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Age estimation of living South African individuals: a multifactorial model

by

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ABSTRACT

Key words: third molar, vertebral ring apophysis, maturation, combined age indicators

Age estimation in living individuals around the legal age of 18 years remains a difficult challenge, with limited options available. In this study third molar development was used, along with the novel method of anterior inferior vertebral ring apophysis development, to assess the age of living individuals and the probability of being 18 years. For third molar development, panoramic radiographs of 705 white and 563 black South Africans were scored using a 10 stage scoring system. Vertebral apophysis development of C2, C3, and C4 of 496 white and 478 black South Africans were assessed from cephalometric radiographs, using a four-stage scoring system. Likelihood values were determined for individuals in each sex and population group being 18 years, based on developmental stages. For apophysis development, the median ages for attainment of stages 0, 1, and 2 were below the 18-year threshold for all ancestry and sex groups, while stage 3 was also below this threshold in some groups. For third molar development, black South African individuals consistently matured earlier than white South African individuals, while for most of the stages the opposite was true for apophysis development. Differences between the sexes were also noted for third molar, but not for vertebral apophysis development. These age indicators were also combined by using a generalised linear model (GLM). The combined sample comprised of 165 females and 122 males aged between 15 and 18 years. Four additional models were obtained from data sets only containing data for third molar and cervical ring apophysis development respectively. The performance of all the models were quantified and compared using the Akaike information criterion (AIC) as an estimator of the relative quality of the statistical models and the prediction error as a mean square error value. The best performance resulted from third molar development, although the vertebral data adds a component related to skeletal development which may better reflect biological reality. These results show that cervical vertebral apophysis development is a valuable, novel addition to the assessment of age in living individuals. Both these methods are easy to use and can be assessed from standard and routinely

used radiographic images. The developed models need to be sex and ancestry specific, as clear differences were noted.

RESEARCH OUTPUTS

Journal articles

1. Uys A, Bernitz H, Pretorius S, Steyn M. Estimating age and the probability of being at least 18 years of age using third molars: a comparison between Black and White individuals living in South Africa. International Journal of Legal Medicine. 2018; 132: 56-61.
2. Uys A, Bernitz H, Pretorius S, Steyn M. Age estimation from anterior cervical vertebral ring apophysis ossification in South Africans. International Journal of Legal Medicine. 2019; 133: 1935-1948.
3. Uys A, Bernitz H, Fabris-Rotelli I, Steyn M. Age estimation combining radiographic information from third molar and cervical ring apophysis development in a South African cohort. Manuscript in progress.

Conference presentations

Oral presentations

1. Anatomical Society of Southern Africa (ASSA), April 2017, Langebaan, Western Cape. Estimating age, and the probability of being at least 18 years of age using third molars. Uys, A[1], S. Pretorius[2], M. Steyn[3]

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2. Forensic anthropology society of Europe (FASE), September 2017. Milan, Italy. Estimating age and the probability of being at least 18 years of age using third molars. Uys, A[1], S. Pretorius[2], M. Steyn[3]

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Anatomical Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

3. Anatomical Society of Southern Africa (ASSA), April 2018, Muldersdrift, Gauteng. Age estimation in a South African population based on vertebral ring apophysis ossification using cephalometric radiographs. Uys, A[1], S. Pretorius[2], M. Steyn[3]

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LIST OF ABBREVIATIONS

°	-	Degree/s
µSv	-	Microsievert
ABFO	-	American Board of Forensic Odontology
AGFAD	-	Study Group of Age Estimation of the German Society of Legal Medicine
AIC	-	Akaike information criterion
ALARA	-	As low as reasonably achievable
BMP	-	Bone morphogenetic protein (BMP).
BSA	-	Black individuals living in South Africa
C2	-	Cervical vertebra C2
C3	-	Cervical vertebra C3
C4	-	Cervical vertebra C4
CA	-	Chronological age
CJA	-	Child Justice act
CJCP	-	Centre for Justice and Crime prevention
CT	-	Computed tomography
CV		Cross validation
CVM	-	Cervical vertebral maturation
CVMS	-	Cervical vertebral maturation stage
DA	-	Dental age
EASO	-	European Asylum Support Office
Eda	-	Ectodysplasin
FGFs	-	Fibroblast growth factors
GLM		Generalised linear model
GPA	-	Greulich and Pyle atlas
IOFOS	-	International Organization for Forensic Odonto-Stomatology
mm	-	Millimetre/s
mSv	-	Millisievert
SD	-	Standard Deviation
THF	-	Tumor necrosis factor
TW	-	Tanner-Whitehouse

UK	-	United Kingdom
WHO	-	World Health Organization
Wnts	-	Wingless-related integration site
WSA	-	White individuals living in South Africa

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DECLARATION

I, André Uys, declare that the thesis, which I hereby submit for the degree PhD at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

ETHICS STATEMENT

The author, whose name appears on the title page of this thesis, has obtained, for the research described in this work, the applicable research ethics approval.

Ethical clearance was obtained from the University of Pretoria, Faculty of Health Sciences (Ethics reference number: 263/2015).

The author declares that he has observed the ethical standards required in terms of the University of Pretoria's Code of Ethics for Researchers and the Policy guidelines for responsible research.

Signature:

Date: 13/11/2019

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Chapter 1: General introduction

1.1 Background and scope

Ageing living individuals is an important aspect of forensic odontology. Age estimation in children and young adults is a relevant medico-legal procedure due to the increase in persons devoid of identification documents – many of these related to issues of migration. Because of the increases in crime, illegal immigration rates and the use of children to perform acts of child labour, the estimation of age for forensic purposes is often required to assess whether individuals should be treated as adults in a court of law.

In most countries the age range for criminal, civil and refugee proceedings fall in the range of between 14 and 22 years [1]. In South Africa, important age categories regarding the criminal capacity of children are under 10 years, 10 - 14 years, and 14-18 years. The Bill of Rights and the Children's Act defines a 'child' as a person under the age of 18 years and the distinction between 17 and 18 years is important with regard to legal and social responsibility.

When ageing the living, a non- invasive and accurate method is required because of the specific legal requirements [2]. Methods used for age estimation should have been presented in peer reviewed journals and accepted by the scientific community as per the Daubert principles. The methods described must be clear and accurate, and the principles used ethical [2]. A variety of age estimation methods exist and it is suggested that the correct assessment of age in the living should include a physical examination, and consider bone and dental development [3]. Several methods of age estimation have been described for both living and deceased individuals. All methods do, however, have limits, mean errors, and some are only suitable for a specific age range [4]. Age estimation methods become less accurate with an increase in age [4]. The chosen method must be provable, transparent and information regarding the accuracy of the method should be accessible [2].

Studies show that slight differences exist between and among populations such that population-specific studies are desirable [5–9]. Currently no standard guidelines and protocols are available for accurate age estimation in a South African population. European age estimation models are followed in South Africa and the age of individuals is estimated by using unrepresentative data. The use of unrepresentative data and models leads to inaccuracy on account of variation in the study design and the characteristics of the samples [5]. Many authors have questioned the applicability of these different age estimation methods to different populations. For example, bone age was significantly overestimated when Asian and Hispanic children were compared with their African American and White peers [6]. The Demirjian method also showed differences between chronological and estimated age in different population groups [7–9]. The differences in growth and maturation patterns among various groups should therefore be recognized [6]. Population differences require detailed assessment in order to determine the adjustments needed for a specific population.

Most age estimation methods in this context can be divided into dental and skeletal methods. The term age refers to the chronological age of an individual, which can be defined as the amount of time that has passed since birth. Without a known birth date the exact chronological age cannot be determined, and forensic anthropologists will use the biological age estimate to predict chronological age [6]. Biological age refers to an individual's physiological state. The investigator dealing with age estimation is thus usually confronted with determining an individual's degree of skeletal and dental maturity.

Dental milestones are used to aid in this process and include all developmental stages from the emergence of the first primary tooth to the mineralisation and completion of the root of the third molar. The tempo of development can be determined by comparing the individuals' chronological age against a specific biological occurrence such as the completion of tooth crown mineralisation.

The foundation of dental age estimation is based on the systematic genetic control of ontogenesis as each development stage has a set limitation [10]. Estimating the age

of an individual after 14 years of age becomes difficult and challenging. Most age estimation methods have advantages and disadvantages and are more or less indecisive after the age of 14, and all that remains are development of the third molars [8, 11]. Age estimation from dental development can be assessed in young children before the completion of root formation [11] and the method by Demirjian is the method most commonly suggested in literature. The seven left mandibular teeth are used in this method and the original model was developed from a French Canadian population [5]. Studies using the Demirjian method demonstrated differences between chronological age and estimated age. Most studies found an overestimation in age when applied to their specific population [7, 8].

A number of factors have an influence on the rate of development including sex, socio-economic status, health, and ancestry. These important modifying factors should be taken into consideration by the investigator, but often these factors are not known [12]. It is important to understand these and other dynamics such as genetics, nutrition and urban/rural considerations and to appreciate their potential influence on dental development [13].

The usefulness of lateral cephalometric radiographs for the assessment of maturation in vertebrae is relatively unexplored [14]. The vertebral bodies of C2, C3 and C4 are visible and can be assessed for maturation on lateral radiographs. Most past studies evaluated skeletal maturation by including assessments of cervical vertebrae development [14–16], however, no study has correlated the change of the cervical maturation stages with the age of the individuals, even though these changes are known to occur during adolescence.

In the development of an age estimation method it is important to use a large sample because the mean age is applied to a group. The range of growth patterns will far exceed the data used to develop any of the available methods. The description of tooth formation at the population level necessitates the inclusion of advanced, normal and delayed children with a sufficient sample size [17]. A combination of age estimation methods using various kinds of data is generally said to be more accurate than the use of a single method [18–23]. Several forensic protocols for age

estimation purposes suggest combining dental and other age estimation methods [4]. Although a wide range of age estimation methods are available, only a few are suitable for estimating age in living individuals. When an age estimation method is considered, the ethical and medico-legal aspects must always be taken into account [2]. To date no clear solution as to how to combine various methods in a statistically responsible manner exists for living individuals [2]. Many different statistical procedures have been applied, but in many studies there is a deficiency regarding the details of the method the analysis was performed [24].

1.2 Motivation for the study

Age assessment in young adults is a relevant medico-legal issue on account of the increase in young individuals devoid of proper identification documents. Internationally, interdisciplinary study groups such as the American Board of Forensic Odontology (ABFO), International Organization for Forensic Odonto-Stomatology (IOFOS) and the Study Group of Age Estimation of the German Society of Legal Medicine (AGFAD) have published guidelines and recommendations for forensic age estimation [25]. Currently Forensic Odontologists are consulted to give an opinion on the age of individuals. In South Africa, odontologists mainly make use of methods and data from European populations to estimate age. New protocols with current data from a South African population thus need to be collected and used to develop age estimation methods. Study groups are needed to support the results and to test the existing and suggested new methods. Methods should be standardised and adapted to establish an accurate usable model for living South African individuals.

This study aims to develop an accurate and usable model for age estimation in a South African population. Standardised age estimation protocols for living individuals in South Africa especially around the key age of 18 years are needed.

1.3 Aim and objectives

The aim of this study was:

- To develop an accurate, workable multifactorial age estimation method for living South African individuals between 15 and 25 years of age.
- To establish the relationship between chronological and dental age in a South African sample of white and black individuals as assessed from third molars, and the likelihood of being 18 years of age at a specific stage of development.
- To establish the relationship between the chronological age of black- and white South African individuals and the timing of ossification and fusion of the anterior inferior vertebral ring apophysis of cervical vertebrae (C2, C3, and C4).
- To use these two methods (third molar and vertebral apophysial development) in combination to establish a method that encompasses both dental and skeletal development.
- To determine the likelihood of being 18 years of age at a specific stage of development, using the above methods, and to determine the differences between ancestry and sex groups.

1.4 Outline of the thesis

The thesis includes six chapters and is focused on the dental (third molar) and skeletal (cervical vertebrae) aspects of age estimation, as well as a combination of these two features. The materials and methods related to each of these aspects are discussed within the relevant chapters, but all findings are discussed in a final discussion chapter. All the chapters are referenced at the end of the thesis.

In Chapter 2 we present the literature relevant to the methods investigated in the study. Literature relevant to third molar development and skeletal age estimation is included.

Chapter 3 presents the materials and methods and the results regarding age estimation utilizing the third molars. The data for maxillary and mandibular left third molars are presented and discussed. We show the differences between attainment of the different stages of third molar development between the different South African populations and sex groups. The probability of being at least 18 years of age was also calculated for each group.

Chapter 4 is the second data chapter, where data for anterior inferior apophysis development are presented. We also present a new classification system for the development of the anterior inferior apophysis. The mean, median and standard deviation values for the development of the anterior inferior apophysis were determined for each population and sex group. The probability of being at least 18 years of age was also calculated for each group.

In Chapter 5 the data from third molar and anterior inferior ring apophysis development were combined into a multifactorial model. We also tested if the use of multiple age indicators will lead to an increase in accuracy of age estimation methods.

Chapter 6 includes the final discussion and future directions related to the research.

Chapter 2: Literature review

2.1 Extent of the problem

A strong relationship exists between age and crime [26], and most of the crime in South Africa is committed by teenagers and young adults. According to research done by the Centre for Justice and Crime prevention (CJCP), the age of the first offence in South Africa is much younger compared with other countries [27]. Young offenders reported that they committed their first crime at ages 10-15 years (43.5%); 16-18 years (35.9%) and 19-25 years (18.7%) [27]. Violence in South Africa is strongly gendered with young men between 15 and 29 years frequently involved in violence as both victims and perpetrators [28]. When serial homicide is considered in South Africa, there were 33 solved cases between 1953 and 2007. The age of the offenders ranged between 18 and 42 years. There were 22 (66.7%) black, eight (24.2%) white and three “coloured” offenders and all were male [29]. Research found that 39% of young females suffered some form of sexual violence before their 18th birthday [30]. Most male rapists do so for the first time before they reach the age of 20 years and 46.5% were between 15 and 19 years [31]. The mean age of male rapists in South Africa is 17 years [32]. The population in South Africa is relatively youthful and ±44% of people were under 20 according to the 2011 census [33]. No national figures are available on criminal age distribution, but the figures show that 98% of sentenced offenders are male. The national institute for Crime Prevention and Reintegration of Offenders Report of 2014 indicated that there were 53 871 prisoners between the ages of 18 and 25 in South African prisons. Most of the offenders in South Africa thus fall into the age group investigated in this study.

According to the UN Refugee Agency, 68.5 million people globally have been displaced from home. This includes 25.4 million refugees of whom more than half are under 18 years of age [34]. Contrary to popular belief, most African migration is directed toward other African countries and not to Europe [31, 32], and South Africa is a particularly popular destination because of its perceived economic wealth. Migration out of Africa is directed towards Europe, the Americas and the Gulf countries [35]. In the two year period between 2014 and 2015 Sweden received

more than 240 000 asylum applications of which 40 000 were unaccompanied minors [37]. In Italy, the number of unaccompanied foreign minors over the past seven years were ± 7 500 per year, and crimes committed until 31 May 2013 by foreign minors that resulted in criminal proceedings numbered 9 529 [38, 39]. The immigration rate has also increased significantly in recent years with reports indicating that 7 066 unaccompanied children registered in February 2013 in Italy [39]. These relate mostly to refugees flooding the European continent from conflict-torn regions of the world. The European Union directive refers to “unaccompanied minors” as stateless persons below 18 years of age who arrive in the territory of the member states unaccompanied by an adult. The main countries of origin for unaccompanied minor asylum seekers in 2017 were Afghanistan (5 460), Eritrea (3 115), Gambia (2 555), Guinea (2 155) and Syria (1 910). Asylum applications submitted by unaccompanied minors in the European Union were 63 245 in 2016 and 31 765 in 2017. The member states with the highest number of applications for unaccompanied minors were Italy (9 945), Germany (9 085) and Greece (2 455). The age distribution of unaccompanied minor asylum seekers in 2016 to the European Union states were 16-17 years (24 375), 14-15 years (5 040) and under 14 years (2 100) [40]. According to the German census of 2008, there were a total of 763 unaccompanied minors in Germany. The minors consisted of 324 individuals younger than 15 years of age and 438 were estimated to have been between 16 and 17 years of age. These minors included a variety of nationalities [41]. The number of expert reports requested in Germany increased considerably and the number of asylum applications by unaccompanied minors in Germany quintupled from 2014 to 2015 [42]. In Spain the census of unaccompanied minors under trusteeship of the Spanish authorities in 2008 totalled 6000 minors [41]. In both countries nearly all unaccompanied minors were male. A migrant considered being “undocumented” and not an “unaccompanied child” has serious consequences for the individual. If the individual is found to be older than 18 years by the age assessment, he/she will not benefit from the privileges granted to these individuals such as lodging, access to healthcare, education, and legal provisions. It is reported that in 2017 most of the European Union member states did not plan or undertake new measures in the field of age assessment for unaccompanied minors applying for asylum. In some cases age assessment was not carried out [40]. In other regions of the world, such as

Australia, investigators rely hugely on the use of forensic bone age assessment to assess the age of migrants and asylum seekers reaching Australia by boat. The crew, usually Indonesian fisherman, can be imprisoned for 5 years if the smuggled individuals are older than 18 years. If the individuals are younger than 18 years they are repatriated. Crew members are often held for months pending age assessment processes [43].

While we have no similar data available in South Africa, the massive problem with refugees and illegal immigrants from all over Africa is well known. According to South Africa's 2011 Census and the 2016 Community survey, an estimated 2.2 million people indicated that they were born outside of South Africa. Statistics South Africa estimates a net immigration rate of 1.02 million people between 2016 and 2021 with most international migrants settling in Gauteng province [44].

In southern African countries the percentage of non-orphans and orphans aged 10-14 years working 20 hours or more per week are 18% and 19% respectively [45]. According to the Basic Conditions of Employment Act, children aged between 15 and 18 years may not be employed to do work inappropriate for their age or that takes place at the risk of the child's well-being, education, physical or mental health. It is a criminal offence to employ a child under the age of 15 years [46]. It is thus important to have population specific data and validated methods available to assist the law in estimating age of vulnerable individuals.

The extent of the problem highlights the importance of age estimation for individuals between 15 and 25 years. The practitioner will be tasked with giving an opinion about the estimated age during age estimation cases. Therefore reference samples (databases) should be available with which age can be estimated.

2.2 Importance of age 18 years

A child is defined by the Convention on the Rights of the Child (1989) as a person younger than 18 years [47]. Adolescence is defined by the World Health Organization(WHO) as the period between the ages of 10 and 19 years [48]. The

age range from 10-24 years are divided into three categories when data are reported: 10-14 years (early adolescence); 15-19 years (late adolescence); and 20-24 years (young adulthood) [49, 50].

In most countries the ages relevant for criminal, civil and refuge proceedings fall in the range of between 14 and 22 years of age [1]. The majority of countries regard 18 years as the legal age and the age where children and adolescents gain adult legal rights [51]. The age of 18 years is in most countries the determining factor for acquiring new rights and obligations [52]. In the South African context, the important ages regarding the criminal capacity of children are under 10 years, 10 - 14 years, and 14-18 years. The Bill of Rights and the Children's Act defines a 'child' as a person under the age of 18 years and the distinction between 17 and 18 years is important with regard to legal and social responsibility. A child becomes a major upon reaching the age of 18 years [53].

On the 1st of April 2010 a new child justice system dealing with children between 11 and 18 years came into operation. The Child Justice act, 75 of 2008 (CJA), caters for children under the age of 18 years. Children under the age of 18 years, who are suspected to have committed a crime, will be dealt with by following the child justice process and not the normal procedures pertaining to adults. The CJA states the following:

- Children up to 10 years of age lack criminal capacity. Children younger than 10 years may not be arrested for committing an offense and will be referred to the Children's Court.
- Children from 11 to 14 years of age have criminal capacity. The state must prove criminal capacity on the part of the child accused of having committed a crime.
- Children above 14 years of age have criminal capacity unless the accused child can prove otherwise.

