very useful in

forensic archaeology and anthropology to determine the age of decaying remains or skeletons (Maret *et al*., 2011).

Saunders (1837) was the first to introduce the correlation between human teeth and chronological age assessment (Stavrianos, 2008). Priyadarshini *et al*. (2015) mentioned that there were three methods for estimation of human age (adults and children) commonly in practice up to now, i.e. morphological, biochemical and radiographic methods. The morphological methods were based on the assessment of teeth (ex-vivo). Hence, these methods required teeth extraction for microscopic preparation. However, these methods may not be accepted due to ethical, religious, cultural, or scientific reasons.

The principle of the biochemical method is that the levels of D-aspartic acid in human enamel, dentine, and cementum increase with age. However, due to the limitations of this method, such as damage for the extracted teeth and its structures, it is considered impractical (Priyadarshini *et al*., 2015).

2.1.1 Radiological method of two and three dimensional

In 1896, a year after Roentgen discovered x-rays, radiology was first used in forensic science to show imaging of lead bullets on a victim's head (Eckert and Garland, 1984). The results of the radiograph are displayed in a negative form in the form of a radioopaque object which is the result of a process that occurs on a sensor that captures x-rays. The radiograph results are more accurate than what has seen visually. Radiographs have advantages, one of which is a more reliable form of dental recording

than dental records (Forrest, 2012). These methods are used for determining age easily, quickly, economically, non-damaging parts of the body and can be used to identify people who are dead and still alive (Vandevoort *et al*., 2004).

The radiographs of the cranium, face, long bones and tooth have been mostly used for forensic identification (Marques *et al*., 2014). Radiological approaches are commonly used in dental age estimation. A 2D radiograph has a projection which is subjected to considerable magnification and distortion errors. This method is based on the use of periapical and orthopanoramic two-dimensional radiography (Alexander and Foote, 1998). In line with this, Jagannathan *et al*., (2011) mentioned that the results of 2D radiographs had considerable magnification and distortion errors and therefore they recommended the mesiodistal and buccolingual assessment of tooth dimensions instead.

Dental age estimation using radiology method was first published in a study by Demirjian *et al*. (1973). As it developed, the method then was based on the correlation of age and changes that occurred in permanent teeth (Solheim, 1993). In previous studies, measurements of dental pulp area and the proportion of secondary deposits of dentine were done with the aid of conventional and digital 2D images, including dental and panoramic radiographs. The reason for this was because the method did not damage any body parts (Murray *et al*., 2002) since it did not involve teeth extraction (Priyadarshini *et al*., 2015).

In previous studies, the determination of age based on the correlation between age and height ratio and tooth width and dental pulp volume has been done with digital

radiographs (Prapanpoch *et al*., 1992; Kolltveit *et al*., 1998; Paewinsky *et al*., 2005; Meinl *et al*., 2007). In other studies, 2D radiographic for measuring age morphology based on ratios of teeth height and width correlation and tooth pulp area have been reported by Kvaal *et al*. (1995), Drusini *et al*. (1997), and similarly, Limdiwala *et al*. (2013) who used panoramic radiograph. In another study, Cameriere *et al.* (2006) utilized the same method to examine an apical foramen in roots. They found that tooth length did not correlate significantly with age on 2D dental radiographs. A similar study by Khorate *et al*. (2014) was done in the Indian population using a panoramic radiograph. In a study by Verochana *et al*. (2016), an equation of age estimation based on regression analysis from lower third molar development in a Thai population was reported.

Based on the previous studies, 2D dental radiographs have been successfully used to estimate tooth age by using the ratios between the pulpal and root size and between the pulpal and tooth size. However, the age was estimated based on the comparison of lengths, widths, and areas in 2D and this was a limitation of these studies, compared to other studies that used 3D measurement methods.

