RADIOGRAPHIC ANALYSES OF SKELETAL AND DENTAL PARAMETERS FOR AGE ESTIMATION IN MALAYSIAN POPULATION

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by

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LIST OF ABBREVIATIONS

ANCOVA	Analysis of covariance
ANOVA	Analysis of Variance
AutoCAD	Automated Computer Aided Design
BA	Biological age
CA	Chronological Age
CBCT	Cone beam computed tomography
CBFS	Combined bone fusion score
CHS	Combined hair score
CI	Confidence Interval
CSS	Combined Skin Score
CT	Computed tomography
DA	Dental Age
DICOM	Digital Imaging and Communications in Medicine
DNA	Deoxyribonucleic acid
HUSM	Hospital Universiti Sains Malaysia
ICC	Intraclass Correlation Coefficient
INTERPOL	International Criminal Police Organization
IPS	Institut Pengajian Siswazah
JEPeM	Jawatankuasa Etika Penyelidikan Manusia
MC	Mandibular canal
MF	Mental foramen
MH17	Malaysia Airlines Flight 17
MicroCT	Microcomputed tomography
MLR	Multiple linear regression
MRI	Magnetic resonance imaging
NRIC	National Registration Identity Card
PDL	Periodontal ligaments
PMCT	Postmortem Computed Tomography
RPV	Root pulp visibility
SD	Standard deviation
SEE	Standard error of estimate

SE	Standard error
SPSS	Statistical Package for the Social Sciences
UK	United Kingdom
UKM	Universiti Kebangsaan Malaysia
USA	United States of America
USM	Universiti Sains Malaysia
VIF	Variance inflation factor

LIST OF APPENDICES

ANALISIS RADIOGRAF PARAMETER TULANG DAN GIGI UNTUK ANGGARAN UMUR DALAM POPULASI MALAYSIA

ABSTRAK

Matlamat penyelidikan ini adalah untuk menghasilkan model ramalan baharu bagi julat anggaran umur antara 17-30 tahun bagi kedua-dua jantina dalam kalangan penduduk Malaysia. Ini dilakukan dengan melihat kepada parameter oral (keterlihatan pulpa akar, keterlihatan ligamen periodontal, foramen mental dan lokasi saluran mandibular) dan peringkat kematangan tulang rawan epifisis klavikular medial. Kajian ini merupakan kajian perbandingan keratan rentas dalam kalangan penduduk Malaysia yang hadir ke klinik di Hospital Universiti Kebangsaan Malaysia dan Universiti Sains Malaysia. Darjah keterlihatan pulpa akar dan ligamen periodontal menunjukkan perbezaan umur yang signifikan terutamanya bagi peringkat 0 dalam kumpulan etnik yang terlibat dalam kajian ini bagi lelaki dan perempuan. Pengukuran antara foramen mental kanan dan kiri, foramen mental hingga sempadan bawah rahang bawah didapati menunjukkan perbezaan purata yang signifikan antara kumpulan etnik selepas diselaraskan mengikut umur dan jantina, Terdapat juga perbezaan yang ketara dalam purata apabila mengukur jarak dari garis tengah ke foramen mental kanan antara lelaki dan perempuan selepas diselaraskan mengikut umur dan kumpulan etnik. Pengukuran dari saluran mandibular kanan dan kiri ke sempadan bawah rahang bawah, menunjukkan perbezaan purata yang ketara antara kumpulan etnik selepas diselaraskan mengikut umur dan jantina, nilai $p < 0.001$. Model ramalan umur menggunakan gabungan pembolehubah rahang bawah, pulpa, dan darjah penglihatan ligamen periodontal menunjukkan $R^2 = 0.561$. Ini bermaksud bahawa model akhir menerangkan 56.1% daripada jumlah varians umur.

Oleh itu, model tersebut dianggap mempunyai kesesuaian sederhana. Sampel radiograf dada (390) menunjukkan perbezaan umur purata yang signifikan di antara lelaki dan perempuan, nilai $p = 0.017$ dengan menggunakan ujian-t bebas. Terdapat perbezaan umur purata yang signifikan antara kumpulan etnik, nilai $p < 0.001$ dengan menggunakan ujian ANOVA sehala. Bagi model ramalan umur menggunakan gabungan klavikular medial kanan, ANOVA menunjukkan $R^2 = 0.767$. Ini bermaksud bahawa model akhir menerangkan 76.7% daripada jumlah varians umur. Oleh itu, model itu dianggap mempunyai kesesuaian yang sangat baik. Penggunaan kaedah radiologi secara multifaktorial memberikan hasil analisa yang baik dalam tempoh masa dan teknik yang cepat. Radiografi dada digital adalah alat yang berguna untuk meramalkan umur.