In terms of the criminal justice system, the Constitution of South Africa and the Child Justice Act will protect the child under the age of 18 years in all regards. If a child under the age of 18 years is detained, the child must be held in the same holding cells as his/her own sex and kept separately from persons older than 18 years. The

detained child must be kept and treated in conditions suitable for his/her age [54]. In any legal case where there is no firm identity documentation, the estimation of age as under or over 18 years of age thus has vast implications.

2.3 Ethics in age estimation

When ageing the living non-invasive, accurate methods are required because of the specific legal requirements. A variety of age estimation methods exists and it is suggested by The Study Group on Forensic Age Diagnostic [18] that the correct assessment of age in the living should consider a physical examination, bone development and dental development [4]. Age estimation methods in living individuals need to take the basic ethical values of autonomy, non-malevolence, beneficence and justice into consideration [55]. Bioethical principles of beneficence and non-maleficence should be followed to assure that the examined person receives the best possible outcome with the least damage [56].

A medical examiner performing a medical investigation needs permission from the patient. This examiner also needs permission from a parent, guardian or caregiver if the patient is a minor. Mentally mature individuals older than 12 years can give permission for an examination or treatment if the parents refuse [57]. During medical treatment patients have the right to complain and this also applies for age estimation examinations. The Children's Act implies that the child's refusal must be respected. According to the National Health Act 61 of 2003, a medical practitioner is responsible to inform health users about their right to refuse treatment. A child with the capacity to give consent is also included in this act and can refuse treatment [58].

During treatment and diagnosis patients should not be harmed. Age assessment using ionising radiation can cause stochastic effects [59]. The European Asylum Support Office (EASO) practical guide states that non-medical methods should be used first, followed by radiation free medical methods and as a last resort radiation methods. When using radiation, the ALARA (as low as reasonably achievable) principle should be adhered to [52]. In forensic age estimation, X-ray examination doses vary from 0.1 µSv (hand radiograph) to 800 µSv in computed tomography

(CT) evaluations [60]. The most commonly used dental age estimation methods use panoramic radiographs to observe the dentition as the degree of dental development can be observed on panoramic radiographs [61]. The effective dose for panoramic radiographs range from 5.5-22.0 µSv [62]. For cephalometric radiographs the effective dose is 2.2-3.4 µSv [63]. To keep radiation doses low, time, distance and shielding can be adjusted and implemented [52]. The natural background radiation a person is exposed to annually range from 1 to 260 mSv (µSv/day). The doses needed for a panoramic radiograph or cephalometric radiograph are equivalent to one day of background radiation, and are thus far below levels that can be considered undesirable. The principle of as low as reasonably achievable (ALARA) should be considered for each radiograph taken. Non ionising alternatives and methods to investigate age related changes are presently not fully developed or verified [61]. Due to the high radiation doses the use of CT is not recommended in children under 18 years of age [60]. Magnetic resonance imaging (MRI) may be considered in the future to reduce ionizing radiation. The use of digital radiography is advantageous because of the lower radiation exposure to the patient and the image can be adjusted for optimal diagnostic quality. When a panoramic radiograph is exposed for age estimation, all pathological changes detected should be reported on and the relevant treatment should be initiated [61, 64]. The benefit involved when exposing an individual to ionising radiation must outweigh the risk, e.g. obtaining better living conditions or treating disease.

In 2011 the Australian Human Rights Commission investigated the unease regarding the age assessment process. The report expressed its disapproval with the use of bone age for age assessment [65]. The concerning factor was related to the radiation dose and the insufficiency of radiographs to distinguish between mature individuals with a wide chronological range. In the United Kingdom (UK) the use of radiographs in age assessment has been contested on the basis that the methods are inaccurate and unethical [66]. In 2006 the UK Government recommended in evidence that hand-wrist radiographs and radiographs of the dentition should be considered when examining an individual. It is currently not the policy of the Home Office in the UK to commission dental age assessments or radiographic reports to advise on an assessment of age. However, if an applicant submits a report it must

be considered together with other evidence. The margin of error and weighting for each method must always be taken into consideration [67].

Age estimation methods must give the most accurate age to benefit the individual. An inaccurate age will not only harm the individual but also the people in the group this individual is incorrectly allocated to [68]. Misclassification must be avoided at all cost for both children estimated to be adults and adults estimated to be children. The examined individual should always get the optimal benefit, if doubt exists. The lowest limit of the predicted age is one way to ensure that optimal doubt is given to an individual. Individuals involved in age estimation procedures should be allowed to be accompanied by their parents, guardians, caregivers and/or lawyers [61].

Age estimation techniques must be able to discriminate between a minor or major (child or adult) as well as above or below certain age thresholds. Likelihood levels must be set for age estimation methods. Combining age predictors may provide the highest level of likelihood. The accuracy to which an age is estimated is less important and far-reaching during adulthood [61].

Age estimation research should be based on reliable and reproducible data collection, similar to good practices in all research. The sample size must be large enough to be representative of the entire range of subjects investigated. Data collection must be standardized and methods should be tested by using intra- and inter observer reliability with a high level of agreement. The developed method must be tested and certified and should provide the smallest difference between the chronological age and the estimated age. Age estimation methods must be described in detail and be presented in peer-reviewed journals to the scientific community [2].

It is recommended that age estimation methods be combined and that the likelihood be calculated to provide added information when age is calculated. Statistical models must be used to calculate the weight contribution of the different age related variables into a workable model [61].

2.4 Age estimation methods

2.4.1 Overview

Currently no method exists that can determine the exact age of an individual. The benefit of doubt and margin of error must be acknowledged, and the age assessment must be determined with the principles of the best interests of the child. No method or combination of methods can provide an exact age, and all methods have advantages and disadvantages. Combining different methods could improve the reliability of age assessment. Current methods are divided into non-medical and medical methods. Non-medical methods include interviews, consideration of documentary evidence, physical appearance and behaviour. Medical methods include dental observation, physical development assessment, psychological interviews, psychological tests, sexual maturity examinations, radiographs of the hand/wrist, radiographs/CT of the clavicle, dental radiographs and radiographs of the iliac crest [69]. Most of these methods are currently accepted for age assessment in Europe.

Literature regarding age estimation provides extensive information about the various methods available, methods of application and statistical analysis [70–72]. Developmental (morphological) methods are utilized in children and adolescents to estimate age and age related developmental features of the dental and skeletal system. With completion of skeletal development and growth, only a few age-dependent features remain which can be used for age estimation in late adolescents, namely development of the third molars and bones of the wrist. The accuracy with which age estimation in the living can be performed decreases with an increase in the age as the accuracy of developmental methods is poor in adulthood. Research found biochemical methods such as aspartic acid racemization to be more accurate in living adults [2].

In living individuals, age estimation methods usually make use of non-invasive radiological approaches. The timing and sequence of defined growth stages of the developing dentition is assessed with the use of panoramic radiographs. Dental

development of all teeth during childhood (0-14 y) [5, 73–79] and third molars (14-21y) [80–83] was assessed by numerous studies making use of radiological examinations. Sex and ancestry differences were found to influence the development of the dentition, with methods becoming less accurate with an advancement of age [5, 73–83] .

Skeletal development can be used for age estimation in living children and adolescence (0-18 y) and makes use of radiological evaluation. With an increase in age, the methods also became less accurate as sex, ancestry, and socio-economic factors influence the results [80, 81, 84, 85].

A radiographic examination of the hand is recommended for age estimation of living individuals [18]. Two methods are commonly used to evaluate the growth of the hand-wrist region. The Greulich and Pyle atlas (GPA) provides radiographic standards of the hand and wrist from birth to 19 years of age. Mean age estimation and an error range can be computed with this method by comparing the standards from the atlas with the proband [86]. The Tanner-Whitehouse (TW) method scores the ossification degree and morphological appearance of the ossification nuclei and bones of the hand and wrist. With this method a maturity score is given which can then be related to mean age and error [87].

Only the three age estimation methods relevant to this study will be discussed in detail.

2.4.2 Dental age estimation

2.4.2.1 Odontogenesis

Tooth formation takes place through chronological and reciprocal inductive signals [88]. Signals are transmitted between the epithelium and mesenchyme from neural crest origin. The tissue layers initiate the differentiation of the other layer and cell differentiation is regulated by epithelial-mesenchymal interactions to form highly specialised structures (teeth). Incisors, canines, premolars and molars are formed

because of the epithelium being present in different parts of the oral cavity [88–90]. Tooth formation begins with the dental epithelium thickening to form the dental lamina. Cells start to proliferate within this thickened band and invaginate in certain areas to form the dental placodes. The different tooth families are initiated by the dental placodes and the site of the placodes is determined by the balance of stimulatory and inhibitory signals. Stimulatory signals come from the fibroblast growth factors (FGFs) and wingless-related integration site (Wnts) and the inhibitory signals from the bone morphogenetic protein (BMP). Transcription factor p63, tumour necrosis factor (TNF) and ectodysplasin (Eda) contribute to the formation and growth of the placodes [91, 92]. Epithelium invagination continues to form the bud, cap and bell stages and contribute to the morphological characteristics of a tooth. Interplay takes place between the epithelium and mesenchyme through inductive signals. Distinct anatomical and functional tooth areas are formed during this interplay as well as the differentiation of the epithelium into ameloblasts and mesenchyme into odontoblasts [93].

During the bell stage the hard tissue of the tooth crown is formed and the specific tooth acquires its specific phenotype [92]. Ameloblasts are responsible for the secretion of enamel and the odontoblasts for dentine. A number of genes have been identified that act at specific stages during the development of a tooth. These genes are responsible for the pattern regulation as well as the differentiation process [93]. The outer enamel epithelium is formed at the periphery of the enamel organ with the inner enamel epithelium bordering the enamel papilla. The inner and outer enamel epithelium is continuous, and the dental papilla accumulates in a concavity formed by the outer enamel epithelium bending inwards and encompassing the dental papilla [92].

A basal lamina separates the dental papilla from the enamel organ. Aperiodic fibrils extend from the basal lamina into an acellular zone. The first secreted enamel matrix protein accumulates in this zone. When the first calcified matrix appears at the cuspal tip of the bell stage, the dental papilla is referred to as the tooth pulp. During the bell stage the separation of the developing tooth from the oral epithelium also

takes place. The inner enamel epithelium completes its folding and the crown of the developing tooth can be recognized [88, 92].

The contour of the tooth is determined by the termination of the mitotic division within the inner enamel epithelium cells. The cells begin to differentiate and take up their role to produce enamel. The inner enamel epithelium and papilla cells arch downward alongside the cusp slopes and dentine and enamel deposition takes place at the cusp tip. The differentiation of a second zone within the inner enamel epithelium will lead to the formation of a second cusp and so forth [88, 92].

The permanent dentition also develops from the dental lamina. The incisors, canines, and premolars are formed by the proliferative activity that takes place at the deepest part of the dental lamina which leads to the formation of another tooth bud lingual of the associated deciduous tooth. The molar teeth do not originate in the same way. Adequate jaw development triggers the dental lamina to tunnel posteriorly below the epithelium lining of the oral mucosa, into the ectomesenchyme. Epithelium outgrowths are formed during the posterior movement of the dental lamina. The epithelium together with the ectomesenchyme forms the tooth germs of the molar teeth. The primary dentition is initiated between weeks 6 and 8 of embryonic growth. Between week 20 in utero and 10 months after birth the successional permanent teeth develop with the permanent molars developing between week 20 in utero and 5 years of age [88, 92].

Ameloblasts and odontoblasts undergo terminal differentiation and are responsible for the formation of enamel and dentine. The formation of enamel already starts during the early crown stage development. Differentiation of the cells of the inner enamel epithelium produces enamel first at the tip of the cusps. The process then progresses downwards until all of the cells of the epithelium have differentiated into enamel forming cells. Enamel formation can be considered as a two-step process: when enamel initially forms it only mineralizes to about 30 %. Later, with the breakdown of the organic matrix and water content loss, the crystals become wider and thicker with a mineral content of about 96%. The high mineral content makes enamel extremely hard but also brittle. Enamel is translucent and varies in thickness

with the maximum enamel thickness being present over the working surfaces of a tooth. The thickness of enamel in these areas can be up to 2.5 mm [92, 94]

Dentine consists of dentinal tubules which is closely packed and extends through the entire thickness of the dentine layer. The odontoblastic extensions are contained within the tubules and the cell bodies of the odontoblasts are aligned within the inner aspect of the dentin and against a layer of predentine. The odontoblasts are responsible for forming dentine as well as to maintain it. Dentine formation starts with the deposition of a layer of unmineralised matrix at the innermost aspect. The thickness varies from 10 - 50 µm and it mainly consists of collagen. The predentine mineralizes to form dentine by the integration of various noncollagenous matrix proteins. Mature dentin comprises of inorganic material (70 %) in the form of hydroxiapatite, organic material (20%), and water (10%). The dentine and enamel is strongly bound at the dentinoenamel junction. The hardness of dentine is more when compared with bone, but less than that of enamel. The difference between enamel, dentine, tooth pulp, and bone can clearly be distinguished on a radiograph. Dentine appears more radiopaque than tooth pulp, and more radiolucent than enamel [92]. Structures such as enamel are very dense and will have greater attenuation of the x-ray beam. The resultant image displayed on the radiograph is described as being radiopaque. An object such as the tooth pulp is a weak absorber of photons and the resultant representative image will be radiolucent [95].

The inner and outer enamel epithelium increases from the cervical loop of the enamel organ to produce a double layer of cells once crown formation is complete. The double layer of cells is known as Hertwig's epithelial root sheath. The epithelial root sheath cells extend around the dental pulp with only the basal portion being open. The inner cells of the root sheath initiate the differentiation of odontoblasts from ectomesenchyme and are responsible for root dentine formation. The formation of multi-rooted teeth is similar with two tongues of epithelium growing towards each other and a primary apical foramen is converted into two or three secondary apical foramina. Hertwig's epithelial root sheath encompasses each apical foramen, forming epithelial tubes and extends from the cervical loop to the apical foramen. As root formation progresses the root sheath starts to do break down and only remains

intact at the advancing root edge. The induction process will continue until root formation is complete. The controlling mechanisms of root development are currently not fully understood but information indicates that the Transforming Growth Factor beta (TGF-β)/Bmp, Wnt, Fgf and sonic hedgehog (Shh) signalling pathways are involved [92, 96, 97].

2.4.2.2 Dental methods of age estimation

Dental age estimation techniques include morphological, radiological and biochemical methods. The dental age estimation methods, relevant to this study, fall into the radiological category. Dental age estimation methods in children and adolescents are based on tooth calcification, development, and eruption. A multitude of studies exist, but established methods are often used. The most frequently quoted dental age estimation methods for children and adolescents include:

- Schour and Massler [98]
- Moorrees, Fanning and Hunt [99]
- Nolla [100]
- Demirjian, Goldstein and Tanner [5, 73]
- London Atlas of AlQahtani *et al.* [101]

2.4.2.2.1 Schour and Massler method

Schour and Massler [98, 102] used an atlas approach to describe 22 chronological stages of dental development starting from 5 months *in utero* to 35 years of age. The stage drawings show the eruption status related to a specific age. Allocating a specific stage drawing to each year of growth, the mean age for each assigned drawing can only be ±6 months and this age range is too small to be reliable [103]. Comparing an individual's dental development with the charts can result in a reasonable estimation of the individual's chronological age. The atlas approach provides a tool which can be used in everyday practice. A radiograph of the maxilla and mandible can be compared against diagrams representing a specific age. The disadvantages of the method are the small sample size used for the development,

narrow age ranges, lack of information regarding the variation within an age range, no development chart drawings for ages 13,14, and 16 -20 years, and the fact that the charts do not distinguish between sexes. There is also no information available on the subjects and the analysing methods used for the development of the charts [4].

Revision of these charts was done by Anderson *et al.* [104] and Ubelaker [105, 106]. Ubelaker [105] developed charts based on data from Native Americans. The error ranges were adjusted, stages were modified and the rates of eruption were described. No differentiation in the data was made between the sexes. The chart was also modified for use on an Australian population [103]. Population specific data were used, sexes were separated and adjustments were made on the drawings representing the corresponding ages. The authors recommended that the developed atlas must be used for an initial age assessment, or "screening tool". When precise age estimation is required, a technique such as Demirjian using population specific datasets is recommended [103].

2.4.2.2.2 Moorrees, Fanning and Hunt's method

The means and variation of 14 clearly defined crown and root formation stages were presented in this method [99]. Lateral or "oblique radiographs" of a small sample of children from Boston, USA, were used. Data were presented for single-and multi-rooted teeth and for both sexes and the development of the third molar was included. The charts are composed of segments, indicating the mean age of attainment and two standard deviations for each stage of development. The study found that female dental development was ahead of male development [99]. The authors concluded that assessment of dental maturation will be affected by the following:

1. Population variation.
2. Variation in developmental rates of different teeth in an individual.
3. The experience of the observer to differentiate between the different stages of tooth development.
4. The availability of records to compare the earlier and later records of the same individual.

5. The amount of time between two stages of development [99].

2.4.2.2.3 Nolla's method

Radiographs of 25 boys and 25 girls from the University of Michigan were studied to develop a grading scale of 0 to 10 for the permanent dentition. Each tooth was described individually by making use of a diagram and a numerical value. Growth-age scales were developed for the calculation of an individual's age. The method can be used with or without the third molar and the charts discriminate between the sexes. The sample size used to develop the charts were however very small. Few developmental differences between the left and right corresponding teeth were found. The rate of development between males and females were statistically insignificant but females started development earlier and finished earlier [100].

2.4.2.2.4 Demirjian *et al.'s* method

The most widely used method to determine chronological age (CA) from dental age (DA) was developed by Demirjian *et al.* [5] and was based on French-Canadian children. The left seven permanent mandibular teeth were evaluated and each tooth was assigned a development stage representing development from the first appearance of calcified points to completion and closure of the root apex [5]. The study identified eight stages of calcification for each tooth. Each stage of calcification was described and a score was allocated. The dental maturity of an individual can be calculated by adding the scores and comparing the score to a scale measuring from 0-100. The tables were constructed for individuals aged 3-16 years and the scores and percentile standards were given separately for both sexes [5]. A detailed description was given regarding the mathematical technique used to calculate the scores for the stages [107]. The original research has two shortcomings. To calculate the score, all seven teeth had to be rated and in many older children not all seven teeth are always present. Using the corresponding tooth on the right side of the mandible is not always possible. A second limitation of the earlier system was the small sample size used in the study to calculate the very young and very old children [5]. A later follow-up study was done by Demirjian *et al.* to overcome the limitations of the first study [73]. The sample size was increased for the older and

younger stages to be more representative and covered the age range from 2.5-17 years. The 3rd and 97th percentiles were calculated and presented in the maturity standards. The authors stated that investigators using their scoring system should remember that the sample used was entirely from French-Canadian origin and that maturity standards may change noticeably for a specific population [73]. The Demirjian stages have been criticised on the basis that the age range is too broad between stages and that an individual's age cannot be assessed with precision [108].

For the estimation of age of infants, children and adolescents, the most accurate methods are dental techniques that make use of progressive morphologic changes [12]. In a study assessing the validity of the five most commonly used dental classification systems, it was concluded that Demirjian ***et al.'s*** classification attained the highest values for correlation between the stages as well as for observer agreement [109]. The method described by Demirjian *et al.* have been used and adjusted in different populations, showing differences between the chronological age and the estimated age [7–9, 81, 110–121].