Detailed measurements of the structure of the tooth can only be obtained from 3D images and this was made possible by micro-computed tomography (micro-CT) imaging (Vandevoort *et al*., 2004; Someda *et al*., 2009) and Vandevoort *et al.* (2004) was the first reported about this study. Micro-CT, which was introduced in the early 20th century has been used to determine age-related three-dimensional changes in the maxillary pulp cavity first of premolar teeth. The results of this study were in line with the beginning histological studies, that decreased pulp volume was not linear (Oi *et*

al., 2004). Micro-CT has been accepted as the reference standard in providing an accurate and precise assessment of volumetric measurements in the oral maxillofacial area, especially of the tooth structure (Peters *et al.*, 2000; Wang *et al*., 2011; Ge *et al*., 2015). In the other study, Someda *et al*. (2009) mentioned that a study by micro-CT on 155 mandibular central incisors in the Japanese population for volume analysis.

Previous studies have compared CBCT and micro-CT measurement results and found that although the micro-CT provided accurate and precise measurements, the average difference between the two was very small (Vandevoort *et al*., 2004).

A previous study by Aboshi *et al*. (2010) mentioned that micro-CT on lower premolar teeth provided significant results for age estimation using multiple regression analysis. These mandibular premolar teeth were chosen because they rarely suffered damage compared to anterior teeth and their structure was simpler than other posterior teeth (Maret *et al*., 2011).

Kvaal *et al*. (1995) conducted a study on upper and lower teeth to compare pulp volume and tooth volume, in which teeth and pulp volume were calculated for mandibular and maxillary teeth. It produced an equation for age determination (Kvaal *et al*., 1995). A similar study was conducted by Someda *et al*. (2009), in which they measured the volumes of 3D structures of the tooth and produced formula regression analysis for age estimation.

However, micro-CT is rarely used for routine dental examinations and if it is, micro-CT involved tooth extraction (Peters *et al.*, 2000; Vandevoort *et al*., 2004; Olejniczak *et al.*, 2007; Someda *et al*., 2009).

In a recent study, the measurement of 3D for teeth images can be developed from people who are still alive (Scarfe *et al*., 2006; Dawood *et al*., 2009). CBCT plays important role in this procedure by helping in unidentified skull digitization and craniofacial model formation (Lee *et al*., 2012). Cameriere *et al*. (2007) mentioned that the CBCT measurement of volume for dental age estimation would be a first choice method.

Recent studies note that CBCT was used for age estimation by calculating the volume of the teeth and pulp chamber. Since then, volumetric reconstruction of CBCT with different software to estimate the dental age of adults has been reported in various studies (Someda *et al*., 2009). The previous study of Jagannathan *et al*. (2011) used CBCT to assess the comparison of pulp volume to tooth volume of lower canines for age estimation in an Indian population. Another study by Pinchi *et al*. (2015) concluded that the study of pulp volume reduction using CBCT can be a useful tool for forensic age estimation in adult.

Asif *et al*. (2018) conducted a study to produce Malay-specific regression equation for age determination using the field of view on apical area lower third molar. As mentioned by Yang *et al*. (2006), who first reported the use of CBCT for age estimation, the new development of CBCT has allowed for finding dental structure in the 3D image with better quality and less radiation. The CBCT systems were designed specifically for dentomaxillofacial imaging. The acquisition of age estimation may offer real potential for exploiting and analyzing volumetric measurements of teeth (De Vos *et al.* 2009).

So far, the periapical radiographs and the orthopantomography used would only demonstrate the real morphological measurements in a restricted way. However, 3D images provide a more accurate measure of the tooth pulp volume and the potential for developing age estimation methods.