RADIOGRAPHIC ANALYSES OF SKELETAL AND DENTAL PARAMETERS FOR AGE ESTIMATION IN MALAYSIAN POPULATION

ABSTRACT

To formulate a new prediction model for the age estimation range between 17-30 years old for both genders in the Malaysian population using oral parameters (root pulp visibility, periodontal ligament visibility, mental foramen and mandibular canal location) and stages of ossification of the medial clavicular epiphyseal cartilage. The study was a cross-sectional comparative study among the Malaysian population attending the clinic in the hospital set up at the Universiti Kebangsaan Malaysia and Universiti Sains Malaysia. Root pulp and periodontal ligament visibility showed a significant mean difference of age, mainly for stage 0 in the ethnic groups involved in the study between males and females. Measurements between right and left mental foramina and mental foramen to the inferior border of the mandible found a significant difference in mean between the ethnic groups after being adjusted for age and sex. There was also a significant difference in mean when measuring the distance from the midline to the right mental foramen between males and females after being adjusted for age and ethnic groups. Measurements from the right and left mandibular canal to the inferior border of the mandible found that there was a significant difference of mean between the ethnic groups after adjusted for age and sex, *p*-value < 0.001. The age prediction model using a combination of mandible variables, pulp, periodontal ligament visibility, mental foramen, and mandibular canal location showed $R^2 = 0.561$. Hence, the model is considered to have a medium fit. Chest radiograph samples (390) showed a significant mean age difference between males and females, *p*-value = 0.017, using an independent t-test.

Furthermore, there was a significant mean age difference between the ethnic groups, p-value < 0.001, by using a one-way ANOVA test. As for the age prediction model using right medial clavicle union, the ANOVA showed $R^2 = 0.767$. This indicates that the final model explains 76.7% of the total variance in age. Hence, the model is considered to have an excellent fit. Using multi-factorial radiological methods showed good results while consuming less time and effort. Digital chest radiographs were a useful tool for predicting age.

CHAPTER 1

INTRODUCTION

Background of the study

1.1 Age estimation in Forensic Dentistry

Forensic Dentistry is a branch of dentistry where the forensic odontologist investigates dental evidence and properly assesses and presents dental findings in the interest of a court of law (Petro, 2013). The roles of forensic odontologists include human identification, bite mark evidence evaluation, dental trauma, fraud, and dental profiling. Forensic odontologists play a vital role in mass disasters when DNA and fingerprints cannot be assessed (Prasad et al., 2012). According to INTERPOL standards, the human identification process utilises reconstructive and comparative analysis steps, whether involving a crime such as the OP Wang Kelian mass grave of alleged human trafficking victims or a disaster such as in air disaster MH17 (Gosling & Ayres, 2015).

In reconstructive identification, one of the aims of forensic odontologists is to provide evidence for identification processes at the time of the death by estimating age (INTERPOL). The estimated age at death facilitates the collation of antemortem records. The identity can be confirmed once both antemortem and postmortem records are available for comparison in the comparative analysis step. In addition, to facilitate searching antemortem dental records, age estimation can help to discriminate the identity between siblings who are involved in the same disaster. For example, in arson cases where individuals are siblings, the deoxyribonucleic acid (DNA) method can successfully identify the maternal lineage. However, in order to discriminate against these siblings, dental age estimation may help determine the elder and younger brothers.

This complementary interdisciplinary teamwork ensures success in the investigation. Furthermore, dental age estimation is of great value since teeth and jaws are resistant to postmortem decomposition changes and environmental factors (Stavrianos et al., 2008).

In Malaysia, every newborn child must be registered, and birth registrations and identity cards are routinely issued and lawfully enforced (Cheong & Baltazar, 2021). However, in scenarios where the deceased is unknown or beyond recognition, birth certificates and identity cards may not be available with the body. Thus, forensic profiling and identification must resort to biological means.

The primary role of age estimation in forensics is to build a biological profile for the skeletal remnants that may assist in the identification process (reconstructive profile). Dental age estimation methods could be assessed either during the development of dentition or after the dentition is fully formed. Moreover, the most frequent methods for estimating dental age can be performed either clinically, radiographically, or histologically (Nayak et al., 2014).

1.2 Biological age estimation methods

Biological age estimation (BA) is a method used to predict age by tracking or measuring changes in size in a particular biomarker, for instance, tooth development and bone formation, or by measuring the degree of molecular and cellular ageing over time (Jia et al., 2017; Waaijer et al., 2012).

1.2.1 Bone age estimation

The commonest method of BA is by utilising the hand and wrist, either by a radiographic technique, such as the posterior-anterior view or by a non-radiographic technique, such as ultrasonography (Mughal et al., 2014). The hand and wrist age estimation methods are limited to the age of 18. Furthermore, iliac and femoral bones can also be used to estimate bone age. However, they may not be as promising or accurate as the hand and wrist methods (Mughal et al., 2014; Schmidt et al., 2011).