The Demirjian method was tested on a variety of populations across the world [7, 9, 110–113, 116, 117, 119, 120], and these mostly found that other populations were more advanced as far as development is concerned than what was reported in the original French-Canadian sample. Girls generally had faster development than boys. Some authors [113, 117] suggested more advanced statistical approaches. It was suggested that the substantial variation among individuals regarding dental maturity support the recommendation that other biological indicators should be used to supplement dental age estimation techniques [116].

2.4.2.2.5 London Atlas of AlQahtani

AlQahtani *et al.* [101] developed an atlas to estimate age, including individuals from 28 weeks in utero to 23 years. The sample aged 2 years and older had a uniform age and sex distribution with 12 individuals in each category. The sample consisted of white and Bangladeshi individuals from the Institute of Dentistry, Barts and the

London School of Medicine and Dentistry. Tooth development was assessed according to the modified Moorrees stages. The results demonstrated that tooth formation is more variable after the age of 16 years. The authors concluded that the atlas covered the entire developing dentition and that all age ranges were represented [101].

2.4.2.3 Third molar development

After the age of 14, age estimation becomes increasingly difficult because most of the dentition is fully developed and only a few age-dependant features can be evaluated by using morphological methods [83, 122]. Third permanent molar tooth development is a valuable tool and should be included for age estimation in late adolescence and in early adulthood [109]. The third molar is the last tooth to initiate development and it may not have yet reached maturity at these ages [11, 122]. The mineralisation tempo of teeth is under strict genetic control and the systematic progression of morphological changes during growth makes “dental age” a valuable measure of an individual’s degree of biological maturity [123]. Evaluating the maturity of the third molar has been described as an appropriate method for estimating age in individuals of unknown age between 12-22 years [124, 125]. Absence of third molar teeth occurs frequently and the size of third molar teeth varies [126]. Various studies have documented the difference in tooth formation and eruption rates between populations [115, 123, 127]. The use of summary data is appropriate to evaluate each of the four third molars [124]. The wide-ranging prediction intervals found when third molar development is used as a single estimation method could be reduced by using a combination of dental methods and skeletal changes [20]. The wide chronologic age ranges observed for each third molar stage, justify reporting on mean ages and age intervals [128].

2.4.2.3.1 Tooth mineralisation stages

Various classifications have been suggested for the evaluation of tooth mineralisation stages [5, 11, 74, 100, 129–134]. These stages can be used for third molar development. Comparing the results achieved by the various classifications

systems is however challenging. When comparing root lengths, the interpretation between roots developed at a ¼, ⅓, ½ or ⅔ of the anticipated future root length is difficult and subjective [127, 135, 136].

Gleiser and Hunt's [129] classification system comprised of 15 stages and described the mandibular first molar based on radiographs (Fig. 2.1). The first molar was chosen due to the fact that its calcification is entirely postnatal. The rates of elongation in this tooth between the two sexes were found to be similar. The development of the crown height accelerates until completion, while elongation of the roots is initially slow, but speeds up from one-third to one-half of root length development. The root length development then decelerates again for an unknown time period. The authors suggested that assessment of calcification stages may be more valuable than emergence for estimating age [129].

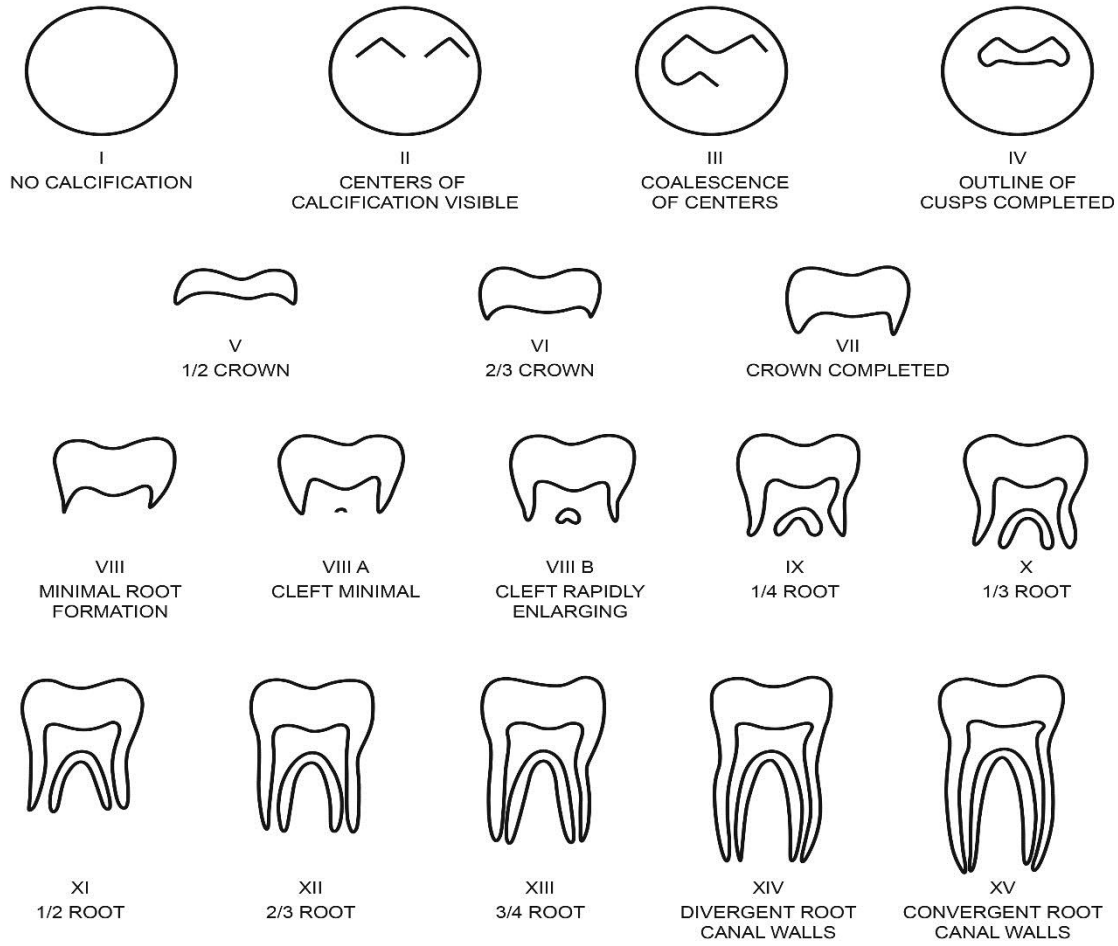


Figure 2.1: Gleiser and Hunt's original stages of calcification diagrams of the permanent mandibular first molar [129]**.**

Nolla [100] illustrated the degree of tooth development in a 10 stage development system (Fig. 2.2). The illustrations separated the central and lateral incisor, the cuspid and bicuspid, and the molars. The left and right side was studied, but the growth rate was found to be similar. The author concluded that the values obtained from one side were representative for the opposite side [100].

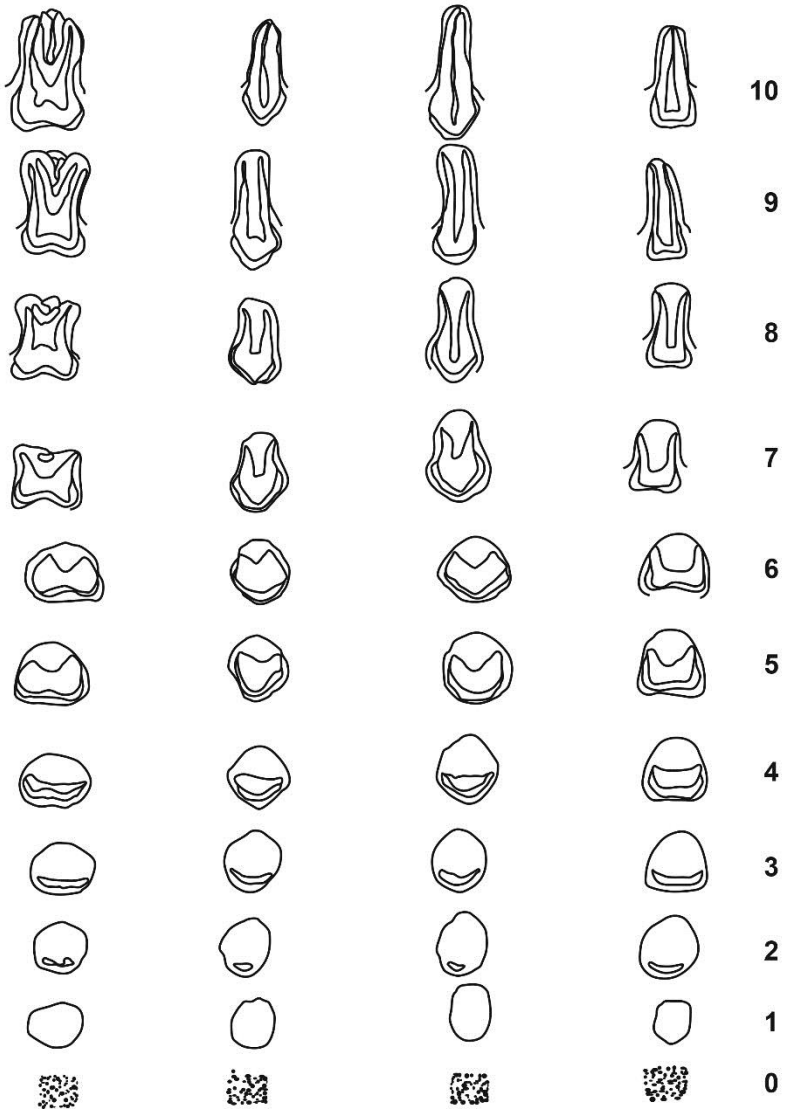


Figure 2.2: Nolla's original stages (0-10) of development of the mandibular and maxillary teeth [100]

Haavikko [130] used a modified Gleiser and Hunt [129] staging system for tooth formation. It comprised of 12 stages, six related to crown formation and six related to root formation. A tooth was categorized as belonging to a specific stage once it has passed the beginning of the stage. Only once the tooth reached the next stage was it regarded as belonging to that particular formation stage. The stages of tooth formation were illustrated for single rooted (Fig. 2.3) and molar teeth (Fig. 2.4) [130].

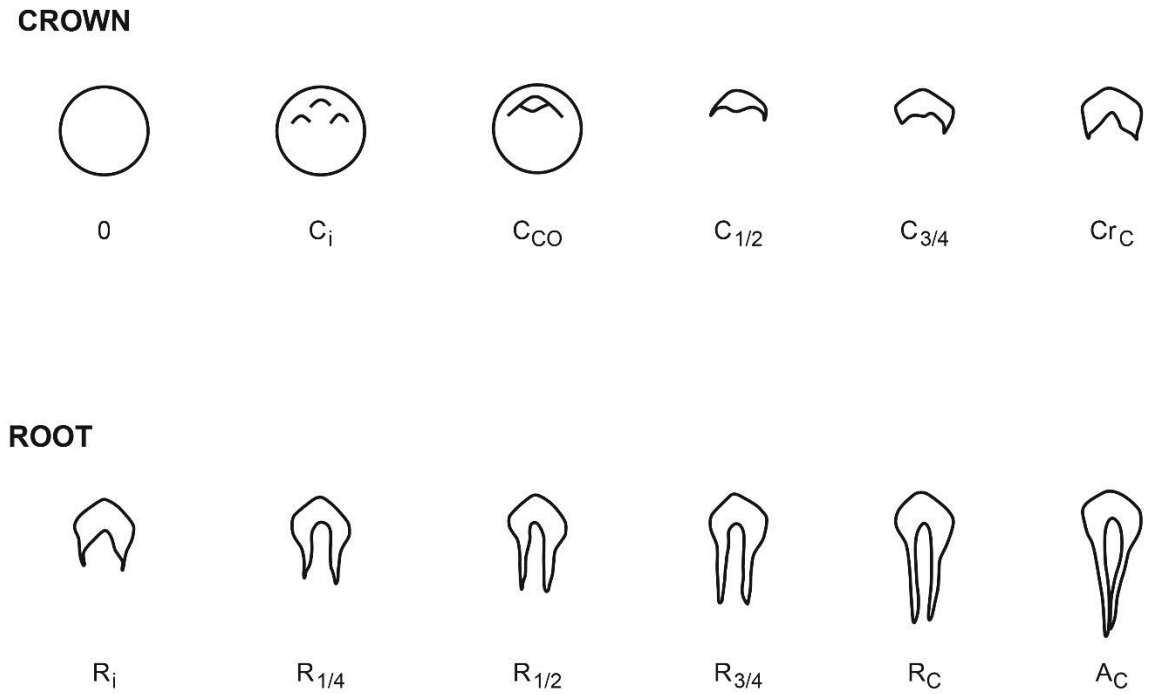


Figure 2.3: Haavikko's original stages of tooth formation for single rooted teeth [130]**.**

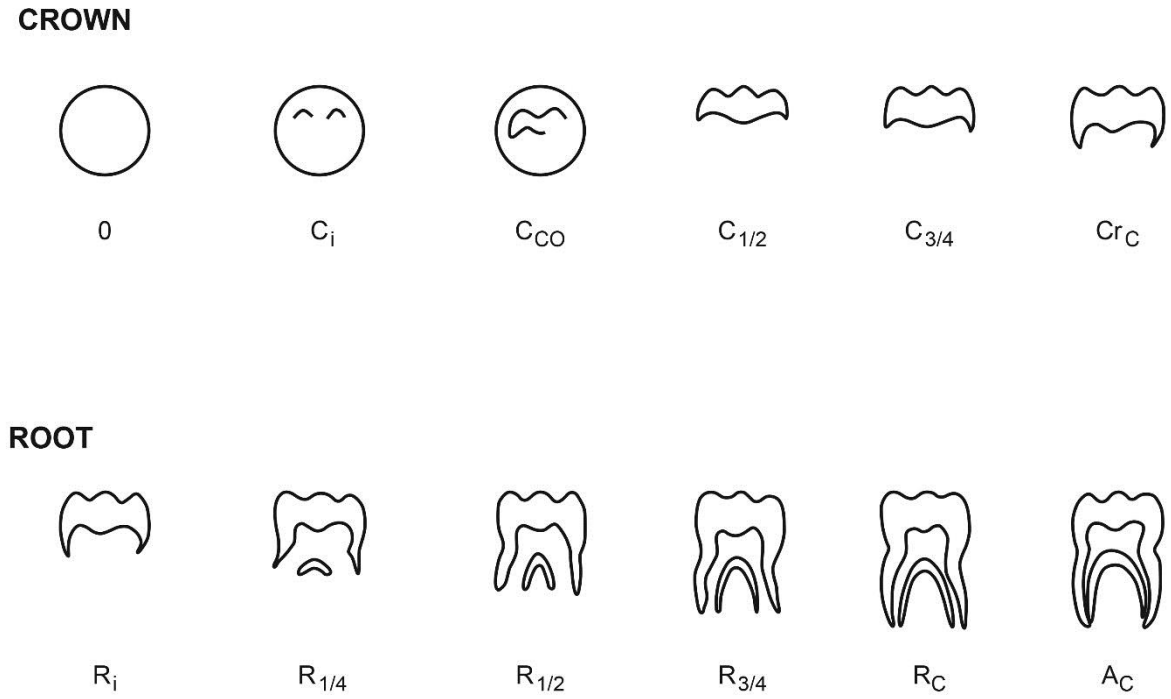


Figure 2.4: Haavikko's original stages of tooth formation for molar teeth [130]**.**

Demirjian *et al.* [5] determined eight stages (A to H) (Fig. 2.5) of tooth mineralisation with an additional stage zero for no sign of calcification. Each stage is described with one, two or three marked criteria. For stages described with one criterion, the

criterion must be met for the stage to be reached. For stages described with two criteria, the first criterion must be met for the stage to be considered reached. In stages described with three criteria, the first two criteria must be met for the stage to be considered reached. At each stage the previous stage criteria must be met in addition to the criteria for that specific stage. In cases with doubt, the earlier stage must always be assigned. The distance between the highest cusp tip and the cement-enamel junction is defined as the crown height. When the crown is slightly angled in the radiograph, the midpoint between the buccal and lingual cusp tips must be taken as the highest point. A rating of 0 is given to a tooth with no sign of calcification. The stages represent the dental maturity of each tooth [5].

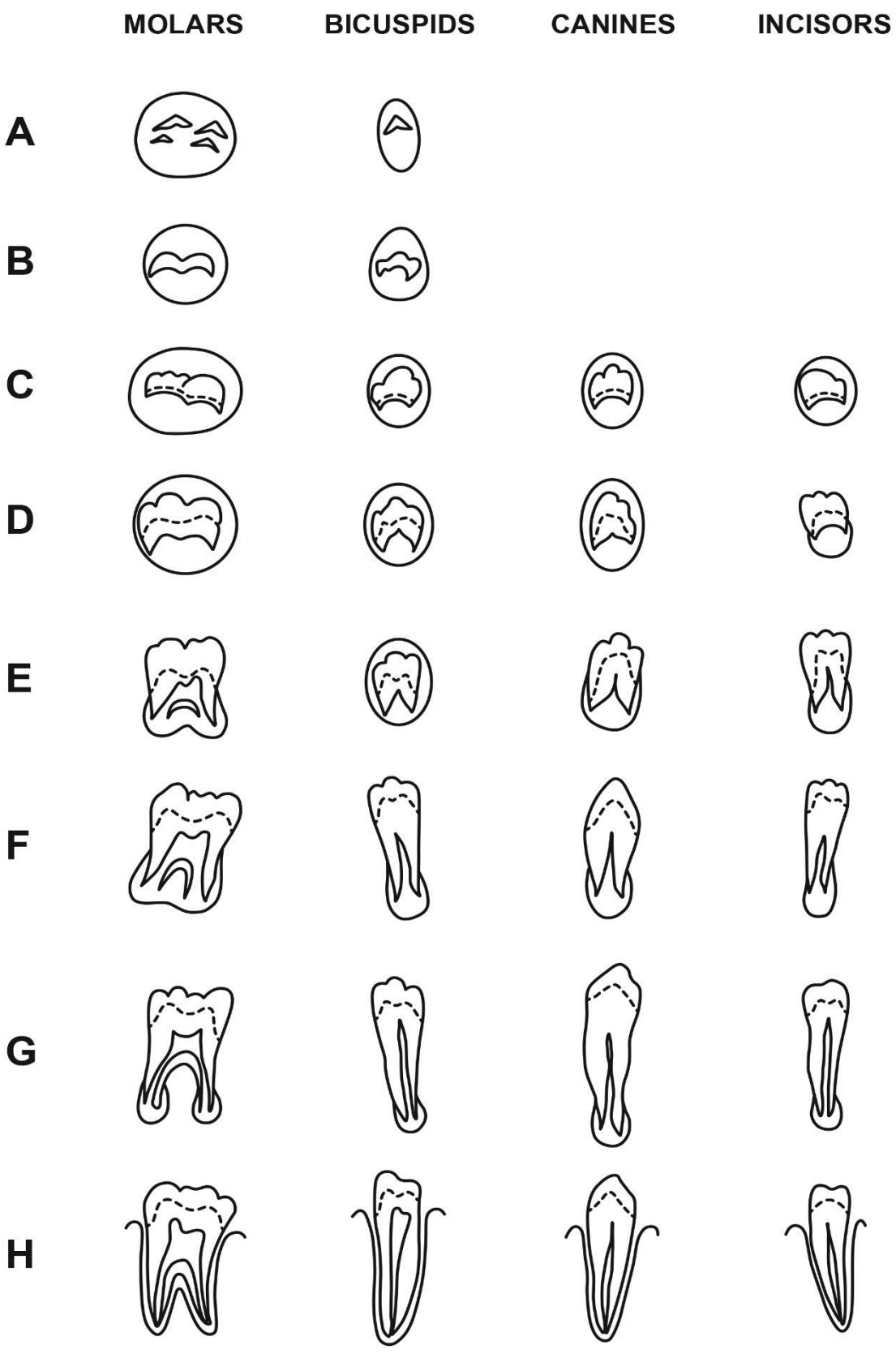


Figure 2.5: Demirjian's original stages of development for the permanent dentition [5]**.**

The stage descriptions of Demirjian *et al*. [5] are shown in Table 2.1.