2.1.2 The age estimation method based on pulp volume using CBCT radiograph

Dental age estimation with the use of non-invasive methods has become the potential method, initiated by the use of 2D radiography such as periapical and panoramic and currently developed into the use of CBCT technology. CBCT is a technology in dentomaxillofacial radiology that was originally developed for angiography in the United States of America in 1982 and subsequently applied for maxillofacial imaging. The introduction of dentomaxillofacial CBCT scanners in the late 1990s has led to an explosion of interest in these devices in the field of dentistry (Dessai *et al*. 2014). CBCT uses four forms of "cone". In principle, CBCT uses multiplanar projections obtained by rotation scanning to produce volumetric data from the image. The CBCT can not be used for patients who cannot stand or sit still, who are always on the move, or who have mental retardation and are unable to follow instructions during the exposure process (Kailash, 2014). CBCT is used to identify and assess root canals, the variation of the root system, root fracture and resorption because it provides good spatial resolution. CBCT can also provide an accurate evaluation of tooth pulp volumes (Ge *et al*., 2015; Pinchi *et al*., 2015).

The potential use of CBCT has long been acknowledged by odontology forensics, pathologists and anthropologists. CBCT technology significantly helps the process of

forensic investigation because it allows identification of identity, trauma, foreign objects, and certain disease quickly and easily without having to involve an invasive procedure (Taylor and Kieser, 2016). CBCT is a non-invasive technique, which is better compared to other techniques used in forensic dentistry (Yang *et al*., 2006). CBCT imaging can fulfil the needs of cases that require more information, more than traditional methods can, especially from 3D information. CBCT in the future will prove to be a great tool for forensic dentistry (Jawaid *et al*. 2014). The method of age estimation from CBCT may become useful in the future for legally sentenced individuals who are indeed above 50 years and whose age cannot be verified by any legal documents (Asif *et al*. 2019).

A previous study by Jagannathan *et al*. (2011) mentioned that age determination can be done based on tooth pulp volume size from CBCT radiographs. However, it used a tooth pulp ratio but did not take into account tooth wear so that the results of the study were inaccurate. Another study on age determination based on comparison of pulp to tooth volume by Someda *et al*. (2009) also explained that significant association was reduced by the various structures of the teeth from CBCT radiographs (Someda *et al*., 2009). Furthermore, the structure of the tooth pulp volume is like dentine but less density and not like enamel. Therefore the exclusion of enamel may be suggested for age estimation analysis that uses pulp volume to tooth volume comparison to obtain higher validity. Besides, studies have mentioned that the secondary dentine directly correlates with tooth pulp volume decrease, whereas decreases in artificial tooth-like enamel attrition are not significantly related.

Limdiwala *et al.* (2013) mentioned that the tooth length observed in CBCT radiographs did not correlate significantly with age. In another study, Asif *et al*. (2018) recommended that for reducing the effects of varying tooth size, people should only use the size of the tooth pulp volume from CBCT radiographs because the size of the tooth pulp volume is protected in the tooth, so it is less likely to be influenced by factors that affect the shape of the tooth.

The previous study by Ge *et al.* (2016) was done on 13 teeth. This study was conducted in the Chinese population of 240 CBCT radiographs aged 16-63 years by measuring the pulp volume and root canals. The results of 13 teeth showed that the tooth pulp volume of upper second molars has a significant correlation with chronological age in all gender group samples and while canines have the smallest correlated coefficient with chronological age. It is assumed that this is affected by the function of the second molar tooth is mill food and canines is ripping food. Single-rooted teeth are counted to the root apex while multiple rooted teeth are counted only in the pulp chamber. The volumes obtained were then analyzed using logarithmic regression analysis to gain the equation for age estimation based on pulp volume (Ge *et al*., 2015 and 2016). The previous study that used only the pulp volume from CBCT radiographs was conducted by Ge *et al*. (2015). They mentioned that dentine deposition is significantly correlated with decreasing tooth pulp volume but this does not occur in enamel because the surface undergoes changes caused by attrition. Therefore, the ratio of pulp volume and tooth volume does not provide accurate results when compared with pulp volume analysis alone. The accuracy of segmentation in the pulp volume is more accurate because the contrast of density between dentine and pulp chamber is very noticeable.

In a further study, Ge *et al*. (2016) reaffirmed the results of their study based on research conducted on thirteen anterior and posterior teeth in the Chinese population.