One of the earliest age estimation methods is using cranial suture closure. With the progression of age, cranial sutures tend to be more fused, with some differences in closure ratio and patterns (Todd & Lyon, 1925). Khandare et al. (2015) used Gustafson's method in which the closure of cranial sutures as an age estimation method was used on the Indian population. The authors reported that total closure of sagittal suture occurred between 61 and 65 years old, 56 to 60 years for coronal sutures, 66 to 70 years for lambdoid sutures, and 66 to 70 years for the temporo-parietal suture.

1.2.2 Dental age estimation

Dental age estimation can be achieved either by morpho-histological method, radiographic method, or biochemical method (Limdiwala and Shah, 2013). The most promising method was the radiological method for estimating children and young adult's age by measuring the extent of dental development. This method is not invasive and can be used for both living and deceased persons (Verma et al., 2019).

Radiographic techniques are easy, simple, and comparatively less time-consuming when used for identification. One of the main age estimation methods in young individuals is the eruption of the third molars, which can be viewed radiographically to validate the ages between 18 and 21 years old (Timme et al., 2017). In addition, complete root formation and closure of apical foramina for the wisdom teeth can also be detected radiographically at the age of 20-23 years old (Olze et al., 2010a).

There are several publications on dental age estimation for the Malaysian population, which cut across different stages of third molar development and other teeth. Johan et al. (2012) assessed third molar development in the Malaysian population by formulating age prediction models. The study, which included Malay and Chinese ethnic groups, had produced an age estimation formula utilising third molar development. Another study, however, was limited only to the Malay ethnic groups between the ages of 4-24 years old and produced an age estimation formula utilising the third molar developmental stages and the developing mandibular permanent teeth (Yusof et al., 2014).

Several other publications related to Malaysians focus on much younger age group by evaluating the reliability and validity of Demirjian, Williams, Nollas, Haavikko, and Cameriere methods of age estimation on Malaysian children 5-15 years old (Kumaresan et al., 2016). The applicability of Demirjian and Willem's method on juvenile Malays has been tested as well (Mani et al., 2008). Cugati et al. (2015) developed an age estimation formula for Malaysian children aged between 5-16 years old across three ethnic groups (Chinese, Malay and Indian) by using Cameriere's method of assessing the mandibular permanent teeth open apices.

The applicability of third molar developmental stages for estimating age is restricted to the age of 21 years old. This leaves a significant gap in which, currently, no age estimation method using third molar teeth is available for forensic application in the age range of 21–30 years old for the Malaysian population.

1.3 Root pulp visibility (RPV) and age estimation

A pulp chamber can be easily seen in radiographs as a less dense radiolucent area within the tooth structure, and it aids in diagnosing and assessing different dental pathologies (Xu et al., 2014). With ageing, as cell death takes place, the pulp becomes less cellular and has a lower cell count. The pulp chamber's size is reduced in older teeth as a result of continuous deposition of dentine along with the calcification of the pulp and stone formation, resulting in complete obliteration of the pulp chamber in some cases (Maeda, 2020). The optical phenomena of pulp fading due to the deposition of the secondary dentine occurs after the completion of root formation (post-development), leading to the narrowing of the pulp and eventually disappearing. The changes to the pulp architecture can be radiographically assessed and can aid in age prediction (Schmeling et al., 2007). Secondary dentine deposition decreases the size of the pulp chamber from 22 years old to 60 years old, after which the deposition rate slows down (Porto et al., 2015; Olze et al., 2010a).

Olze et al. (2010a) assessed root pulp visibility stages (RPV) of the lower third molar in the German population using a classification and scoring system for the assessment that they introduced. They classified the stages of visibility into four stages (0, 1, 2, and 3). However, no age prediction model was produced from these two studies.

Asif et al. (2019) utilised the post-developmental method pulp/tooth ratio of matured maxillary canines and central incisors. The study was done on a wide range of ages, i.e., 16-65 years old. It produced an age estimation model with a mean absolute error of 6.48 for maxillary central incisors and 8.58 years for maxillary canines.

The pulp/tooth volume ratio was not significantly correlated with the young age group between 16-25 years old in Malaysians. The findings suggest that the pulp/tooth volume ratio may not be good enough to be used for Malaysian young adults.

We intend to evaluate RPV using Olze et al.'s scoring system to estimate the age of the Malaysian population and to fill the literature gap, as no study has been conducted on the Malaysian population with this approach. In turn, by assessing the RPV of the lower third molars, an age estimation model will be formulated.