Table 2.1: Stage description criteria according to Demirjian *et al.* [5].

Stage A:		Applies to single and multi-rooted teeth. In the superior aspect of the crypt, calcification resembling the shape of an inverted cone or cones is present. No fusion between the calcified segments has taken place.
Stage B:		Fusion between the different calcified segments has taken place with formation of the cusp/cusps. The occlusal surface of the tooth is outlined.
Stage C:	**1.**	The occlusal surface enamel formation is complete. Convergence and extension of the enamel towards the cervical margin is seen.
	2.	The start of dentine formation is seen.
	3.	The shape of the pulp chamber is curved towards the occlusal border.
Stage D:	**1.**	The formation of the crown is complete down to the level of the CEJ.
	2.	In uniradicular teeth the superior border of the pulp chamber appears concave. Pulp horns are present giving the pulp an umbrella shape. A trapezoidal shape appearance of the pulp in molars is present.
	3.	The start of root formation can be seen in the form a spicule.
Stage E:	**Single rooted teeth:**	
	1.	The pulp chamber walls are straight and the profile is only broken by the pulp horn. The pulp horn appears larger than in the previous stage.
	2.	The crown height is more than the root length.
	Molars:	
	1.	The start of the radicular bifurcation calcification can be seen in the form of a semi-lunar shaped calcification.
	2.	The crown height is more than the root length.
Stage F:	**Single rooted teeth:**	
	1.	The pulp chamber walls form an isosceles triangle. The apex is funnel shaped.
	2.	The crown height is less or equal to the length of the root.
	Molars:	
	1.	The radicular bifurcation calcification extends further down the root. The roots end in a funnel shaped outline.
	2.	The crown height is less or equal to the length of the root.
Stage G:		The root canal walls are parallel with an open apex(Distal root in molars)
Stage H:	**1.**	The apex of the root is closed (Distal root in molars).
	2.	The periodontal ligament has a uniform width around the root apex.

The stage system developed by Gustafson and Koch [74] was based on four easily recognized stages of tooth development. The aim of their study was to collect data to develop a practically useful schematic representation. The stages included:

I. Start of mineralisation
II. Crown completion
III. Eruption (Cusp(s) piercing through the gingiva)
IV. Completion of root formation

Deciduous and permanent teeth were used to develop this new stage diagram of Gustafson and Koch [74] (Fig. 2.6). The authors considered it important to present the stages practically and easily useable. It was emphasised that it was only possible to evaluate mineralisation on good quality radiographs.

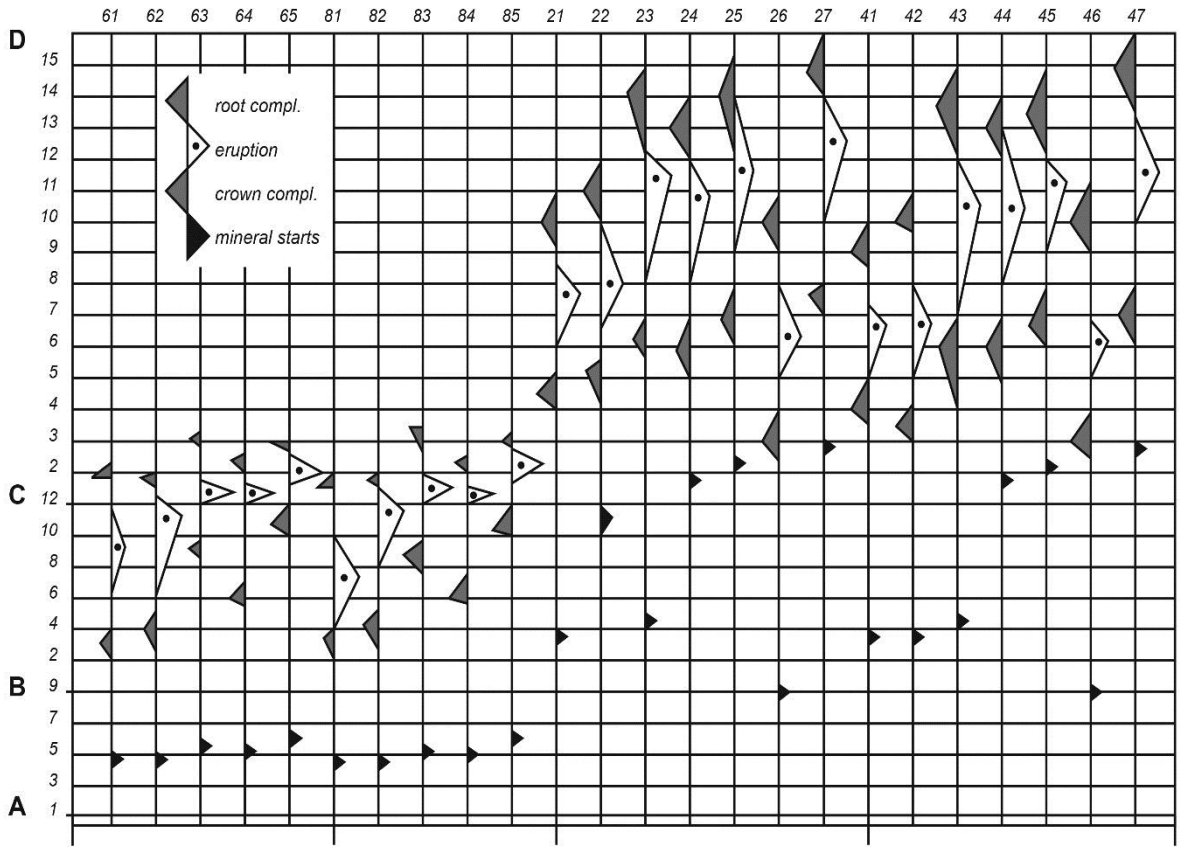


Figure 2.6: Gustafson and Koch's original dental development diagram. A-B: intrauterine life, B-C: first year of life and divided into 2 monthly intervals, C-D: 2-16 years of age and divided into 1 year intervals. The base of the triangle represents range and the peak mean age [74]**.**

The x axis (Fig. 2.6) represents the tooth number and the y axis the development time.

In a South African study, Nortjé [131] categorized the development of the right mandibular third molar root according to the cemento/enamel apical root length (Fig. 2.7). Five hundred panoramic radiographs of “Coloured” patients between the ages of 15 and 21 years were used. The study population was referred to as “Coloured” patients and was not clearly defined. In the Western Cape two distinct (non-European) cultures are present namely Cape Coloured and Cape Malay. The Coloured population in South Africa have mixed ancestry from various populations including Khoisan, Bantu-speakers, Afrikaner, English, Austronesian, East Asian and South Asian[137]. Root development was classified according to an eight grade classification system with marked criteria as follows:

Grade I: Root formation is visible with about 5 mm of root formed and the cleft present.

Grade II: The root has reached a ¼ of the anticipated root length.

Grade III: The root has reached a ⅓ of the anticipated root length.

Grade IV: The root has reached a ½ of the anticipated root length.

Grade V: The root has reached ⅔ of the anticipated root length.

Grade VI: The root has reached ¾ of the anticipated root length.

Grade VII: The root has reached the anticipated length but the apex remains open.

Grade VIII: The apex is closed with a uniform periodontal ligament surrounding the apex.

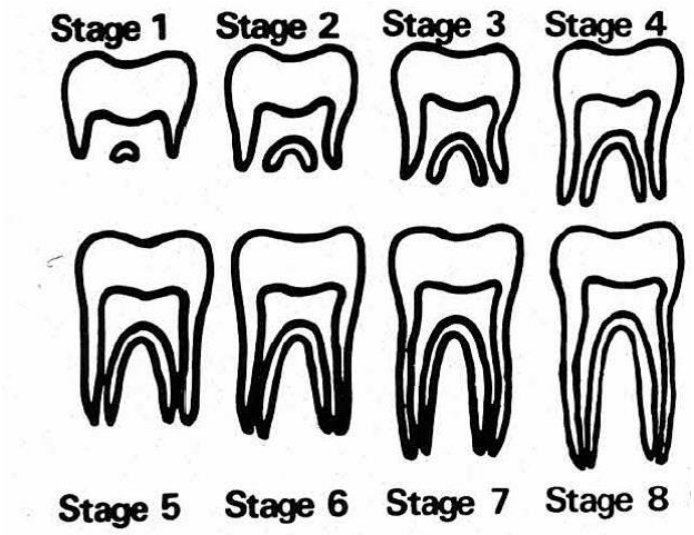


Figure 2.7: Nortjé's original classification stages for lower third molar development [131]

The results of the study displayed a large standard deviation with some age differences between the estimated age and the true age as large as 29 months. The reason for the large age discrepancy was believed to be due to an error of the classification system. It was suggested that fewer stages should be used in future to eliminate the discrepancies. A five stage system was proposed [131].
Harris and Nortjé [132] used the proposed five stage classification system to classify 407 Coloured individuals on panoramic radiographs.

Kullman *et al.* [133] categorized the development stages for the third molars into seven stages (Fig.2.8).

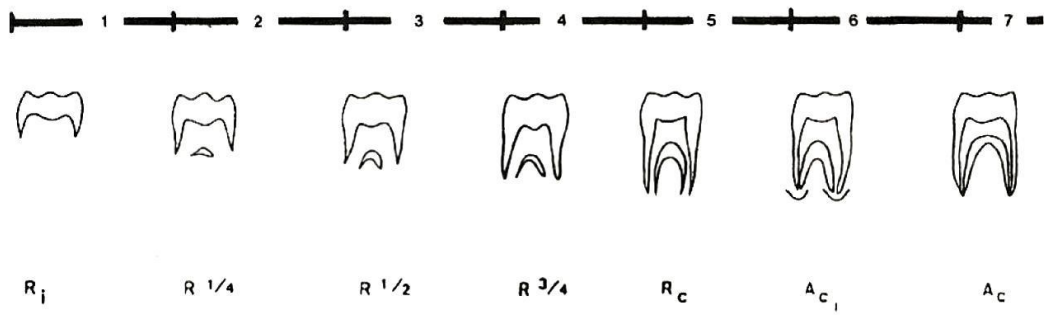


Figure 2.8: Kullman *et al's.* original classification stages for lower third molar development [133]**.**

In stage 1 the root development was initiated but the length was less than a ¼ of the anticipated root length. Stage 2 represents a root length of more than a ¼ but less than ½ of the anticipated root length. Stage 3 represents a root length of more than ½ but less than ¾ of the anticipated root length. In stage 4 more than ¾ has been formed but not the entire anticipated root length. In stage 5 the root length is complete but apex closure has not started yet. Stage 6 represents the start of apex closure. In stage 7 the apex is closed end root development is complete [133].
Köhler *et al.* [134] used a modified version of the original Gleiser and Hunt [129] development stages (Fig. 2.9). The stages comprised of 3 crown formation and 7 root formation stages.

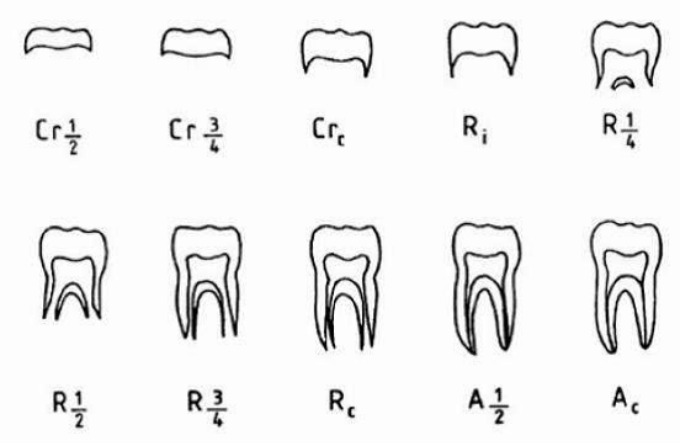


Figure 2.9: Köhler *et al's.* original classification stages for lower third molar development [134]**.**

The staging chart used by Demirjian [5] has been modified by Solari and Abramovitch [11] by adding intermediate sub stages to the latter stages of tooth development (Fig. 2.10). By adding two additional stages to the latter stages of dental root formation, higher accuracy can be achieved [11]. Difficulties were found to accurately distinguish between stages “F” or “G”. Two additional stages were added, F1 and G1, to make the transition towards apex closure easier to define and to achieve a higher level of accuracy when assigning a developmental stage to a developing tooth. Stage F1 is defined as having a root length of at least twice the crown length with a funnel-shaped opening at the root end. Stage G1 represents a tooth with parallel root canal walls and apices still slightly open. The periodontal

ligament space surrounding the apices is wider than 1 mm. By adding the two additional stages, a 10 stage scoring scheme was developed [11].

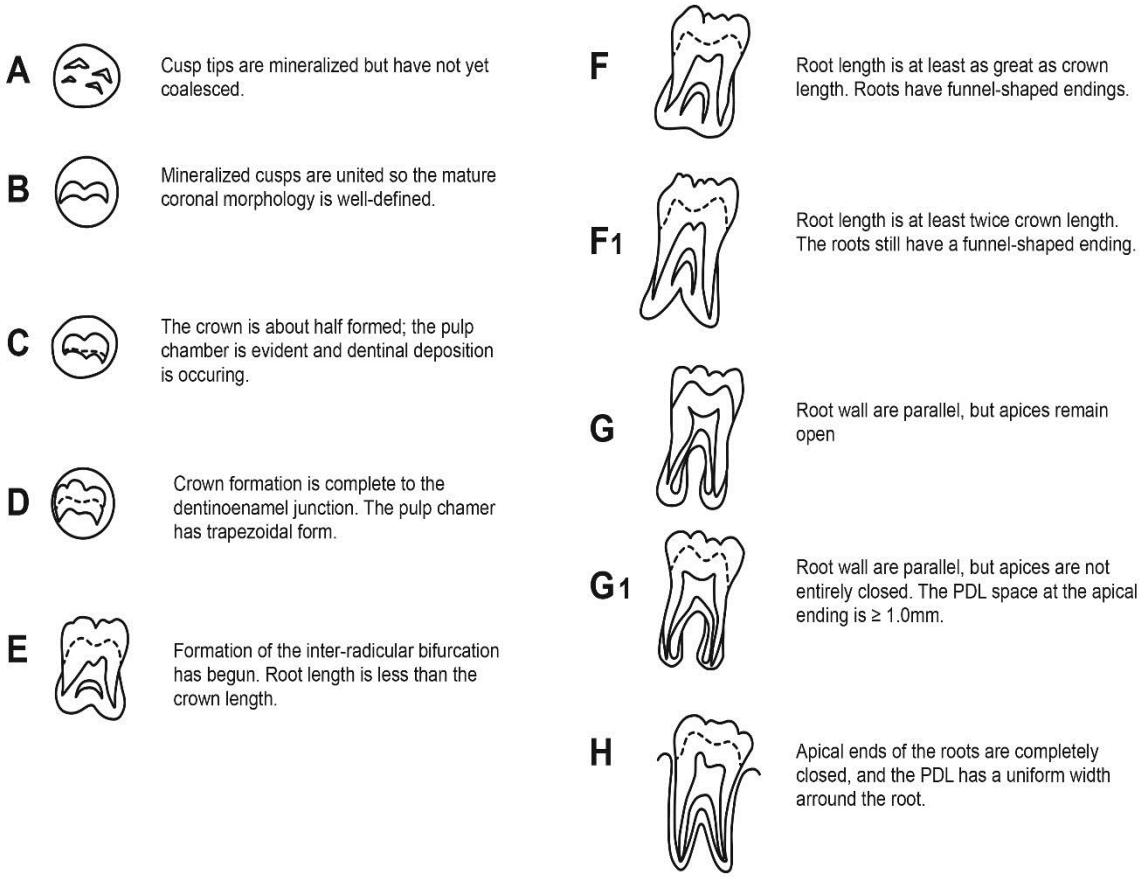


Figure 2.10: Solari and Abramovitch [11] original development stages modified from Demirjian *et al*. [5]**.**

2.4.2.3.2 Dental age estimation studies utilizing third molar development

In a study validating the common classification systems, the left mandibular third molar was assessed by using five different classification systems: Gleiser and Hunt [129], Demirjian *et al.* [5], Gustafson and Koch [74], Harris and Nortjé [132] and Kullman *et al.* [133]. Demirjian *et al.'s* [5] classification was found to be the most accurate [109]. The method of Demirjian *et al.* displayed the highest intra-class coefficient with the methods by Gustafson and Koch, and Harris and Nortjé yielding the lowest scores. Excluding Demirjian *et al.'s* stages, all the other methods used stages dependant on fractions of the length or anticipated future length. The use of ratios and measurements when evaluating third molar development were found to be less accurate in predicting age than the use of stages [138]. The stages described by

Demirjian *et al*. [5] are dependent on the changes and not the speculative estimates of root development lengths. Demirjian's stages can be used directly as variables, decreasing statistical errors and simplifying the statistical process [113].The classification methods with only a few stages has an undesirable impact if a wrong stage is selected. The result for an incorrect stage results in a greater corresponding error. The authors stated that Demirjian's stages should be used to evaluate third molar development in ageing individuals for forensic purposes [109].

Numerous researchers have investigated third molars to assess age in various population groups [11, 82, 108, 123, 127, 128, 133, 139–150]. The classification system by Demirjian *et al.* [5] was used in most of these studies to describe third molar development [82, 108, 128, 143, 145, 147–150]. Left and right mandibular third molars showed a uniform rate of development [147, 149] with the maxillary third molars developing faster compared to the mandibular third molars [82]. In general individuals with closed third molar apices (stage H) was found to be older than 18 years [146, 148] and the mean age of attainment for stage H was different among different populations [11, 140, 145]. Male third molar development tended to be faster compared to females [11, 82, 108, 123, 128, 140, 141, 145, 147, 149] and different population groups displayed variation in the degrees of sexual dimorphism. Studies comparing black American and white American individuals found that the black Americans achieved mineralisation stages faster than their white counterparts [82, 108, 123]. The group difference between black and white samples was found to be greater during crown formation (12%) than during root formation (5%). The formation of the lower third molar started about a year earlier in black individuals and the time spent on formation was about 1.5 years less compared with the white individuals [123]. The statistical analysis pertaining to these studies included the determination of probability values [11, 82, 108, 140, 142, 149], multiple and stepwise regression analysis [82], linear regression analysis [150], and logistic regression [140]. A cut-off of 0.08 for the third molar maturity index significantly increased the test sensitivity with regard to phase H. It was suggested that a maturity index of <0.08 was the most suitable method to determine if an individual is older than 18 years of age [142]. Suggestions from these studies were that more categories of crown-root formation are needed with finer discrimination between

stages [146]. More accurate results for estimating chronological age can be achieved by using multiple teeth rather than a single tooth [82] and the sex should be taken into account [144]. An improvement in accuracy and precision could also be achieved by making use of additional age indicators besides teeth from other skeletal maturity indicators such as hand radiographs [133].