Kumar *et al*. (2016) also examined the measurement of age estimates of the volume of pulp chambers in first molar teeth by looking at radiographic images of CBCT in the Indian population using ITK-SNAP 2.4.0. The sample size used was 130 males and 190 females. The method used in the study was the linear regression analysis. The age range in the study population was 10-70 years with the exclusion criteria for teeth with no caries, teeth with no restoration, teeth that did not use crowns and bridges, teeth in good condition and teeth that were not endodontic treatment without pulp calcification. The results show the coefficient of determination (R^2) is the maxillary male $R^2 = 0.782$ and the mandible $R^2 = 0.763$ while the maxillary females $R^2 = 0.705$ and the mandible $R^2 = 0.580$. Therefore, it can be concluded that the pulp volume can determine the chronological age of humans in the Indian population.

The previous study by Marroquin *et al*. (2017) mentioned that the tooth length was not significantly correlated with age increase because it was influenced by attrition from enamel, bruxism and bad chewing habits, but the estimation of age was more significantly correlated with the physiological process of ageing. However, for predicting age, the best method is to measure pulp volume and analyze its correlation with chronological age in 3D like CBCT. This conclusion was made to avoid the presence of external variables that could interfere with the accuracy of the measurement results such as changes in the tooth enamel surface due to attrition, caries and other pathological abnormalities.

2.2 Age estimation based on secondary dentin deposition

Dental age estimation is one of several measurements of physiological development based on permanent tooth growth. The early study on age estimation was based on tooth development. The first method was published by Schour and Masseler, (1941). However, age estimates are carried out only below the age of 24 years based on the time of the eruption and the phase of tooth development and the age determination in adults after the eruption of the third molar is still a problem.

Other studies based on morphological methods were done by Gustafson (1950), Dalitz (1962), Bang and Ramm (1970), Johanson (1971), Maples (1978), Solheim (1993), and Priyadarshini *et al*., (2015). However, cases of age estimation from morphological changes such as stages of tooth eruption, erosion and abrasion rates are considered to be susceptible for errors because their contents were not always accurate.

The biochemical method using aspartic acid was used to be an age estimating solution. This method is recommended for the racemization of amino acids using aspartic acid. Ritz *et al*., (2000) used the method of racemization with dentinal biopsy in living individuals. This method was useful for determining age but destructive. Although the results were accurate, this method needed high standards in the procedure and damage the teeth (Priyadarshini *et al*., 2015).

In recent studies on age estimation, dentinal deposition has become the main focus. The estimation of age from radiographic imaging assessment of tooth pulp volume is distinctive because it is related to the characteristics of secondary dentine apposition. Previous studies have described that dentine deposition is responsible for the decrease

in tooth pulp volume (Kvaal *et al*., 1995; Drusini *et al*., 1997; Kolltveit *et al*., 1998; Cameriere *et al*, 2004; Yang *et al*., 2006; Meinl *et al*., 2007; Someda *et al.*, 2009; Aboshi *et al*., 2010; Maret *et al*., 2011; Jagannathan *et al*., 2011; Gulsahi *et al*., 2018; Ge *et al*., 2015, 2016; Asif *et al*., 2018). Dentine deposition starts from the crown of the tooth and spreads to the apex of the root area for teeth that function normally. The dentine apposition was also more common in the pulp wall of the molars than other teeth, including canines (Ge *et al.*, 2015).

The study argues that this type of dentin is called physiological secondary dentine, which occurs because of the ageing process. The formation of secondary dentin starts from the apical area and then spreads to the coronal area as for teeth affected by traumatic injury. Secondary dentine deposition is responsible for the reduction in the volume of the pulp cavity with age. Secondary dentine deposition is initiated after odontogenesis and develops after root completion. Odontoblast lining the pulp chamber continually forms a secondary dentine layer which deposited along with the pulp chamber. The deposition of dentinal matrix follows the circular pattern of tubules and produce layer which causes size reduction of the pulp chamber, this continuous process caused asymmetrical pulp shape and pulp obliteration due to deposition of matrix throughout life (Meinl *et al*., 2007).