1.4 Periodontal ligament (PDL) and age estimation

The periodontal ligament (PDL) thickness ranges between 0.15 and 0.38 mm; it is thinnest in the middle third of the root and can be clearly detected around the roots of teeth in radiographs (Lai & Basrani, 2012). Therefore, PDL space is visible and well-recognised in panoramic radiographs (Shah & Angadi, 2021). Destruction of the PDL occurs with age progression (due to gingival recession and chronic periodontal disease). There is also a decrease in the number and activity of cells due to reduced physiological stimulation of the PDL (Lim et al., 2014). The width of the PDL can also noticeably decrease and become less visible in radiographs (Guo et al., 2020).

Olze et al. (2010b) introduced a scoring system for periodontal ligament (PDL) visibility and found it helpful in predicting age in different populations. Subsequently, many studies tested Olze et al.'s PDL visibility scoring method on different populations worldwide, which yielded a variety of results (Sequeira et al., 2014; Timme et al., 2017; Guo et al., 2020; Lucas et al., 2017; Chaudhary & Liversidge, 2017). No previous study has been conducted to generate age prediction models using PDL visibility and RPV.

In this study, we intend to evaluate the PDL visibility using Olze et al.'s scoring system to estimate the age of the Malaysian population to fill the gap in the literature, as no study was held in the Malaysian population assessing PDL visibility of the lower third molars and formulating age estimation models.

1.5 Mental foramen and age estimation

Mental foramen is a funnel-like opening that allows the mental nerve to exit the mandible. It is a critical feature that must be detected prior to any surgical operation in this region to prevent risks such as neurovascular bundle injury. The position of MF changes numerous times throughout life, from birth to old age. During early life stages, it is positioned closer to the mandible's inferior border and posterior to the bud of 1st molar. The MF shifts anteriorly towards the first and second premolars after the beginning of emergence of the permanent dentition. In older ages, however, as result of loss of teeth and bone resorption, the MF moves nearer to the mandible's alveolar ridge (Kanchan and Krishan, 2015). According to a number of studies, the portion of the bone from the MF to the lower boundary of the lower jaw is not impacted by resorption of bone and leftover unchanged throughout life (Wical and Swoope, 1974; Lindh et al., 1995; Singal and Sharma, 2017), and thus is not affected by biological changes before death (Guler et al., 2005). Because of this, the position of MF is also crucial in determining age and gender (Kanchan and Krishan, 2015).

Our intention here is to evaluate and detect the changes in the location of MF and its relationship with age. This notion is justified due to the continuous remodelling of the mandible throughout life and because no previous study has assessed the vertical position of MF to formulate an age estimation model in the Malaysian population.

The formation of an age prediction model based on the vertical position of MF may be useful as MF was shown to be a useful marker to predict age in other populations.

1.6 Mandibular canal (MC) and age estimation

The mandibular canal (MC) begins from the mandibular foramen and extends to the median plane across the body of the mandible. In panoramic radiographs, the MC shows a black radiolucency flanked by two opaque lines. Its location may be used to predict the age of individuals as there is a significant change in its location as age advances (Bhardwaj et al., 2014). A statistically significant difference in the mandibular canal location between the three ethnic groups and the gender of the Malaysian population was reported (Abdullah, 2012).

Our intention here is to detect and measure the vertical position of the mandibular canal (MC), relying on its position with age advancement due to continuous mandibular remodelling. No study covering the Malaysian population studied the relationship between age and MC position.

1.7 Clavicle and age estimation

The age estimation method using the analysis of the medial clavicular epiphysis through the computed tomography images is considered the gold standard method as it increases the accuracy of age estimation compared to other skeletal bones (Schmidt et al., 2011). The bone of the clavicles typically completes their union at around 22 to 27 years old and has different stages of growth (Kreitner et al., 1998; Ufuk et al., 2016; Singh & Chavali, 2011; Mansourvar, 2014). Conventional chest radiographs, ultrasound, and magnetic resonance investigation (MRI) can easily detect the stages of the medial clavicular union but with less accuracy than computed tomography (CT) and ultrasound. Moreover, the MRI techniques require professional skills and adequate training to use the device. Still, they are accounted to be less invasive (Schmidt et al., 2017).

Therefore, it can provide additional value for age estimation of young Malaysian adults aged 17-30 by combining methods for age estimation using oral structures and clavicle bone union. Furthermore, the clavicle bone is the last to decompose among the long bones of the body (Mansourvar, 2014).

1.8 Malaysian population, ethnic groups, and culture

Malaysia is made up of 13 states situated in the southeastern part of Asia. Malaysian ethnic-based records showed that Malays, Chinese, and Indians were the largest ethnic groups, with a distribution of 62%, 23%, and 7% respectively (Department of Statistics Malaysia 2023). The age group from 15 to 64 years old is the most prominent and counts for 69.5% of the total population (Department of Statistics Malaysia 2023).