2.4.2.3.3 Dental age estimation utilizing third molar development – the southern African context

A number of studies focusing on southern Africans have been done including two from Botswana [151, 152]. Cavrić *et al.* [151] used 1294 panoramic radiographs from Botswana individuals to determine the age of majority. The individuals were aged between 13 and 23 years and the left mandibular third molar was analysed. The aim of the study was to evaluate the cut-off value of the third molar maturity index (I_{3M})=0.08 as proposed by Cameriere *et al.* [142]. The value of 0.08 was used as a method to discriminate between minors and adults. The socioeconomic status or the ethnicity of the different groups was not evaluated. Individuals with an I_{3M}<0.08 and older than 18 years, are considered as true positives. Individuals with an I_{3M}<0.08 and younger than 18 years, are considered as false positives. Individuals with an I_{3M} ≥0.08 who are 18 years and older are considered being false negatives, and those with I_{3M} ≥0.08 who are younger than 18 years are considered to be true negatives. The likelihood ratios were determined for the positive and negative tests for the cut – off value of I_{3M} to determine the probability that an individual is older or younger than 18 years. The Bayes post-test was suggested to discriminate between individuals older and younger than 18 years. An accurate classification was possible for 1183 of the 1294 individuals and the I_{3M}<0.08 was considered to be positive. In males, the overall accurately classified fraction was 0.91 (95 % CI, 0.86 to 0.90) whereas it was 0.92 (95%CI, 0.90 to 0.93) in females. The cut-off value of I_{3M}=0.08 demonstrated the greatest specificity and Bayes' post-test probability. Less than 10 % incorrect classifications were made with the use of the cut-off value [151]. No statistically significant differences for the mean ages were found between males and females across all the evaluated I_{3M} ranges (p>0.05).

The same authors performed a cross-sectional study on a Botswana population comprising of 1760 individuals aged 6 to 23 years. Demirjian ***et al's*** [5] method was used to stage all permanent left maxillary and mandibular teeth including the third molars. A *t*-test was performed to compare the means of the chronological age across the developmental stages as well as between the sexes. In the maxilla, development in males for the third molar at stage F was significantly earlier compared with females. No statistically significant differences were found in the mean age within the developmental stage of lower third molars between the sexes. For the crypt stage of third molar development, the mean ages of attainment were 7.42 ± 0.22 and 7.49 ± 0.91 years in the maxilla and 7.18 ± 0.90 and 7.54 ± 1.08 years in the mandible in males and females, respectively. A wide age range for the final stage of third molar development was noted. The ages ranged from 14.67 to 22.51 years and 14.74–23.72 years in the maxilla and 14.67–22.60 years and 15.30–23.07 years in the mandible in males and females respectively [152].

Nortjé [131] evaluated 500 panoramic radiographs of Coloured South African individuals between the ages of 15 and 21 years. The right mandibular third molar was evaluated according to an eight stage grading system. Irregularities were found between stages 4, 5 and 6 in the upward projection of the mean ages according to the stages. The combining of these stages removed the irregularities. When stages 1 and 2 were combined the mean age for these two stages was 16.5 years (SD=1.3 years). Stage 3, and the combination of stages 4, 5, and 6 showed a mean age of 17.5 years (SD=1.3 years) and 17.8 years (SD=1.3 years) respectively. For Stage 7, when the root is fully developed but the apex remains open, the mean age of attainment was 18.5 years (SD=1.1 years). For Stage 8, when root formation is complete, the mean age of attainment was 19 years (SD=1.2 years). The author suggested that a five stage system rather than the eight stages should be used to classify third molar mineralisation and to minimize the standard deviation. The age of Coloured South Africans could be ascertained with 95% confidence within 2.4 years of the correct age. With a 99% confidence interval, the correct age could be determined within 3.6 years. The conclusion was that third molar root development was valuable to determine age in the age period of 15 to 21 years [131], but that it was highly variable.

Following on this, Harris and Nortjé [132] recorded measurements of the mesial root of the right mandibular third molar with a calliper on the same population. A five stage classification system was used and the descriptions for stage 1 and 2 were changed slightly [131]. The description for stage 1 was changed to "the root had reached one third of the final length", and for stage 2 one third was changed to half. The results showed that the direct measurement method compared favourably to the previous study by Nortjé [131] where a visual interpretation method was used. The five stage classification system eliminated the discrepancies and misclassification of stages compared with the eight stage classification system. With the direct measurement method, age could be ascertained with 95% confidence within 31 months, and at a 99% confidence level within 40 months of the correct age [132].

Olze *et al.* [153] examined 595 panoramic radiographs of black South Africans aged between 10 and 26 years. Demirjian ***et al.'s*** [5] eight stage classification system was used to evaluate third molar mineralisation. They found that mandibular third molars reached stage F 0.8 years earlier in men. No statistical difference was noted between the left and right third molar teeth. Sex-specific differences were found regarding tooth 38 for stage G: females reached this stage 1.5 years earlier compared with their male counterparts. Stages F, G, and H for all four third molars were reached earlier by females [153].

Phillips *et al.* [154] developed dental age estimation methods based on Moorrees *et al.* [99] and Demirjian *et al.* [5] on a South African population sample. This study was, however, restricted to 91 Zulu children from Durban, Kwazulu Natal, 472 males and 442 females of white and coloured South Africans from the Western Cape (called the Tygerberg sample) and 153 Indian South Africans. The samples were not separated into males and females. The data from each group were used to determine the error between the chronological age and the estimated age calculated with the Moorrees *et al.* and Demirjian *et al.* methods. The results showed that the Moorrees *et al.* method consistently underestimated the three groups compared with the Demirjian *et al.* method which overestimated the age [154].

Phillips *et al.* [155] then constructed the Phillips tables which incorporated the correction factors required to more accurately estimate the ages of three South African groups. Moorrees *et al.'s* [99] classification system was used to evaluate third molar mineralisation. The age range ranged between 7 and 16 years. Differences between the various groups regarding third molar development were incomplete because of the cut-off at 16 years. Third molar root formation is not complete at age 16 years and comparisons in development is difficult to make with other studies. The sexes were also grouped together for the study. The sample size in some of the age groups was very small with the Nguni group comprising of only 4 individuals between the ages of 16 to 17 years. The validity and accuracy of the age related tables should be questioned when such a small sample was used to construct the tables [155].

In order to assess whether there are differences between tooth development of children in various parts of the world, Liversidge *et al*. [156] compared White and Bangladeshi children from London with black South African and Coloured South African children from South Africa. Moorrees *et al.'s* [99] descriptive stage formation for third molar development was used to stage crown and root mineralisation. The average age of third molar stages was significantly later for the three groups compared with black South African (SA) children. In 44 out of the 45 comparisons made for combined sex group, black children displayed a significant difference in mean age compared with the other groups. No significant sex differences were found in third molar development of Bangladeshi individuals. Significant differences between the white South African males and females were found for the last root development stages. White South African males attained the root completion stages earlier than the females. Cape Coloured males attained the last seven stages before the females, with the age differences in two of the last three stages being statistically significant. An unusual finding was that black South African girls developed on average earlier for all the third molar formation stages compared with black SA boys [157]. The number of black South Africans used was 390 boys and 335 girls and the sample size for girls in the age range 21.00-21.99 and 22.00-22.99 years was only 10 and 1 individual, respectively. For a large number of black South Africans used in this study (n=431) the age was only recorded in years. The assumption was made by

the author that the individuals were on the half year. For example, an eight year old child was assigned an age of 8.50 years [157]. The low number of individuals used in some of the age categories and the inaccurate assumptions made while calculating the age of the individuals leads to inaccurate data for determining age in the population. The minimum age to reach stage Ac, distal apex closed based on Moorrees *et al.'s* [99], was 13.98 years for black males and 13.50 years for black females. The mean age to attain stage Ac was 19.31 years (SD=1.00 year) for black males and 19.27 years (SD=0.98 years) for black females. White males achieved stage Ac at a minimum age of 17.13 years and the mean age of attainment was 19.26 years (SD= 0.87 years). White females achieved stage Ac at a minimum age of 16.23 years and the mean age of attainment was 20.88 years (SD=1.31 years) [157].

The probability of an individual to be at least 18 years of age by root stage was calculated in black and Cape Coloured individuals. Males and females were compared by calculating the 95 % confidence interval (CI) of this ratio. A significant difference between black South Africans and the other reference groups were found at the group level. Because of the level of the standard deviation the authors concluded that a single individual from one group does not significantly differ from any other group. When estimating age at the individual level population differences at the group level is of no consequence. Features such as the range of age distribution, size and selection of radiographs of the reference sample was found to be more important than the ancestral or geographic group. In black South African males, the probability was calculated as 0.992 to be at least 18 years old for a fully developed left third molar. In black South African females the probability was 0.901 [156].

Recently, Esan *et al.* [158] constructed the WITS atlas from the results of a prospective cross-sectional study of 642 black South African children aged 5 to 20 years. The objective was to develop a population-specific atlas of tooth emergence and formation. Demirjian *et al.'s* [5] eight tooth formation classification stages were used and tooth stage tables were constructed for males and females in each age cohort. To construct the atlas, the relationship of the occlusal surfaces were checked

and compared between the radiographs and the intraoral findings. The authors justified the decision to combine males and females by stating that the difference between the attainment of a specific stage was not more than one developmental stage. The results showed that the most prominent difference between the London atlas and the WITS atlas was the timing of the third molar development. Third molars emerged at age 15.5 years in a black South African population and were in occlusion at age 17.5 years. The results are in contrast to the London atlas where emergence of the third molars was four years later and the roots were only fully formed at age 21.5 years. In the age cohort 17 to 17.99, a total of 45 individuals were included. In the maxilla and mandible, 37 and 39 out of the 45 individuals completed third molar root development, respectively. The next age cohort extended over two years and included 17 individuals between 18 to 20 years. In the maxilla and mandible, 11 out of the 17 individuals had completed third molar root development. Third molar root development completion for the 17 to 17.99 year age group was ± 80% compared with the older 18 to 20 year age group with 64%. The sample size in the age range 18 to 20 years was very small and the development of third molars will extend past the cut-off age of 20 years used in this study [158].
The atlas can be used as a tool to do an overall assessment of a child but not to accurately estimate age and especially around the critical age of 18 years.

2.4.2.3.4 Male–female differences

Dental maturity is more advanced in girls compared with boys when the permanent left seven mandibular teeth are individually evaluated [7, 13, 159, 160]. The pattern of mean difference between dental age (DA) and chronological age (CA) when girls and boys are compared also differs by age group [9]. Up to five to six years of age, dental development was similar in boys and girls for French Canadian children. This changed in the older ages where girls were always more developed than boys [159].

Males tend to reach third molar mineralisation stages at earlier chronological ages compared with females [11, 82, 108, 123, 128, 133, 139–145, 147–149, 157, 161]. In South African black individuals, however, females reached development stages earlier compared with males [153, 157]. No significant differences were found in third

molar development between males and females in Turkish [150], black Americans [82], and Botswana [151, 152] populations. Steroid –mediated adolescent growth phases can be a possible explanation for the unique pattern of earlier third molar development in males [123]. Two other postulations are that factors modulated by the X chromosome slow down third molar development in females or that the factors modulated by the Y chromosomes enhance the rate of third molar mineralisation in males [123]. Sex differences become less evident when a population is stressed and the size of males projects downwards to that of females. The hypothesis behind this phenomenon is that two X chromosomes provide better safeguarding against stress than the XY chromosomes in males [162].

2.4.2.3.5 Population differences

The faster tempos of growth in black Americans compared with white Americans [163, 164] and black South Africans compared to Japanese and German subjects have been widely researched [135]. Other common measures of physiological age such as hand –wrist development and bone age indicate that black populations develop earlier than white populations [84, 165–167]. Black South African children compared with French-Canadian children showed an average advancement of 0.8 years for boys and 0.5 years for girls when the dental age was compared for individuals between 6 and 16.9 years [13].

Olze *et al.* [153] reported that mineralisation stages were reached earlier by a black South African sample when compared with a German sample [153]. The study presented means and standard deviations, median values and the lower and upper quartiles separately for both sexes [153]. The validity of the values should be questioned due to the distribution of the sample size. Some ages only had two individuals in the sample size making the values statistically insignificant.

Noticeable differences between black American and white individuals in third molar development were also observed [108, 163] with black American individuals achieving developmental stages significantly earlier compared with white individuals [115, 168].

The black individuals tend to be more advanced in permanent tooth emergence with the largest absolute differences displayed by the mandibular third molars (5.6 years) compared with maxillary third molars (3.7 years) [163]. Black individuals achieve the early developmental stages noticeably faster than white individuals [108, 115]. It is suggested that ancestral differences are greater during the earlier crown formation stages. The results showed that black males achieve a tooth formation stage 4% ahead of white males, and black females 6% ahead of white females [115].

Liversidge [157] described population differences as the result of earlier initiation and completion of third molar development in black South Africans. Third molar formation stages and descriptive criteria by Moorrees *et al.* [99] was used. The mean age difference between black individuals and other groups including a white British group living in London was significant in 44 out of 45 comparisons. The mean age for entering the crypt stage for the black and white groups was 7.16 years and 9.06 years, respectively. Black individuals in a combined sex group reached stage Ac (apex closed) at age 19.27 years compared with the white group reaching this stage at age 20.16 years. The cumulative frequency distribution for all stages demonstrated distinct patterns for stage Rcl (beginning of root furcation visible) and stage A1/2 (apex of distal root partially open, periodontal ligament slightly wider at distal aspect) in the black individuals. The slope for stage Rcl was far steeper compared with the other stages and stage A1/2 had overlap with stage Rc (walls of the distal root canal are parallel and full length with rounded/blunt edges). The author concluded that stage A1/2 might not be a suitable separate stage for this group.

The studies quoted above, in general, have shown a great variability in the dental maturation process when different population groups are compared. Results from several authors showed inaccuracy when another population is assessed using Demirjian's method [8, 9, 13, 116, 169]. The creation of representative databases for each population is necessary to reach a better understanding of human dental maturation related to age. In the South African context only one study assessed third molar development in a black South African sample [153]. The validity of the study is however questionable due to the low number of subjects used. A study using a large

representative sample size is necessary to develop an accurate age estimation method based on third molar maturation.

2.4.2.3.6 Probability of being 18 years of age

Several studies have determined the probability of an individual being 18 years, including Japanese [149], Austrian [170], Korean [143], German [171], Hispanic [11], U.S Hispanic [128], American [82], American black and white [108] populations. Most of these studies [143, 149, 170, 171] made use of the Demirjian developmental stages for classification. The probabilities differed between the maxillary and mandibular third molars [149]. The results showed that most populations had a probability of more than 97 % of being 18 when third molar development has reached stage H [149, 170, 171].

Reference material was established for third molar development in a Japanese population by Arany *et al*. [149]. Panoramic radiographs of 1282 Japanese individuals between the ages of 14 to 24 years were used. The probability that a Japanese juvenile would be older than 14, 16, 18 and 20 years was predicated. The results showed that for most stages males reached the indicated stages earlier. The probabilities also differed between the maxilla and mandible. The probability for a Japanese juvenile to be older than 18 years when the mandibular third molar is considered was 98% for males and 99% for females [149].

The probability for an Austrian individual to be older than 18 years was determined for medico legal purposes [170]. The cross-sectional study included 610 panoramic radiographs of 275 males and 335 females between the ages of 12 and 24. The likelihood values were calculated using Demirjian's stage H. When the right mandibular third molar is considered the likelihood to be older than 18 years based on Demirjian's stage H was 100% for both sexes. For the left mandibular third molar the likelihood was 99.1% and 98.7% for males and females respectively to be older than 18 years based on Demirjian's stage H [170].

Digital panoramic radiographs of 2360 German individuals between the ages of 15 to 22 years were used to determine the usefulness to which the third molar calcification stages can be used as additional criteria for age estimation. The probability for an individual with stage H (Demirjian's method) being older than years, as well as the corresponding confidence interval was calculated. The results showed that all individuals with the lowest calcification stage H of all present molars were older than 18 years. The authors concluded that even if the lowest calcification stage of all present third molars is stage H, additional methods must be applied when estimating age for forensic purposes [171].

Cameriere *et al.* [142] used a different method to evaluate the left mandibular third molar of an Italian population. Root development was recorded as root completely closed (maturity index = 0), or if the roots were open the sum of the distances between the inner sides of the two open apices divided by the tooth length were calculated. The results showed that if the root apices of the third molar are closed then there is a high probability that the individual is at least 18 years old. For a terminal stage H the probability of the individual to be 18 years or older is 0.98. The study concluded that a maturity index of < 0.08 is the most suitable method for determining if an individual is older than 18 years of age [142].

The Cameriere *et al*. [142] cut-off values were used to discriminate between minors and adults in a black African population in Botswana [151]. The results showed that the cut-off value of 0.08 may be recommended for discriminating 18 years or older. The 0.08 cut-off value showed the best specificity and Bayes' post-test probability and an accurate classification of this cut-off, showed less than 10 % incorrect classifications [151].

Liversidge *et al.* [156] included black and Coloured South Africans in a reference sample of 1663 panoramic radiographs. The root stage was used to calculate the probability of an individual in the sample to be at least 18 years. The data revealed no significant differences between the sexes and the data were combined. The positive and negative test results were calculated for each mandibular molar stage. The results demonstrated that a mature third molar apex is more than 13 times more

likely to occur in an individual of at least 18 years of age compared with someone younger than 18 years of age. The probability of being at least 18 in the reference sample if the third molar was mature was 0.945. Early root stages had high sensitivity and ruled out being at least 18, while the two last stages had high specificity (third molar mature and being age being at least 18 years was 0.96) ruling in the age category of being at least 18 years of age. The authors concluded that once the third molar apex is mature, age cannot be estimated and that likelihood calculation of being at least 18 years is an appropriate measure at the individual level [156].

2.4.3 Skeletal age estimation in living individuals

Age can also be estimated with making use of other methods:

- A physical examination to determine the weight and signs of sexual maturation
- Radiographic examination of the left hand
- Radiographic or computed tomography of the clavicles if skeletal development of the hand is complete [18, 172]

Useful parameters in age estimation include anthropometric data in combination with tabulated values for body height and weight, as well as the development of sex characteristics and the development of epiphyseal/apophyseal joints [144]. The guidelines recommend that when results are interpreted, the data should be compared with reference studies. The results of each assessment should be analysed separately and the age estimate should be recorded as the degree of probability. Age estimation protocols can combine methods to achieve a higher accuracy. Skeletal methods are non-invasive and make use of radiographs and computed tomography to determine the degree of ossification of the hand-wrist [173, 174], medial aspect of the clavicle [175–177], and costal cartilage of the first rib [178, 179]. In this study a novel method of using the cervical vertebrae will be introduced, and is the only skeletal feature that will be discussed in detail.

Maturation of the skeleton follows clear and distinct stages for all population groups. The decisive rate of skeletal maturation is, however, strongly associated with the socio-economic status of a given population [180].

2.4.3.1 Joint structures

Joints can be divided into diarthrodial joints, fibrous synarthroses and amphiarthrodial joints or symphyses. The diarthrodial joint is cavitated to form a freely moving unit connecting two bones. Their articulating surfaces are covered by hyaline cartilage; two exceptions are the temporomandibular and sternoclavicular joints which are covered by fibrocartilage. Fibrous synarthroses are non-movable joints with dense collagenised fibrous tissue [181].

The amphiarthrodial joints or symphyses are characterised by limited movement and typical examples are the intervertebral disc and pubic symphysis. The intervertebral disc consists of a fibrocartilaginous complex, and is situated between the two articulating surfaces of the vertebral bodies. The intervertebral discs add to the stability and mobility of the spine as well as the spread of the weight load. Disc height varies between segments of the spine, with the cervical and thoracic discs being flatter compared with the lumbar region. Disc height also varies from anterior to posterior with discs becoming thinner with age. Intervertebral discs are divided into two components: the annulus fibrosus or the outer ring and the nucleus pulposus - the gelatinous core. Collagen fibres extend from the vertebral body to the adjacent vertebral body in an oblique manner and the fibres of the annulus are attached into the bony plates by Sharpey's fibres. The position of the nucleus pulposus is eccentric and positioned more towards the posterior with the nucleus tissue separated from the adjacent vertebral bone by hyaline cartilage. The hyaline cartilage extends to the inner margins of the annulus [181–183].