The old odontoblast cells, also called resting odontoblasts, continue to form secondary dentine after the primary dentin is formed. The formation of dentin was slow because the odontoblast has been decreased secretory function (Couve and Schmachtenberg, 2016). Odontoblast cells secrete primary dentin at a speed of 4 µm/day, while the rate

of formation of secondary dentine is 10 times slower (Stavrianos *et al*., 2008; Goldberg *et al*., 2011).

Secondary dentine deposition was physiologically stimulated by mastication (Goldberg *et al*., 2011). The formation of secondary dentine may be caused by attrition, abrasion, erosion, caries, changes in osmotic pressure throughout the pulp chamber or ageing (Philippas, 1961; Bang, 1989; Solheim, 1992; Berkovitz, *et al*., 1992) and decreases the volume of the dental pulp chamber. The form of dentine formation was different for each tooth, in the maxillary anterior, the largest dentine deposition lies on the palatine wall of the pulp cavity in the incisal point. In molar teeth, the largest deposition occurs based on the pulp chamber, slightly on the occlusal and lateral walls (Meinl *et al*., 2007).

Changes in the volume of the pulp space in the teeth are considered to be predictors of tooth age. Although the secondary dentin apposition is not homogeneously distributed over all the walls of the pulp cavity and even differs with the type of tooth examined, buccolingual and mesiodistal pulp width and pulp cavity height decrease with ageing (Morse *et al*., 1991; Oi *et al.*, 2004).

Degenerative changes by the ageing of pulp tissue can occur histologically and morphologically. Degenerative changes by age factors were morphologically in the form of a reduction in the number of blood vessels and nerves, as well as shrinkage of the pulp space due to the gradual formation of secondary dentin which lasts a lifetime could affect the dental pulp volume (McKenna *et al*., 2002).

So far, the age determination after the eruption of the third molar is still a problem, cases of age estimation from morphological changes contents are not always accurate, the biochemical method needs high standards in the procedures and secondary dentine deposition is responsible for the reduction in the volume of the pulp cavity with age. Therefore, dentinal deposition from radiographic evaluation of pulp volume becomes the focus for age estimation study.