To our knowledge, there are no publications that show the association of age changes with medial clavicle union developmental variations, RPV, PRL visibility, MF, and mandibular canal position between Malaysian ethnic groups.

The reported age changes and association with the MF and mandibular canal position, pulp, PDL variations, and medial clavicle union among young adults are observable using radiological approaches. Furthermore, radiological approaches have an advantage over other methods because of the non-destructive nature of the method.

Thus, this study aimed to combine the third molar post-developmental methods (and not developmental stages), MF location, and mandibular canal location, complemented by the medial clavicular union for age prediction for the Malaysian population.

1.9 Problem Statement and Study Rationale

Johan et al. (2012) utilised the lower third molar developmental stages through the Demirjian method observed on panoramic radiographs of the Northern Malaysian population.The prediction model derived in this study has been used cautiously for assisting alleged homicide cases in Kelantan state (Dewi, 2015), to determine the age of suspects, and for human identification in the OP Wawasan mass grave at the Perlis-Thailand border for reconstructing postmortem identification profiles of young victims. The drawback is that the application of the developmental stages of third molar for estimating age is restricted to the age of 21 years old, and most Northern Malaysian samples have completed their tooth development by this age (Johan et al., 2012).

Asif et al. (2019) utilised the post-developmental method pulp/tooth ratio of matured maxillary canines and central incisors. The study was done on a wide range of ages, i.e., 16-65 years old. The pulp/tooth volume ratio was not significantly correlated with the young age group between 16-25 years old in Malaysians. These findings suggest that the pulp/tooth volume ratio may not be good enough to be used for Malaysian young adults.

Therefore, to improve the applicability of age estimation for young Malaysian adults, a wider sampling, which includes archived data from Universiti Sains Malaysia Kota Bharu and Universiti Kebangsaan Malaysia, was recruited. The study also embarked on utilising multiple parameters from panoramic radiographs and chest radiographs.

1.10 Alternative research hypothesis

There is a statistically significant association between chronological age and post-developmental changes in the third molar (RPV, PDL visibility), MF location, mandibular canal location, and the union of the medial clavicular epiphysis.

1.11 Research Questions

1. Do age changes have any effect on root pulp and PDL visibility in the post-developed third molar teeth between males and females and both sides of the mandible among Malaysian ethnic groups (Malays, Chinese and Indians)?
2. Do root pulp and PDL visibility in the post-developed third molar teeth between males and females and both sides of the mandible among Malaysian ethnic groups (Malays, Chinese and Indians) vary with age changes?
3. Can the right and left root pulp and PDL visibility in the post-developed third molar teeth among the Malaysian population predict age with excellent accuracy?
4. Does age change have an association with mental foramen location and mandibular canal location parameters (i.e. MFr-MFI, MFl-Line A, MFr-Line A, MFr-MID, MCl-Line A, and MCr-Line A) after controlling the effect of genders and ethnic groups among the Malaysian population?
5. Does the clavicular union stage differ between genders among Malays, Chinese and Indian ethnic groups?
6. Do the clavicular union stages of the right clavicle of chest radiograph samples across males and females and Malaysian ethnic groups (Malays, Chinese and Indians) vary with age changes?
7. Do the combined root pulp visibility, periodontal ligament visibility, mental foramen location and mandibular canal position using panoramic radiographs predict age with excellent accuracy?
8. Does clavicular union stages' using chest radiographs predict age with excellent accuracy?

1.12 Objectives

1.12.1 General objective

To formulate a new prediction model for the age estimation range of 17-30 years old Malaysian population

1.12.2 Specific objectives:

1. To determine the mean difference of age on root pulp and PDL visibility in the post-developed third molar teeth between males and females and both sides of the mandible among Malaysian ethnic groups (Malays, Chinese and Indians)
2. To determine the age range across root pulp and PDL visibility in the post-developed third molar teeth between males and females and both sides of the mandible among Malaysian ethnic groups (Malays, Chinese and Indians).
3. To formulate an age prediction model for right and left root pulp and PDL visibility in the post-developed third molar teeth among the Malaysian population.
4. To determine the mean difference of age of mental foramen location and mandibular canal location parameters (i.e. MFr-MFI, MFl-Line A, MFr-Line A, MFr-MID, MCr-Line A, and MCl-Line A) on gender and ethnic groups after controlling the effect of age among the Malaysian population.
5. To determine the mean difference of age on gender across clavicular union stages of Malays, Chinese and Indians.
6. To determine the age range across the clavicular union stages of the right clavicle of chest radiograph samples between males and females among Malaysian ethnic groups (Malays, Chinese and Indians).

7. To formulate an age prediction model for the combined root pulp visibility, periodontal ligament visibility, mental foramen location and mandibular canal position using panoramic radiographs.
8. To formulate an age prediction model for clavicular union stages using chest radiographs.