2.4.3.2 Development of the vertebrae

Vertebral development is initiated by the movement of sclerotomal cells round the neural tube and type II collagen signal expression during the blastemal stage. During

stage 17 of vertebrae development, chondrification initiates and one cartilage anlage forms each centrum. Initiating from the base, each half of the neural arch is chondrified. The process extends dorsally into the laminae and vertically into the pedicles. When stage 23 is reached, 33 cartilaginous vertebrae are present but the spinous processes have not developed yet. Fusion of the spines only occurs after the fourth month in utero with the chondrification of the transverse and articular processes occurring in continuity with the neural arches [184].

The arches unite in the lumbar region first followed by the thoracic and cervical regions. The centra of the upper cervical vertebrae unite with the arches in the third year. The superior and inferior surfaces of the bodies and the apices of the transverse and spinous processes consist of cartilage until puberty. Five additional secondary ossification centres appear: one in each transverse apex, one in the spinous process, and two annular epiphyses (ring apophyses) associated with the upper and lower circumferential parts of the vertebral body. The annular epiphyses have articular facets closely related to the vertebrae and fusion takes place at age 25 years with the vertebral body [184].

Information regarding the maturation of the vertebral epiphysis is limited, and most anatomy and osteology textbooks merely state that vertebral ring epiphyses appear at puberty and complete their union by the age of 25 years [185, 186]. The vertebral ring apophysis is described as a thin cartilaginous mound that encircles the borders of the inferior and superior surfaces of the vertebrae. These rings develop outside of the epiphyseal plates [187]. The branching fibres of the longitudinal and intervertebral ligaments insert into the individual vertebrae at this site. Traction takes place at the point of insertion by these fibres. The development of the ring takes place within the peripheral osseous depression and cells concentrate within this area. The depression is only evident radiographically and histologically. Calcification of the circle ring begins at about six years of age. Ossification of the ring begins at about age thirteen, and it fuses with the vertebral body at about seventeen years. Histological sections of the area showed that fusion was complete at age eighteen, and that the ring could not be identified histologically at age twenty [187, 188]. As these vertebral rings fuse relatively late, they can provide valuable information

regarding the age of young adults [189]. The fusion of the superior and inferior epiphyses of the thoracic and first lumbar vertebral centra, for example, has been shown to be practically usable to establish skeletal age in teenagers and young adults [189, 190].

2.4.3.3 Radiology of the cervical vertebrae

The use of the cervical vertebrae in growing subjects as a biological indicator to assess skeletal maturity has gained interest during the past few decades. Most studies compare the cervical vertebral maturation (CVM) in lateral cephalometric radiographs to hand-wrist radiographs [14–16, 191, 192]. The effective radiation dose for a cephalometric radiograph varies between 2- and 3 µSv [193]. The aim of these studies is to correlate skeletal maturity on the radiographs of two different regions and to assess whether hand-wrist radiographs can be replaced by using lateral cephalograms. Cervical vertebral maturation is assessed to identify the optimal treatment timing in dentofacial orthodontics [194]. Skeletal maturity can be evaluated in an objective manner with the use of cephalometric radiographs [16].

The vertebrae are visible from birth on conventional plain spine radiographs (anterior-posterior and lateral views), CT, and MRI. Conventional radiographs are one of the preferred modalities to view the bony spine. For finer evaluation of the bone, CT can be used. Soft tissues of the spine (ligaments, discs) and the spinal cord are visible on CT images. To evaluate the soft tissue structures MRI is the modality of choice but is not the modality of choice to demonstrate bone detail [195, 196].

At birth some ossification is present in all vertebrae from C1 to the sacrum. These ossification centra are visible on conventional radiographs. The odontoid process, the body of C1 and the coccyx are not seen at birth [197]. At age 2 to 3 years the anterior-posterior diameter of the vertebral body is greater than the intervertebral disc height. An increase in density can be observed due to the continuation of ossification. At age 5 to 8 years, step like recesses appear on the superior and inferior anterior surfaces of the vertebral bodies. The protrusions appear as an

anterior lip on lateral radiographic projections. The protrusions are referred to as the ring apophyses and represent the annular rim of cartilage. The cartilage rim develops outside of the cartilaginous end plate and extends into the upper and lower borders of the vertebral body. The ring apophyses do not take part in the growth of the vertebral body. During ossification the annular ring apophysis is visible on radiographs at the superior and inferior borders of the vertebral body. During calcification the ring apophysis begins to unite with the vertebral body. This process takes place over the time period of age 18 to 25 years. The anterior lip of the vertebral body disappears by the age of 10 to 13 years but the calcification and later ossification can remain to the age of 18 to 25 years [187, 195, 196]

Modifications in the size and shape of the cervical vertebrae can be used to assess growth and this can be analysed on lateral cephalograms [14, 194, 198]. Lateral cephalograms form part of the evaluation process for orthodontics patients and the shape of the vertebral bodies of C1 to C5 is clearly visible.

2.4.3.4 Cervical vertebrae maturation

The shape of the vertebral bodies changes with maturation [14, 198]. During the first stages of maturation the vertebral bodies are wedge shaped and the superior border is tapered from posterior to anterior. The vertebral bodies then change in shape from rectangular, with the greater length in the anterior to posterior plane, to square and lastly to rectangular with the height of the body greater than the width. The concavity of the inferior border during cervical vertebral maturation stages has also been used in the classification of vertebral maturation [14, 198]. It was found that the more concave the inferior border, the greater the cervical vertebrae maturation [15, 199]. Using the criteria defined by Lamperski [198] and developed by Hassel [200] and Hassel and Farman [14], CVM can be categorized into six stages (Figs. 2.11, 2.12).

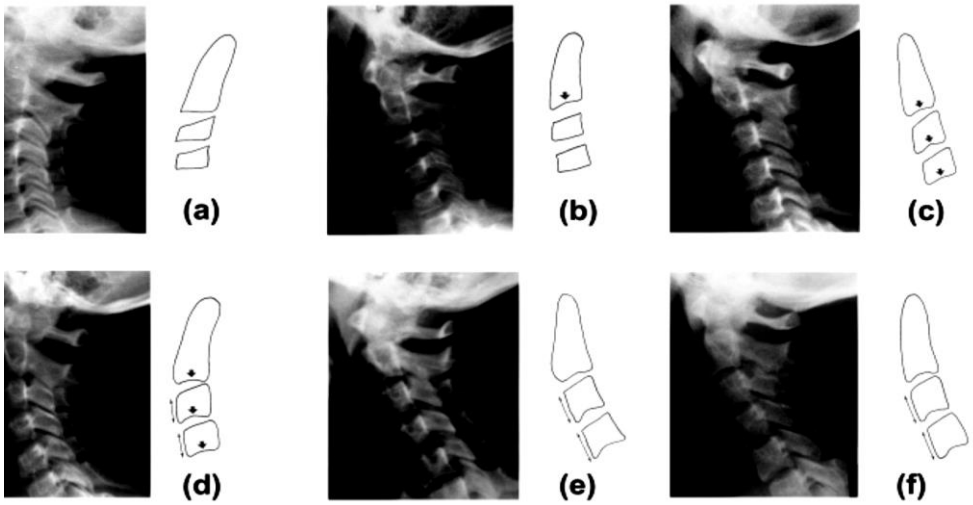


Figure 2.11: Categorisation of the cervical vertebrae maturation into six stages [199].

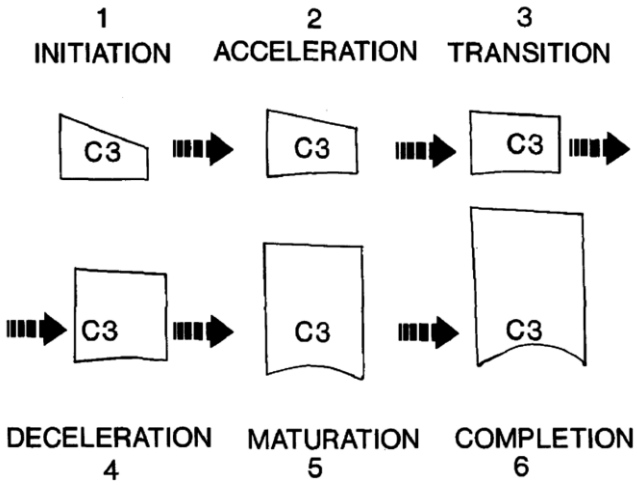


Figure 2.12: Cervical vertebrae maturation using C3 as a guide [14].

In a study sample of 220 white subjects aged 8 to 18 years from Northern European descent, skeletal maturation was assessed by comparing hand-wrist radiographs with lateral cephalograms. Three areas of the cervical vertebrae were assessed and included the dens, the body of cervical vertebra (C3) and cervical vertebra (C4). The results showed that six categories of the cervical vertebrae maturation index (CVMI) could be identified and categorised. During skeletal maturity the vertebral bodies of C3 and C4 changed from wedge shaped, to rectangular, to square with the dimensions increasing vertically compare to the horizontal dimensions. As the individual matured the inferior borders of C2 and C3 became more concave. The

conclusion of the study was that the orthodontist can use the CVMI to establish if the individual will still grow and to factor this into the treatment plan [14].

The Cervical Vertebral Maturation (CVM) method was also used to detect the peak in mandibular growth based on the analysis of C2 to C4. The morphology of C2 to C4 on six consecutive cephalograms was evaluated for the presence of a concavity at the lower border and the shape. The results revealed that no significant difference was present between cervical vertebral stages 1 and 2 (Cvs 1 and Cvs 2) as defined by the CVM method. The inferior border of the second cervical vertebra being concave was found not to be a distinctive feature. The newly described Cervical Vertebral Maturation Stage 1 (CVMS I) merged the two formally described stages Cvs 1 and Cvs 2. A concavity at the lower border of C3 is the characteristic associated with the stage preceding peak mandibular growth (Cvs 3, actual CVMS II). The method described CVMS III as having concavities at the lower borders of C2, C3 and C4. The shape of C3 and C4 are rectangular horizontal in shape. For stage CVMS IV, the concavities for C2 to C4 remains but at least one of the bodies of C3 or C4 is squared in shape. During stage CVMS V the concavities at the lower border of C2, C3 and C4 are still evident with at least one of the bodies of C3 and C4 being rectangular in shape. The new classification system reduced the number of stages from six (Cvs 1 through Cvs 6) to five (CVMS I through CVMS V). Peak pubertal growth was reached between stages CVMS I and CVMS II. The authors concluded that the method can be used to evaluate skeletal maturity on a single cephalogram if cervical vertebrae C2 to C4 is visible [201].

Cephalometric radiographs of 176 Japanese girls aged 7 to 14.9 years were used to develop a formula to obtain cervical vertebral bone age. A second group of 66 girls were used to determine the reliability of cervical vertebral bone age compared with bone age by the Tanner-Whitehouse 2 method. The aim of the study was to establish a new method for evaluating skeletal maturation using cephalometric radiographs. The following measurements were taken: anterior vertebral body height, vertebral body height, posterior vertebral body height, and antero-posterior vertebral body length of the third and fourth cervical vertebrae. The ratios of these parameters were calculated and a formula for obtaining cervical vertebral bone age

was determined from the ratios. Chronological age using a stepwise multiple regression analysis was also determined. The authors concluded that an atlas approach cannot be used to evaluate growth in an objective and detailed manner and that the method used in this study is more impartial. Only the C3 and C4 were evaluated in this study and ratios were used to calculate cervical vertebral bone age. The results showed that the correlation coefficient between cervical vertebral bone age and bone age according to the Tanner-Whitehouse 2 method was significantly higher than between cervical vertebral bone age and chronological age. Cervical vertebral bone age on cephalometric radiographs was found to be as reliable as the Tanner-Whitehouse 2 method on hand-wrist radiographs [16].

The relationship between chronological age and maturation of cervical vertebrae was investigated in a Turkish population consisting of 213 males and 290 females aged 5.3 to 24.1 years. The Hassel and Farman [14] method was used to evaluate the development. Stage 1 was found in females younger than 12 years of age. Stages 1 and 2 were found in males younger than 15 years of age. Stages 2 and 3 were found in females younger than 15 years. Stage 6 was found in females older than 12 years of age and in males older than 15 years of age. The results demonstrated a high correlation coefficient between chronological age and cervical vertebrae skeletal maturation. The authors concluded that the cervical vertebrae stages method can be used as a maturity indicator to determine the pubertal growth spurt [191].

In a Brazilian population, the cervical vertebral bodies of 128 females and 110 males were traced and measured, and regression formulas were developed to determine cervical vertebral bone age. The reliability of the results was verified by comparing the results to another sample of lateral cephalograms and hand-wrist radiographs. The Tanner et al.[202] method (TW3) was used in the hand-wrist radiographs to determine the bone age. The cephalometric radiographs were manually traced and the following measurements were taken: anterior vertebral body height, vertebral body height, posterior vertebral body height, and antero-posterior vertebral body length on the third and fourth cervical vertebrae. A multiple regression analysis was developed for males and females to determine cervical vertebral bone age. In the female sample the anterior vertebral body height, vertebral body height and posterior

vertebral body height increased in an accelerated manner between the ages of 10 and 13 years for cervical vertebra C3. Anterior vertebral body height, vertebral body height and posterior vertebral body height increased in an accelerated manner between the ages of 11 to 13 years for cervical vertebra C4. In the male sample the anterior vertebral body height, vertebral body height, posterior vertebral body height, and antero-posterior vertebral body length of C3 increased in an accelerated manner between the ages of 12 to 15 years. No increase was noted for C4. The authors concluded that cephalometric radiographs are reliable and can be applied to both female and male subjects to evaluate skeletal maturation [203].

In a Chinese study, cephalometric radiographs of females aged 12 to 15 years, and males, aged 12 to 17 years, were evaluated to determine the validity of the cervical vertebral maturation method as an indicator of skeletal age. The results were correlated to the hand-wrist method. The Bacetti *et al.* [194] method was used to evaluate the cervical vertebral maturation and the hand wrist method was evaluated according to the Hägg and Taranger [204] method. The authors concluded that the cervical vertebral method showed a high correlation with the hand wrist method in their population. Cervical vertebral stage 3 was found to be around the peak of the growth spurt [192].

In a longitudinal study lateral cephalograms were evaluated of Turkish females aged between 9 and 16 years. Cervical vertebral maturation was assessed according to the six categories described by Hassel and Farman [14]. The greatest amount of growth occurred in C2 with a length increment of 11 mm and C1 and C4 reached their peak growth rate at 11.5 years. C3 reached its maximum growth rate at age 10.5 years. A linear growth rate curve was displayed by C2. By age 15.5 years the length increment rate decelerated to 0-0.2 mm/year. After stage 6 (15.5 -16 years) length increases ceased. The study concluded that the cervical vertebrae demonstrate morphological changes according to the six stages and thus can be used to determine skeletal maturation [199].

2.4.3.5 Vertebral ring apophysis

The later fusing superior and inferior vertebral rings can produce valuable information regarding age estimation of young adults [189]. The fusion of the superior and inferior epiphyses of the thoracic and first lumbar vertebral centra is a practical method to establish skeletal age estimation in teenagers and young adults [189, 190].

Vertebral rings have also been used in ageing young adult American males [205] and active union was found to occur between ages 19 and 21 years. Union was complete at age 24 years for all cases. This study was limited to males and ancestry differences were not mentioned [205].

The first two lumbar vertebrae have been used for age estimation. The cervical vertebrae were not included due to the difficulty in obtaining the specimens during autopsy [189]. The scoring for the progress of union was done according to a classification system (stage 0-stage 3) with set criteria. The scoring system included the following criteria for each stage:
Stage 0: No union has taken place. The superior and inferior surfaces of the centrum are billowed and striated with rounded edges. The surface of the bone is irregular with no evidence of ring adhesion.
Stage 1: Union of the epiphysis has begun or is in the process of uniting to the central part of the vertebra. The epiphyseal ring is only attached in some places. The appearance of the ring is thin.
Stage 2: Union is almost completed or recently completed. In the early phase the spaces between the epiphysis and vertebral centrum are reduced. A shallow groove is present between the epiphysis and the centrum.
Stage 3: Union is completed and the vertebra appears as one piece. Obliteration of the line demarcating the epiphysis from the centrum has taken place with only a faint scar occasionally still remaining [189].

The results demonstrated that complete union was found in 30 % of the 17 to 18 year olds, in 18% of the 19 to 20 year olds and in 22% of the 21 to 22 year olds. The

high percentage for the 17 to 18 year age group was due to one individual who showed complete union for all the investigated vertebrae. For the individuals 29 years and older, 99.4 % showed complete union. No attachments of epiphyseal rings were found before 16 years and 4 months in males and 14 years in females. For stage 2 the youngest female to show almost complete union was 17 years and 3 months and the youngest male 17 years and 8 months. The oldest female was 26 years and 10 months and the oldest male 26 years and 4 months. Complete union (stage 3) in any vertebrae was observed in the youngest female at age 18 years and the youngest male showed complete union at age 18 years and 9 months. The youngest female to demonstrate complete union for all vertebrae was at age 25 years. The youngest male to demonstrate complete union for all the vertebrae was age 24 years and 2 months. No statistically significant differences were found between the tempo and timing of union between sexes and the reason was attributed to the small sample size. During stage 2, the researchers observed that union began earlier in females but the males “caught up” before stage 3 was reached. Comparison between ancestry groups was not possible due to the small sample size. No significant differences were found between the sequence of union between the superior and inferior ring epiphyses. The authors concluded that vertebral ring epiphyseal union correlate well with age. Age could be calculated with 99.9% confidence within a range of plus and minus 2.566 years for vertebral ring epiphyseal union [189].

Ring epiphyseal union were studied in skeletal samples using the four-stage McKern and Stewart [205] method. A Pearson’s correlation showed that epiphyseal vertebral ring union were fairly highly correlated with age at death. The correlation was higher for females and the authors attributed the findings to the age distribution of the sexes. Significant sex differences were found but the sample sizes were too small to analyse ancestry differences. In females, for the age range 14 to 18 years, stage 0 (no union) was present up to age 18 years. Stage 2’s earliest appearance was at age 14 years with stage 3 (complete) being absent for the age range 14 to 18 years. In males for the age range 17 to 22 years epiphyses were present for stages 0, 1, 2, and/or 3. Inter-observer agreement was good using the four stage method and the

method fared well compared to other skeletal and dental age estimation methods [206].

Age estimation was studied from the stages of epiphyseal union using the pre-sacral vertebrae of Portuguese individuals. A three-stage scoring method was used to score the degree of fusion of the epiphysis: 1) no union, 2) partial union, and 3) completed union. For the cervical vertebrae C2, C3, and C4, stage 1 was present in samples younger than 18 years of age. The age range for stage 2 was between 14 and 21 years and stage 3 was only present in samples older than 15 years. The sexes were pooled together and no sex differences were observed for the cervical vertebrae. The conclusion was that the data provide additional information that can be used in a variety of settings [207]. Other studies regarding the vertebra-, used skeletons and the stages of epiphyseal ring union were not addressed [208, 209].

2.4.3.6 Combining age estimation methods

The wide prediction intervals obtained with age estimation methods can possibly be reduced by combining dental observations with skeletal information. The Study Group on Forensic Age Diagnostics, with reference to the legal and ethical implications, recommends that a forensic age estimate should combine the results of a physical examination, anthropometric analysis, sexual development, radiograph of the hand–wrist, and a dental examination on a panoramic radiograph [4]. Most methods give standard deviations and standard errors and the estimation will range, for example, from 17 to 18.5 years. The probability of a person actually having reached age 18 years is, however, a more practical and helpful method to make decisions on reaching age of legal responsibility [4].