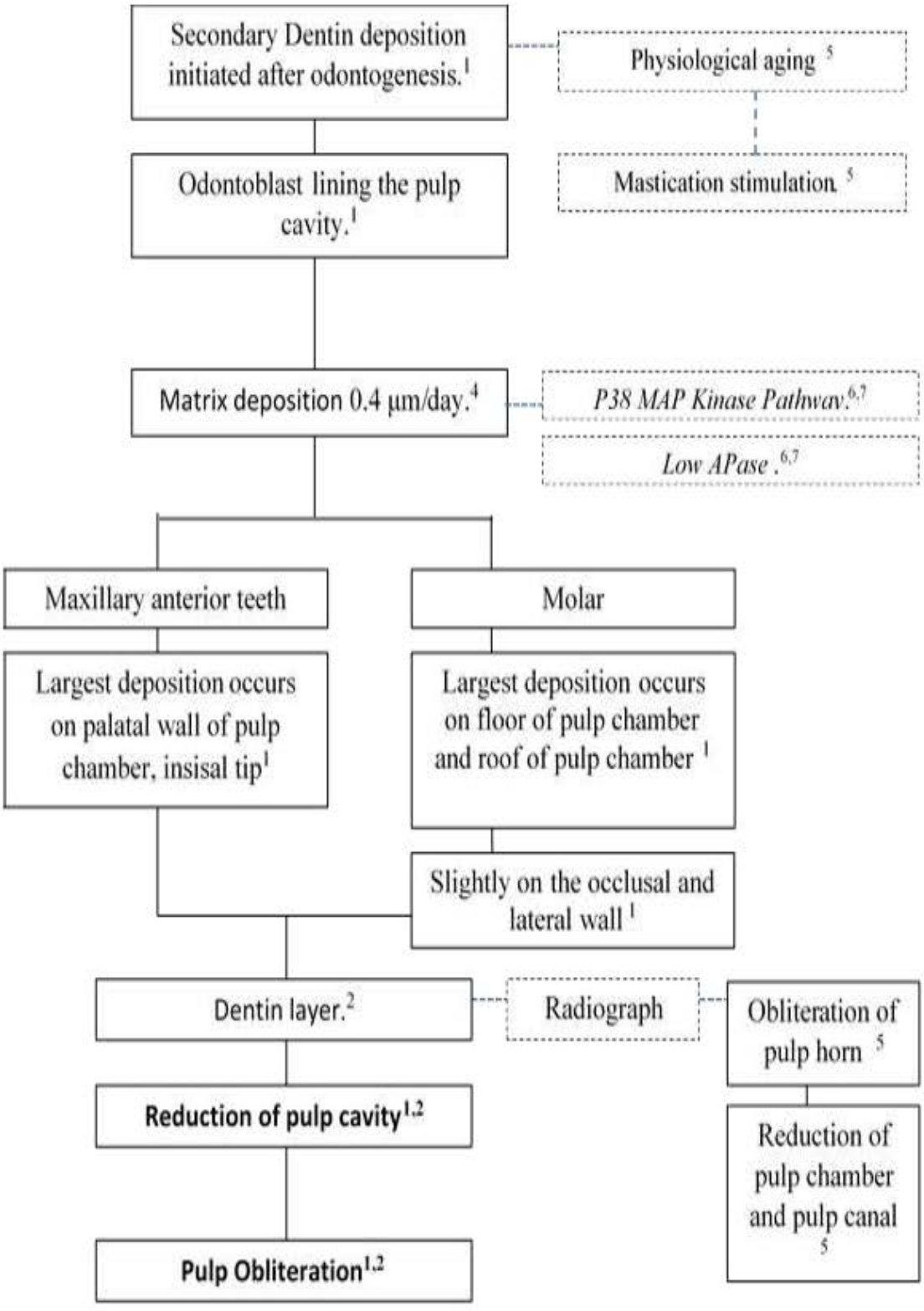

Figure 2.1 Secondary dentine deposition

2.3 The number and type of teeth for age estimation on the previous studies

The studies to determine the age of the teeth in the future must be carried out on all teeth simultaneously to get more accurate results (Yang *et al*., 2006; Cameriere *et al*., 2007).

Previous studies for age estimation were done only on one tooth type, such as Jagannathan *et al.* (2011) on 140 the mandibular canines, Pinchi *et al.* (2015) on 148 the upper left central incisors, Porto *et al.* (2015) on 118 the upper central incisors, De Angelis *et al.* (2015) on 91 the maxillary right canine, Tardivo *et al.* (2011 and 2014) on 101 and 840 the canines respectively, Sakuma *et al.* (2013) on 136 the mandibular first premolars, and Someda *et al.* (2009) on 158 the mandibular central incisors.

Besides, studies conducted on two types of teeth were done by Agematsu *et al.* (2010) on 147 the lower central incisors and 110 the lower second premolars, Aboshi *et al.* (2010) on 100 mandibular first and second premolars and Ge *et al.* (2015) on 373 maxillary first molar and 372 mandibular first molars.

In other studies by Vandevoort *et al.* (2004) on 43 single-rooted teeth and Yang *et al*. (2006) on 28 single-rooted teeth, namely incisors, canines, and premolars. The more recent study done by Ge *et al*. (2016) was done on 13 types of teeth with a total number of 3120 teeth. Pinchi *et al*. (2015) mentioned that the age estimation in future studies should be carried out and the prosecution of the research is ongoing on larger with larger sample size and with even distribution of age.