CHAPTER 2

LITERATURE REVIEW

Previous literature review was focusing on the published studies utilising population data for the parameters, including their scoring method and accuracy, parameters on the third molar in age estimation, and parameters on the mandible and clavicle bone ossification.

2.1 Chronological Age (CA) and Dental Age (DA)

In a scenario where the date of birth is known, the chronological age can easily be calculated. However, in a forensic application where the date of birth is unknown, age estimation using biological indicators, such as tooth and bone, has been commonly used with different ranges of accuracies.

2.2 Third molar tooth in age estimation

Age estimation in forensic dentistry, with the help of dental parameters, covers tooth development and the post-development period. The dental method during the developmental period produces better accuracy of age estimation with 2-4 years variation (Nayak et al., 2014). However, the dental method during the development period was limited. For instance, the last permanent tooth developed is the second molar at 14 years of age (Almeida et al., 2013), followed by the third molar at around 21 years of age (Johan et al., 2012). Several publications were using the post-development method of the third molar with promising outcomes for the young adult group. Therefore, this review further elaborates on the assessment and variation of the third molar tooth's role in age estimation for young adults.

The development of third molars is focused on their crucial role in dental age estimation and prediction (Lee et al., 2009; Thevissen et al., 2009; Thevissen et al., 2010; Johan et al., 2012; Soares et al., 2015; Chhaparwal et al., 2021), despite being the most variable teeth in the human dentition. Most populations displayed sexual dimorphism pattern of the third molar, in which the tooth developed earlier in males than in females (Mesotten et al., 2002; Blankenship et al., 2007; Martin-de las Heras et al., 2008; Karatas et al., 2013). However, few populations in Northern India and American Black showed the reverse pattern (Blankenship et al., 2007; Rai & Anand, 2009).

Legović et al. (2010) studied the reliability of Nolla's method in Croatian children, which is a modification of Demirjian's method. They concluded that it was accurate and reliable in chronological age estimation in children. Similar results were found in a study conducted for age estimation in the Caucasian population between 16 and 22 years of age (Mesoten et al., 2002), and in the Japanese population, with ages ranging from 14 and 24 also using the Demirjian method (Arany et al., 2004). Bagherpour et al. (2012) used the scoring system of Gleiser and Hunt, modified by Köhler, to predict chronological age in the Iranian population. The prediction model for the age of 18 was successfully obtained. Furthermore, they suggested a population-specific standard for forensic age estimation using the third molar developmental stage.

Kumaresan et al. (2016) evaluated the reliability and validity of Demirjian, Williams, Nollas, Haavikko, and Cameriere methods of age estimation on 426 panoramic radiographs of the Malaysian population. The study was limited to Malaysian children 5-15 years old. They produced an age estimation formula utilising the development of mandibular permanent teeth.

The findings indicated that the methods used by Nolla overestimated age by 0.97 years, whilst the methods used by Willems and Demirjian inflated age by 0.5 years. However, Haavikko and Cameriere's methods underestimated age with a mean of 1.31 years and 0.4 years, respectively.

Yusof et al. (2014) studied the feasibility of William's dental age estimation method on selected Malay children aged between 4 and 24 years old. They produced an age estimation formula utilising third molar development and the mandibular permanent teeth. The results showed that William's age estimation method overestimated dental age for the selected Malay children with a mean error of around 0.5 years.

Cugati et al. (2015) aimed to create an age estimation formula for Malaysian children between 5 and 16 years old, including three ethnic groups (Chinese, Malay and Indian), by using Cameriere's method. The study assessed the mandibular permanent teeth and considered the open apices. The generated formula showed a -0.01 year difference between the observed and predicted.

Naik et al. (2017) evaluated the reliability of Demirjian and Nolla's method of age estimation on the Malaysian population between 14 and 22 years old. The study consisted of 100 panoramic radiographs. Results showed that the estimated age by Demirjian's method was more reliable and closer to chronological age than Nolla's.

Mani et al. (2008) studied the applicability of Demirjian and William's age estimation method on a selected Malay population (214 boys and 214 girls). To assess the mandibular teeth maturity, panoramic radiographs were utilized. William's age estimation method overestimated age with a mean of 0.55 years for boys and 0.41 years for girls. Similarly, Demirjian's age estimation technique overestimated age with a mean of 0.75 years for boys and 0.61 years for girls.