Hand-wrist development stages have been shown to be closely associated with the pubertal growth spurt and can be used as an indirect method to assess maturity [204, 210]. Cameriere and Ferrante [211] used a combination of hand-wrist and tooth development on a group of Italian children and developed a regression equation:

$$\text{Age} = 4{:}619 + 0{:}401g + 0{:}551N_0 - 0{:}647s + 7{:}163Bo/Ca - 0{:}123N_0s$$

where s equals the sum of the normalized open apices, (N_0) the number of teeth with complete root development and Bo/Ca the ratio between carpal bones area and carpal area. The mean prediction error was 0.553 years and a standard error of estimate was 0.73 years.

Cervical vertebrae bodies were evaluated on cephalometric radiographs according to the methods described by Baccetti *et al*. [194], Seedat *et al*. [212], Caldas *et al*. [203] and Rai *et al*. [213]. The inclusion of information obtained from the cephalometric radiographs to third molar development according to the modified Köhler *et al.* [134] method improved the explained variance in age by 48%. The study concluded that, by adding the registrations obtained of the cervical vertebrae development to third molar development stages, age predictions can be significantly improved [20].

In a Japanese sample, panoramic radiographs, lateral cephalometric radiographs, frontal cephalometric radiographs, and left hand and wrist bone radiographs were retrospectively collected. The staging technique of Demirjian *et al*. [5] was used to stage the left seven permanent mandibular teeth and the third molars were staged additionally according to Köhler *et al*. [134]. The third cervical vertebral body was staged according to the Hassel and Farman [14] technique and the cranial measurements obtained from the frontal cephalometric radiographs. The Greulich and Pyle [86] method was used to register the hand and wrist bone development. The classification system was condensed into four stages from the original stages and the Bayes' rule was applied. The root mean square error values obtained from the continuation ratio model were significantly reduced with the addition of the skeletal features to the dental development. The authors concluded that combining dental and all the skeletal indicators improved the age prediction in both sexes [21].

Overall, very few studies in living individuals employ a combination of methods for age estimation, similar to what is the case in the field of forensic anthropology involving skeletal remains [214, 215]. No clear guidelines exist as to how to statistically combine different indicators of age [215–218]. While some authors, such as Konigsberg *et al.* [219] and Sironi *et al.*[220] argue that a Bayesian approach should be used, others [214, 216, 221] are of the opinion that multivariate regression

models should be employed and that a Bayesian approach holds no advantage over regression.

In a recent paper Shi *et al.* [214], for example, combined DNA methylation markers with skeletal and dental ages and the age estimation model was build using multivariate linear stepwise regression. The accuracy of the age estimation method was evaluated by the mean absolute error (MAE), the root mean square error (RMSE) and the coefficient of determination (R^2). Cardoso *et al.* [215] used logistic regression to determine the probability of an individual being 16 years of age or older. Regression formulae thus seem to be one of the preferred methods, mainly because it is easy to use and clearly understandable by most practitioners.

Kumagai *et al.* [21] used Bayes' rule with a multivariate continuation ratio model for the distribution of the dental scores. The authors concluded that the age estimation performance increased but also added that a limitation to the study was the small, heterogeneous sample. A Bayesian approach has been used, for example, in the ADBOU programme [222] that is very often used by forensic anthropologists, and has also been advocated by Konigsberg *et al.* [223] in a paper evaluating the status of third molar development. Thevissen *et al.* [224] compared third molar information using classical regression models and a Bayesian framework. The authors concluded that that Bayesian approach comes with higher computational complexity and that in general the Bayesian approach did not outperform the classical approach. No strong reduction in differences between the observed and predicted age were found.

From the literature it seems as that, if the aim is to obtain an actual estimate of the age of an unknown individual, a multivariate regression model should be considered [70, 140, 142]. A Bayesian framework can be considered to obtain the probabilities that an individual is younger or older than a given age threshold [225, 226], which may be more appropriate in living individuals. Both methods are extensively used.

Chapter 3: Estimating age and the probability of being at least 18 years of age using third molars: a comparison between Black and White individuals living in South Africa

3.1 Introduction

Examination of the dentition with accompanying radiographs is a suitable method for age estimation [18, 227]. Chronological age can be defined as the amount of time that has passed since birth, while biological age refers to an individual's degree of somatic development. Forensic anthropologists use the biological age estimate to predict chronological age [228]. Tooth development is relatively independent of exogenic factors such as disease or malnutrition, making it a better measure of chronological age than e.g. skeletal development [229]. All methods used in forensic anthropology and odontology do, however, have limitations. Limitations include the difficulties in the standardization of different methods, the lack of consensus between different methods, mean error, age range discrepancies and the variation present among individuals and populations; the individual variation being the most important.

Estimating the age of an individual after 14 years of age becomes difficult and challenging. All age estimation methods have both advantages and disadvantages and are more or less indecisive after the age of 14. Age estimation from dental age can be used to predict chronological age in young children before the completion of root formation [11] and the method by Demirjian is the method most commonly suggested in literature [7, 9, 116]. The left seven mandibular teeth are used in this method and the original model was developed from a French Canadian population [5]. Studies using the Demirjian method demonstrated differences between chronological age and estimated age. The staging chart used by Demirjian [5] has been modified by Solari and Abramovitch [11] by adding intermediate sub stages to the latter stages of tooth development. By adding these two additional stages higher accuracy can be achieved [11]. Difficulties were found to accurately distinguish between stages "F" or "G". The two additional stages, F1 and G1, make the transition towards apex closure easier to define and to achieve a higher level of accuracy. By adding the two additional stages a 10 stage scoring scheme was

developed [11]. Most studies found an overestimation in their particular population [7, 8, 169, 230]. A modified Demirjian method will be used in this study.

3.2 Materials and methods

Digital panoramic radiographs of 1268 individuals taken from 2013 to 2016 at the School of Dentistry, University of Pretoria, with known age and sex were selected by using a quota sampling method. The sample comprised of 705 White South Africans (WSA) and 563 Black South Africans (BSA) aged between 15 and 25 years (Table 3.1). The different categories (sex and ancestry) were divided into groups according to their chronological age and each age was calculated to two decimal points. The 15 year-olds included individuals of ages ranging from 15.0 to 15.99, 16-year olds from 16.0 to 16.99, etc. The individuals treated at the School of Dentistry are of different social groupings and include individuals living in the city as well as referrals from neighbouring rural areas and thus include the entire socioeconomic spectrum. The identification of WSA and BSA was made according to self-classification information present in the patient's hospital records. All panoramic radiographs were part of the patient's routine dental treatment and no panoramic radiographs were taken primarily for this research project.

Table 3.1: Age and sex distribution. Numbers in brackets represent samples with closed apices (Stage H) of the left third maxillary and mandibular molars respectively. Age 15 indicates all individuals aged 15.00-15.99 years, etc.

	BSA		**WSA**	
Age (last birthday)	Females	Males	Females	Males
15	33	30	31	33
16	30	35(4, 1)	36(2, 1)	30
17	31(6, 3)	30(7, 7)	32(5, 2)	33(2, 1)
18	30(10, 8)	30(15, 12)	34(7, 7)	34(17, 11)
19	30(20, 14)	32(19, 17)	32(12, 7)	31(19, 13)
20	30(16, 14)	37(31, 29)	36(26, 19)	31(29, 27)
21	30(29, 26)	30(28, 27)	32(23, 21)	30(27, 25)
22	31(30, 30)	32(31, 31)	32(27, 25)	31(29, 27)
23	31(31, 31)	31(31, 31)	34(29, 29)	32(29, 30)
24	-	-	30(28, 28)	31(30, 30)
25	-	-	30(30, 30)	30(30, 30)

Total	276	287	359	346
Total	563		705	

3.2.1 Exclusion criteria

Exclusion criteria included the presence of systemic diseases; presence of congenital anomalies; unclear panoramic radiographs; the absence of the left maxillary and mandibular third molars; non- South African citizens; and if the age was less than 15 years or above 25 years at the time the panoramic radiograph was performed.

3.2.2 Assessment of dental development

All panoramic radiographs were taken in a digital format using Instrumentarium, OP200 D/ OC200 D units and Sidexis, Orthophos XG5 units. Care was taken so that the individuals were positioned correctly during panoramic radiography and that all radiographs were of good image quality.

The maxillary and mandibular left third molars were scored according to a ten stage scoring system from ‘A’ to ‘H’ (Figs. 2.10, 3.1 - 3.4) [11]. Each stage represents a developmental period, ranging from the initial calcified points to the closure of the tooth apex. To improve the evaluation of third molar development stages a modified version of the Demirjian *et al*. [5] root formation classification was used. Two additional stages were added namely stages F1 and G1 (Figs. 2.10, 3.1) [11]. Stage F1 represents a root length that is twice the crown length and the roots still have a funnel shaped opening at the apex. Stage G1 represents a tooth with parallel root walls, where the apices are not completely closed and the periodontal ligament space at the apex is ≥1.0mm. These additional stages were found to be of particular value to fine-grade the final stages [11].

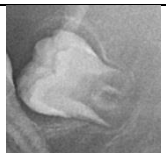
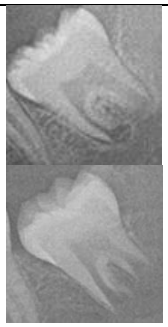
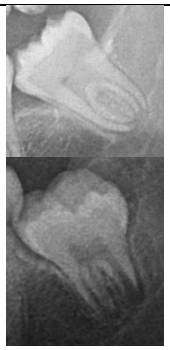
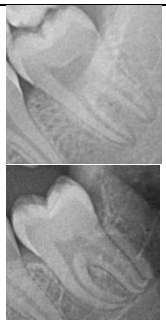
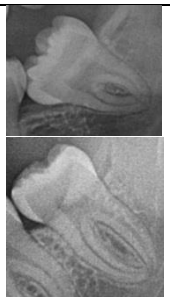
Stage	Radiograph of third molars	Criteria
F		The root length is at least as long as the crown length. The root endings have a funnel shape.
F_1		The root length is twice the length of the crown. The root endings still have a funnel shape.
G		The walls of the radicular pulp (root canal) chamber are parallel, and the apical foramen remains open.
G_1		The walls of the radicular pulp (root canal) are parallel, and the apical foramina are not completely closed. The periodontal ligament space surrounding the apical ending is ≥1mm.
H		The apical ends of the roots are completely closed. The periodontal ligament surrounding the roots is uniform in width.

Figure 3.1: Demirjian third molar development staging F-H as modified by Solari *et al*. [11]

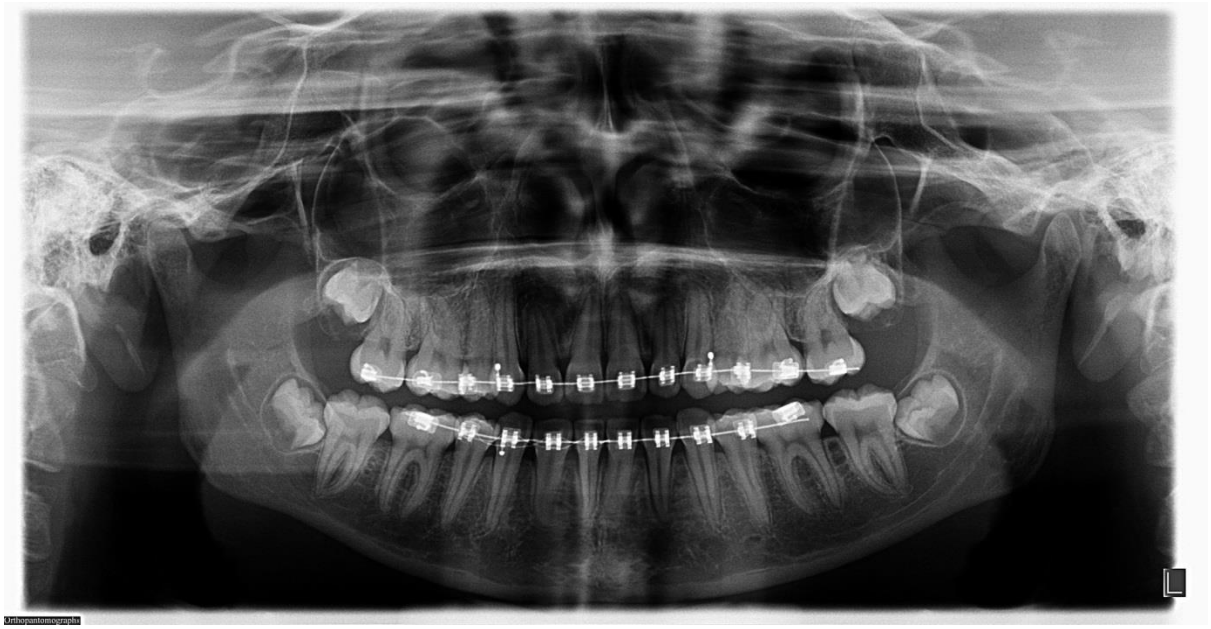


Figure 3.2: Digital panoramic radiograph of a 17.62 year old white South African female. According to the classification system the left maxillary third molar can be classified as a stage E and the left mandibular third molar as stage D.

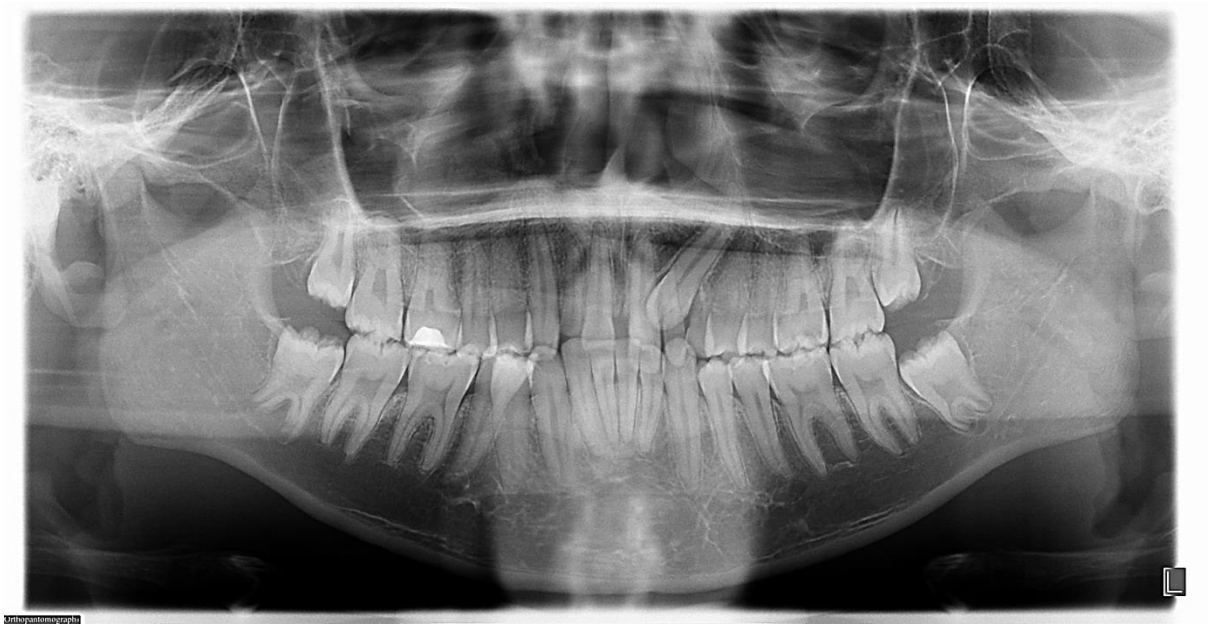


Figure 3.3: Digital panoramic radiograph of a 19.92 year old black South African male. According to the classification system the left maxillary third molar can be classified as a stage G1 and the left mandibular third molar as stage G.

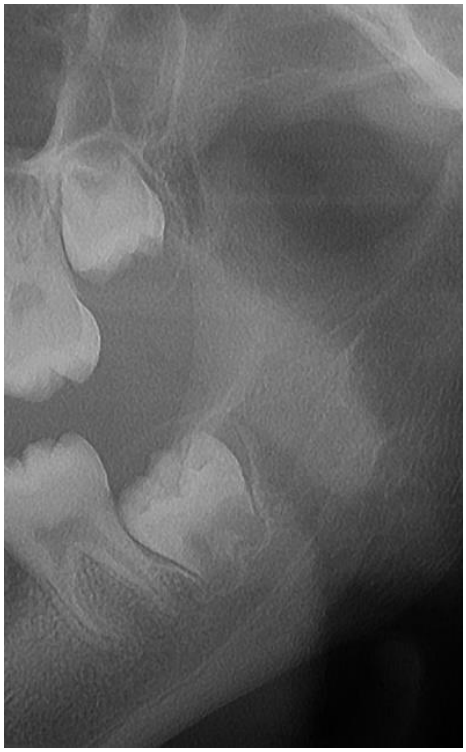


Figure 3.4: Cropped panoramic radiograph of a 15.17 year old male. According to the classification system the left maxillary third molar can be classified as a stage D and the left mandibular third molar as stage E.

3.2.3 Data analysis

All the examinations were carried out by the researcher. One hundred and thirty randomly selected cases were re-examined to determine intra- examiner reliability. Fifty randomly selected cases were also re-examined by a second observer with experience in Forensic Odontology to determine the level of inter-examiner reliability. Cohen's kappa coefficient was determined to assess both intra- and inter-observer repeatability.

Median, maximum- and minimum- values, together with the means and standard deviations were calculated for ages at each stage of development for males and females separately, using Matlab and Excel. Skewness was calculated for each stage classification and for each population group. Outliers were not excluded from the calculations. Both Wilcoxon rank sum tests and t-tests were conducted but the Wilcoxon rank sum test was preferred since we were unable to properly establish the normality of the distributions with the relatively small samples, notably in early

categories/stages. A non-parametric Kruskal Wallis test was also performed (as a one-way ANOVA) to indicate significant differences for each stage.

The likelihood of an individual being at least 18 years at a specific third molar stage was calculated. A similar method to that of Liversidge *et al.* [156] was used where by the number of observed individuals, per category, older than age 18 were divided by the total number of observations in that category to establish likelihood estimates. The combined likelihood was estimated by the number of individuals who were classified jointly in both stages. The number of individuals older than 18 in that stage combination was then divided by the total number of individuals in that stage combination.

3.3 Results

The results for the intra-observer repeatability indicated substantial agreement for scoring the mandibular and maxillary teeth with a Cohen's kappa value of 0.8511 and 0.9263, respectively. The results for inter-observer repeatability indicated substantial agreement between observers for the evaluation of the maxillary third molars, with a Cohen's kappa value of 0.6287, and moderate agreement for the mandibular third molars with a Cohen's kappa value of 0.5107. The highest rates of disagreement between the observers for both the maxillary and mandibular third molars were between stages F and F1 and between G and G1 in the mandible.

The data were separated into ancestry groups and sex and then further subdivided into the maxilla and mandible. Table 3.2 displays the median ages of the left maxillary and mandibular third molar tooth development stages for each ancestry group. The *p* – values were determined to determine significance between ancestral groups.

Table 3.2: Median, mean, minimum, and maximum ages and standard deviations (SD) of third molar crown-root formation at the given stages of development for Black South African (BSA) and White South African (WSA) individuals.