Johan et al. (2012) assessed the lower third molars of the Northern Malaysian population with the aim of generating age prediction models. They examined 1080 panoramic radiographs of Malays and Chinese aged between 14 and 25, equally split between both genders. They found notable differences in the third molar development among genders of the Northeast Malaysian population. Roots of third molars were developed in males before females around six months. Multiple regression analysis confirmed a relationship between tooth development stages, age, and sex. It showed that 71.1% ($R^2$0.711) of variance in age could be verified by sex and developmental stage of wisdom teeth. The age prediction model is found to be applicable in Northeast Malaysians, but it was limited to stages C to H, while stages A and B should be re-considered due to the study design (Johan et al., 2012). The prediction of age using third molar developmental stages was restricted to late teens. In the forensic identification process, the age of victims may fall in the range of 20-30 years old, and the estimation methods should consider other parameters. Unfortunately, only a few studies assessed the third molar post-development changes to explore its potential to estimate age for young adults older than 18 but less than 30 years old.

2.2.1 Root pulp visibility of third molar tooth and age estimation

Olze et al. (2010a) conducted a study on the German population to assess the stages of RPV of the third molar with a classification and scoring system for the assessment which they introduced. They classified the stages of visibility into four stages (0, 1, 2, and 3). The research involved 1198 panoramic radiographs

(569 male and 629 female), with ages ranging from 15-40 years old. No statistical differences between genders were mentioned in the study. Olze et al. (2010a) reported that the first appearance of score 0 in pulp visibility was at the age of 17.6 for males and 17.2 for females. While score 1 was reported at the age of 21 for males and 21.6 for females. Score 2 was seen at 22.3 years old for males and 23.4 for females. Finally, score 3 was first noted at the age of 25 for both sexes.

Another study conducted in Germany by Timme et al. (2017) covered 2,346 panoramic radiographs (1,167 male and 1,179 female) with an age range between 15 and 70 years old. The study aimed to assess the RPV of the third molar and validate Olze's classification. Timme et al. (2017) reported no statistical significance between different sides of the jaw and between different sexes. The first appearance of score 0 was at the age of 16.9 for males and 16.7 for females. Score 1 early appeared at the age of 21.0 for males and 20.6 for females. While score 2 was noted at 25.3 for males and 22.1 for females. The first appearance of score 3 was at the age of 29.5 years old for males and 24.8 years old for females.

The above two studies conducted on the German population revealed that any panoramic radiograph showing a score of 1, 2, or 3 RPV is most likely to be above 18 years old for both sexes. Participants scoring 2 or 3 are likely to be above 21 years old for both sexes. These two studies also showed variations in the age for each score despite recruiting a similar population. No age prediction models were produced from these two studies.

Pérez-Mongiovi et al. (2015) conducted a study on Portuguese to determine the suitability of the RPV of the lower third molars to predict age by panoramic radiographs. The study included 487 panoramic radiographs (259 male and 228 female), with individuals aged between 17-30 years old. Pérez-Mongiovi et al. (2015) revealed a statistical significance between the increase in stage and the increase in age for both sexes. The minimum appearance for score 0 was noted at 18.2 years old for males and 17.0 for females. Considering score 1, it was first noticed at 18.4 years old for males and 17.4 for females. Score 2 first appeared at 18.1 for males and 18.8 for females. Score 3 root pulp visibility was early seen at 19.1 for males and 21.2 for females. The findings on the Portuguese population were not as promising as in the German population studied by Olze et al. (2010a) for younger sample pools, as they recruited four samples females only from age groups 17.0 to 17.9 years and 20 samples, six females and 14 males from age group 18.0 to 18.9 years. They produced an age estimation formula utilising the RPV scoring system separately for each gender. Formula generated for females showed 96.2% accuracy for participants older than 21.0 years old, while it showed 19.9% for participants below 21.0 years old.

Formula generated for males showed 96.9% for participants older than 21.0 years old, while it showed 27% for participants below 21.0 years old, with a mean error between -0.17 and 3.14. However, the method could accurately predict those who were older than 21 years old.

Lucas et al. (2017) carried out a study in England (Oxford) to identify whether the RPV is reliable for identifying participants under or above 18 years old.

The research included 2000 dental panoramic images (1000 male and female each) from ages 16-26 years old. No statistically significant difference among the sexes was reported in this study. Lucas et al. (2017) stated that the minimal achievement of score 0 was at 17.16 years old for males and 16.33 for females. Regarding score 1, the first appearance was at 17.0 years old for both sexes. Score 2 was minimally achieved at 18 years old for both sexes. Score 3 RPV first appeared at 20.19 for males and 22.45 for females. It was clearly mentioned that the scores of participants 2 and 3 were equal or slightly above 18 years old.

Guo et al. (2018) studied the RPV scoring system in the Chinese population. Participant's ages were between 15 and 40 years old, with a total sample of 1300 panoramic radiographs (650 male and 650 female). The main goal of the research was to test Olze's scoring system for the Chinese population and evaluate its effectiveness. No statistically significant difference between the sexes was reported in the study. The study by Guo et al. (2018) showed that the first appearance of score 0 was at the age of 17.05 for males and 18.76 for females. Considering score 1, it was minimally achieved at 19.25 for males and 20.73 for females. Score 2 was first reported at the age of 22.0 for both sexes, score 3 was noticed first at 26.45 for males and 27.66 for females. The study showed the probability of scores 1, participants were older than 18 years old, while for scores 2 and 3, participants were older than 21 years old. No age prediction model was produced from this study.

Two studies were conducted in Turkey to evaluate Olze’s RPV system and determine the cut-off effectiveness for 18 and 21 years old. The first study was conducted by Akkaya et al. (2019) using 463 panoramic radiographs (199 male and 264 female) aged between 16 and 34 years old. Cohen's Kappa intra-rater results were excellent, while the inter-rater agreement was substantial. Akkaya et al. (2019) showed that the minimal achievement of score 0 was at 16 years old for both sexes. Score 1 was early recognized at 17.9 for males and 16.93 for females. Considering score 2, the first appearance was at 18.0 years old for both sexes. Score 3 was first seen at 22 years old for both sexes. The cut-off point was determined using Youden’s index to ensure accuracy and sensitivity.

Gok et al. (2020) assessed 1511 panoramic radiographs for Turkish subjects (747 male and 764 female) aged between 15 and 40 years old in Turkey. No significant differences regarding sex were found in the study. However, there was a statistically significant relation between age and increasing stage. Score 0 was first noticed at 17.0 years old for both sexes. The first appearance of score 1 was at the age of 19.0 for both sexes. Score 2 was first recognized at the age of 20.0 for both sexes. Finally, score 3 was noticed early at the age of 25.0 for both sexes. In the Turkish population, there is a variation when assessing Olze's scoring system as a cut-off point for 18 years old, as Gok et al. (2020) reported that any participant from panoramic radiograph scores 1, 2, 3, the pulp visibility was above 18 years old.

Akkaya et al. (2019) studied the same Turkish population and reported that any participant with panoramic radiograph scores 2 and 3 was equal to or above 18 years old. When determining the cut-off for 21 years old, both Akkaya et al.

(2019) and Gok et al. (2020) stated that individuals from the Turkish population with a score of 3 were considered more than 21 years old.

Al-Qattan et al. (2020) evaluated the RPV among the Maltese population, with ages ranging from 16-30 years old. The study consisted of 662 (288 male and 374 female) panoramic radiographs from Malta to test Olze's scoring system for RPV and determine the cut-off point for 18 years old. The study reported a statistically significant difference between the sexes at a score of 0. Regarding score 0, Al-Qattan et al. (2020) reported that the first appearance was 16.0 years old for both sexes. The minimal appearance for score 1 was at the age of 16 for both sexes. Score 2 was first noticed at the age of 18.2 for both sexes. Score 3 early appeared at 24.0 for males and 22.03 for females. Al-Qattan et al. (2020) showed that the cut-off point for the 18 years old in the Maltese population was when panoramic radiographs showed scores of 2 and 3 RPV for both sexes. Regarding the cut-off point for 21 years old, it was apparent in the Maltese population that any participant with a score of 3 was above 21 years old. Al-Qattan et al. (2020) study stated that the minimal appearance of score 1 showing changes in RPV appeared at the age of 16.0 years old for both sexes.

RPV is found to be a useful tool in estimating cut-off points for 18- and 21-year-olds, with some variations reported in the literature. No age estimation formulas using RPV were produced except for the Portuguese population. All RPV studies are summarised in table 2.1.

Table 2.1 Minimum age by years of root pulp visibility in different populations

Study	Year	Population	Gender distribution		Age range	Minimum age for stages of pulp visibility			
						0	1	2	3
Males									
Olze et al.	2010	German population	M= 569 F=629	Conventional panoramic radiographs	15-40	17.6	21	22.3	25.2
Perez Mongiovi et al.	2015	Portuguese population	M=259 F=228	panoramic radiographs	17-30	18.2	18.4	18.1	19.1
Lucas et al.	2017	England	M=1000 F=1000	Dental Panoramic Radiographs	16-26	17.16	17.71	18.16	20.19
Timme et al.	2017	German population	M=1167 F=1179	Conventional panoramic radiographs	15-70	16.9	21	25.3	29.5
Guo et al.	2018	Chinese Population	M=650 F=650	panoramic radiographs	15-40	17.05	19.25	22.33	26.45
Akkaya et al.	2019	Turkey	M= 199 F=264	panoramic radiographs	16-34	16.61	17.91	18.13	22.36
Gok et al.	2020	Turkey (Barsa)	M= 747 F=764	panoramic radiographs	15-40	17.2	19.1	20.4	25.7
Al-Qattan et al.	2020	Maltese population	M= 288 F=374	panoramic radiographs	16-30	16.3	16.5	18.2	24