Stages		D	E	F	F_1	G	G_1	H
Maxilla Males								
BSA	Median	16.00	16.05	16.61	16.23	17.22	18.68	21.28
	Mean	15.89	15.81	16.54	16.45	17.43	18.53	21.06
	SD	0.85	0.67	1.20	1.19	1.69	1.49	1.87
	Min	15.00	15.01	15.00	15.00	15.09	15.76	16.32
	Max	17.18	16.77	20.02	18.77	22.61	21.27	23.84
WSA	Median	15.84	16.36	15.73	16.85	17.6	18.18	21.85
	Mean	15.95	16.46	16.16	17.17	18.37	18.62	21.73
	SD	0.81	1.19	1.18	1.50	2.26	1.63	1.91
	Min	15.02	15.00	15.00	15.18	15.59	16.17	17.26
	Max	18.11	19.61	18.77	21.19	23.59	24.09	24.90
Median difference in months (years)		1.92* (0.16*)	-3.72 (-0.31)	10.50 (0.88)	-7.50 (-0.63)	-4.56 (-0.38)	6.0 (0.5)	-6.90* (-0.58*)
Females								
BSA	Median	16.05	15.97	16.89	16.34	17.33	18.23	21.67
	Mean	16.14	16.29	16.82	16.78	17.81	18.45	21.29
	SD	0.99	1.20	1.18	1.31	1.94	1.48	1.76

	Min	15.00	15.00	15.00	15.00	15.00	15.33	16.37
	Max	18.30	19.68	19.51	20.26	22.02	21.48	23.86
WSA	Median	15.77	16.73	16.43	17.35	18.93	18.85	22.60
	Mean	16.25	17.07	17.26	17.65	19.37	18.94	22.45
	SD	1.20	2.03	2.29	2.14	2.24	1.59	2.22
	Min	15.00	15.01	15.00	15.44	15.59	15.76	16.17
	Max	19.10	23.51	24.69	23.35	24.02	22.77	25.99
Median difference in months (years)		3.36* (0.28*)	-9.06 (-0.76)	5.52* (0.46*)	-12.12* (-1.01*)	-19.20 (-1.6)	-7.38 (-0.62)	-11.16* (-0.93*)
Mandible **Males**								
BSA	Median	16.11	15.51	16.84	16.56	17.6	19.43	21.42
	Mean	16.12	15.74	16.71	16.79	17.94	19.03	21.23
	SD	0.68	0.71	1.30	1.36	1.65	1.57	1.76
	Min	15.00	15.01	15.00	15.00	15.27	16.18	16.51
	Max	17.18	17.09	20.02	20.34	22.61	21.36	23.84
WSA	Median	15.59	15.97	16.59	17.36	17.67	19.01	22.18
	Mean	15.69	16.26	16.56	17.27	17.93	19.30	21.97
	SD	0.57	1.11	1.18	1.00	1.72	1.83	1.82
	Min	15.02	15.17	15.00	15.43	15.59	16.17	17.26
	Max	16.77	19.61	19.44	18.77	23.25	24.09	24.90
Median difference in months (years)		6.30 (0.53)	-5.52 (-0.46)	3.00 (0.25)	-9.60* (-0.8*)	-0.84 (-0.07)	5.04* (0.42)	-9.06* (-0.76*)
Females								
BSA	Median	16.02	16.31	16.36	16.59	17.94	19.02	21.85
	Mean	16.05	16.43	16.71	17.18	18.19	19.12	21.55
	SD	0.86	1.39	1.26	1.73	1.70	1.22	1.64
	Min	15.00	15.00	15.00	15.00	15.00	16.51	17.43
	Max	18.30	19.68	20.26	20.77	22.02	21.93	23.86
WSA	Median	15.77	16.34	16.43	17.72	18.75	20.27	23.01
	Mean	16.21	17.04	17.05	18.29	18.81	20.30	22.77
	SD	1.02	1.96	2.07	2.12	1.56	1.84	2.07
	Min	15.00	15.00	15.00	15.44	15.76	16.17	16.84
	Max	18.52	23.51	24.69	23.35	23.02	24.02	25.99
Median difference in months (years)		3.00 (0.25)	-0.42 (-0.04)	-0.84 (-0.07)	-13.50* (-1.13*)	-9.75 (-0.81)	-15.00 (-1.25)	-13.92* (-1.16*)

*statistically significant at $p < 0.05$

The analysis began at a stage D because of the small sample sizes for stages B and C for the sample age range of 15 to 25 years. The median and mean age increased for all ancestry and sex groups with progression through the development stages except for WSA males between stages E to F1 in the maxilla and for BSA males in the mandible between stages D to F. Statistically significant differences were noted between ancestry groups for 11 out of the 28 stages (Table 3.2), with the South African black individuals consistently maturing earlier than the white individuals. The median ages when BSA females were compared with WSA females were lower for

BSA females for stages E, F1, G, G1 and H in the maxilla and for stages E, F, F1, G, G1 and H in the mandible. The median ages when BSA males were compared with WSA males were lower for BSA males for stages E, F1, G and H in the maxilla and for stages E, F1, G and H in the mandible (Table 3.2). The greatest median difference (> 1 year difference) between ancestries were for BSA and WSA females for stage F1 and G in the maxilla with a difference of 1.01 and 1.6 years, respectively. In the mandible the greatest difference was between BSA and WSA females for stage F1, G1 and H with a difference of 1.13, 1.25, and 1.16 years, respectively. For stages D, E, and F in the mandible the median differences between BSA- and WSA females were 0.25, 0.04, and 0.07 years respectively. The greatest median difference between BSA and WSA males were 0.88 years for stage F in the maxilla. The smallest median difference between BSA and WSA males was 0.07 years for stage G in the mandible.

Statistically significant differences were noted among sex groups for some of the stages, mostly those near the final stages of root development (Table 3.3). This indicates that male third molars completed their development faster than those of females. The median ages when BSA males were compared with BSA females were lower for BSA males for stages D, F, F1, G and H in the maxilla and for stages E, F1, G and H in the mandible. The median ages when WSA males were compared with WSA females were lower for WSA males for stages E, F, F1, G, G1 and H in the maxilla and for stages D, E, F1, G, G1 and H in the mandible (Table 3.3).

Table 3.3: Median ages and median differences comparison of third molar crown-root formation at the given stages of development for males and females.

Stages	D	E	F	F_1	G	G_1	H
Maxilla BSA							
Males	16.00	16.05	16.61	16.23	17.22	18.68	21.28
Females	16.05	15.97	16.89	16.34	17.33	18.23	21.60
Median difference in months (years)	-0.60 (-0.05)	0.96 (0.08)	-3.42 (-0.29)	-1.38 (-0.12)	-1.26 (-0.11)	5.40 (0.45)	-4.74 (-0.40)
WSA							
Males	15.84	16.36	15.73	16.85	17.6	18.18	21.85
Females	15.77	16.73	16.43	17.35	18.93	18.85	22.60
Median difference in months (years)	0.84 (0.07)	-4.38 (-0.37)	-8.40 (-0.70)	-6.00 (-0.50)	-15.90 (-1.33)	-7.98* (-0.67*)	-9.00* (-0.75*)

Mandible BSA							
Males	16.11	15.51	16.84	16.56	17.6	19.43	21.42
Females	16.02	16.31	16.36	16.59	17.94	19.02	21.85
Median difference in months (years)	1.08 (0.09)	-9.54 (-0.80)	5.76 (0.48)	-0.36 (-0.03)	-4.08 (-0.34)	4.92* (0.41*)	-5.16 (-0.43)
WSA							
Males	15.59	15.97	16.59	17.36	17.67	19.01	22.18
Females	15.77	16.34	16.43	17.72	18.75	20.27	23.01
Median difference in months (years)	-2.22 (-0.19)	-4.44 (-0.37)	1.92 (0.16)	-4.26 (-0.36)	-12.96* (-1.08*)	-15.12 (-1.26)	-10.02* (-0.84*)

Black South African (BSA) and White South African (WSA) individuals.
*statistically significant at $p < 0.05$

The greatest median difference between BSA males and BSA females was for stage E with a 0.80 year difference. The smallest median difference was for stage F1 with a difference of 0.03 years. The greatest difference between WSA males and WSA females (> 1 year difference) was for stage G in the maxilla with a 1.33 year difference and for stages G and G1 in the maxilla with 1.08 and 1.26 years difference, respectively.

Figures 3.5.1-3.5.8 display the median ages of all the individuals at which developmental stages D through H were attained for all the ancestry and sex groups. In the maxilla and mandible, for black South African females (Figs. 3.5.1 and 3.5.3), stage B and C was not recorded for any individual with only one individual presenting with stage D older than 18 years. For white South African females (Figs. 3.5.2 and 3.5.4), one individual presented with stage B in the maxilla and mandible and three individuals were older than 18 years with a stage D presentation for the maxillary third molar development. All female individuals from both ancestry groups were older than 16 years when stage H was reached for the maxillary and mandibular third molar development. In the maxilla (Figs. 3.5.5 and 3.5.6) stage D was only present in black South African males and not in white males. For white South African males (Fig. 3.5.6), only two individuals were older than 18 years when stage E and stage F was reached for the maxillary third molar development. In black South African males (Fig. 3.5.5) all the individuals in stage E, and only one in stage F, were younger than 18 years of age for the maxillary third molar development. All the male individuals were older than 16 years when stage H was reached for maxillary third molar

development. In black South African males, in the mandible (Fig.3.5.7), stage C was not present and all the individuals with stage D and E were younger than 18 years. By the time stage H was reached all black South African males were older than 16 years for mandibular third molar development. In white South African males (Fig. 3.5.8.), one individual was in stage C and two individuals were older than 18 years in stage E for mandibular third molar development. All the white South African males were older than 17 years when stage H was reached for mandibular third molar development. Figures 3.6.1 and 3.6.2 display the median ages at which development stages D through H were attained in the maxilla and mandible for all the ancestry and sex groups.

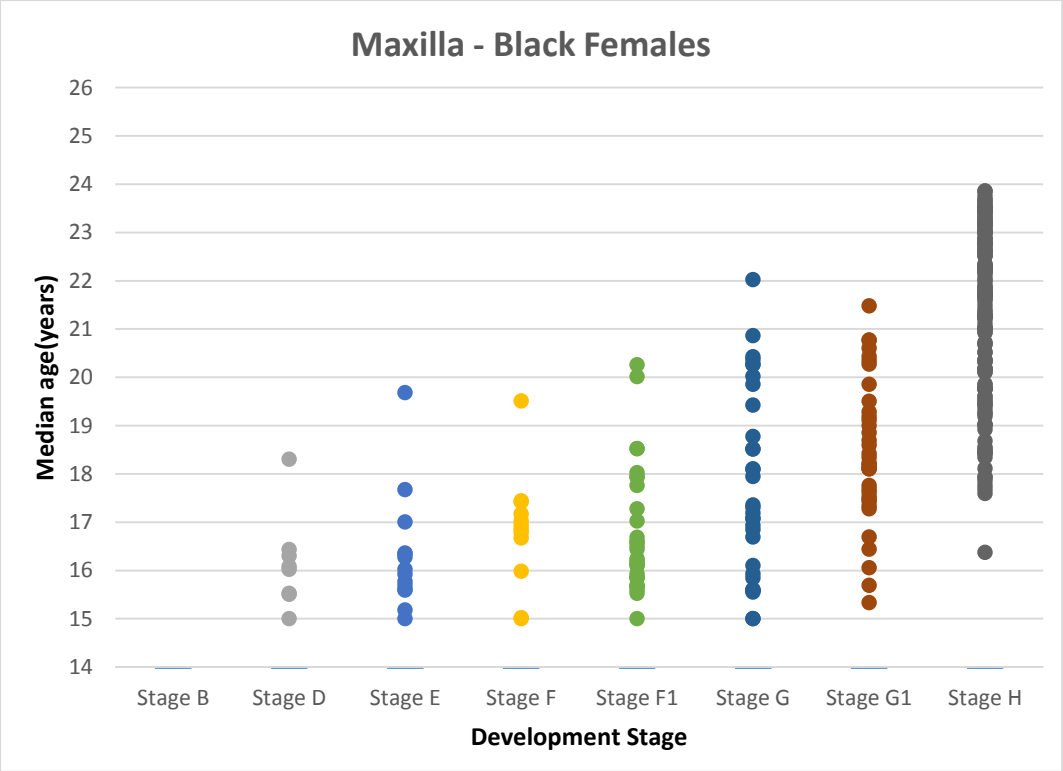


Figure 3.5.1: Dot plot of the median ages at which developmental stages D through to H were attained in the maxilla for black South African females.

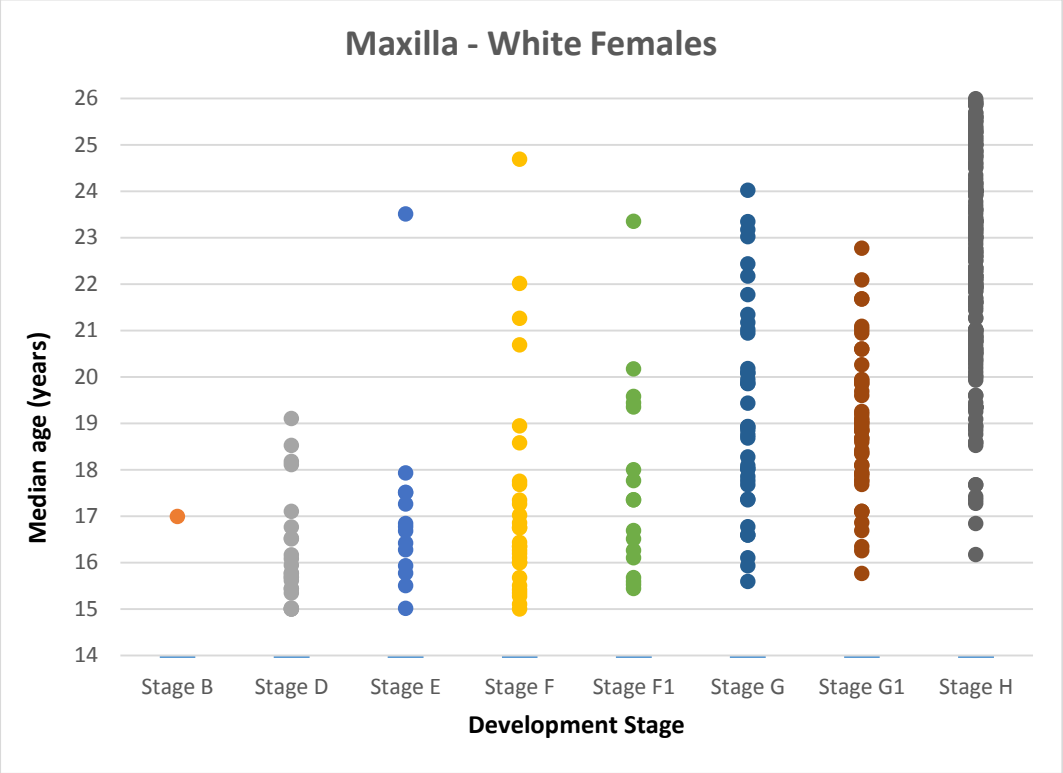


Figure 3.5.2: Dot plot of the median ages at which developmental stages D through to H were attained in the maxilla for white South African females.

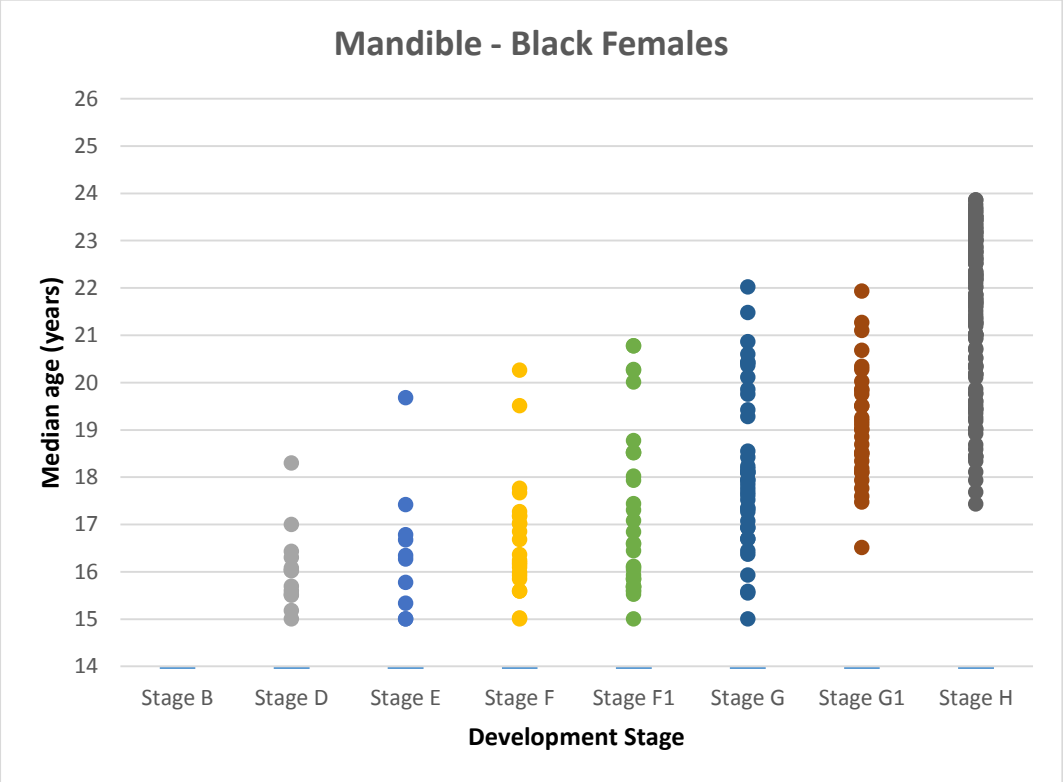


Figure 3.5.3: Dot plot of the median ages at which developmental stages D through to H were attained in the mandible for black South African females.

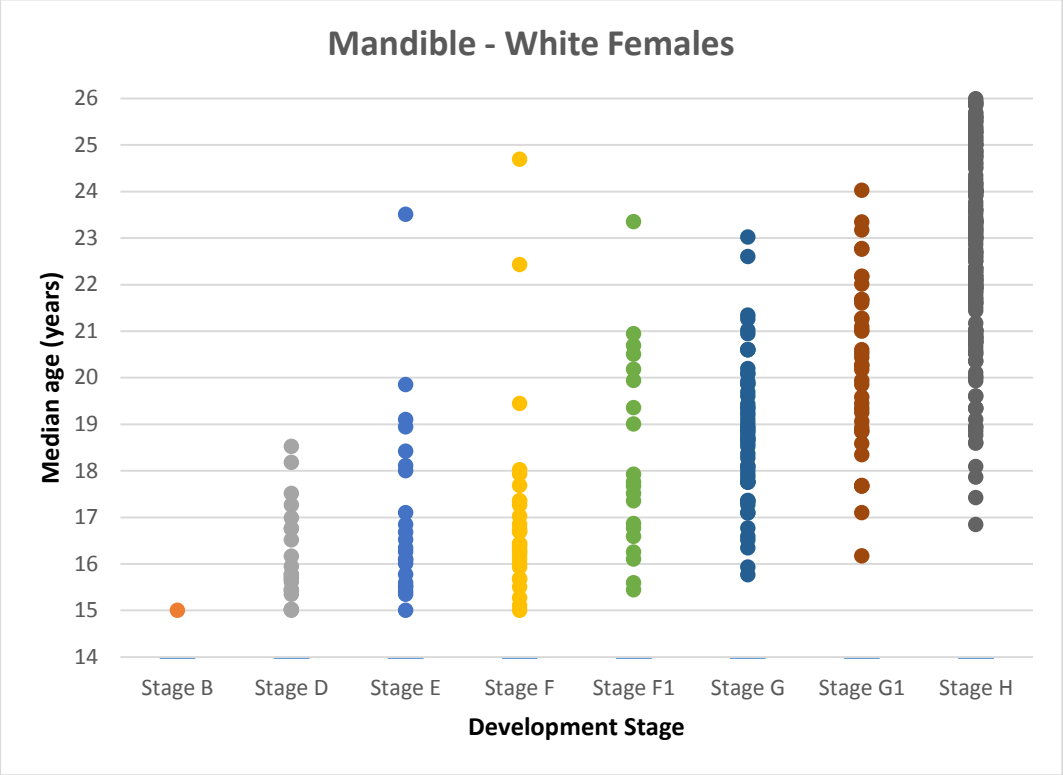


Figure 3.5.4: Dot plot of the median ages at which developmental stages D through to H were attained in the mandible for white South African females.

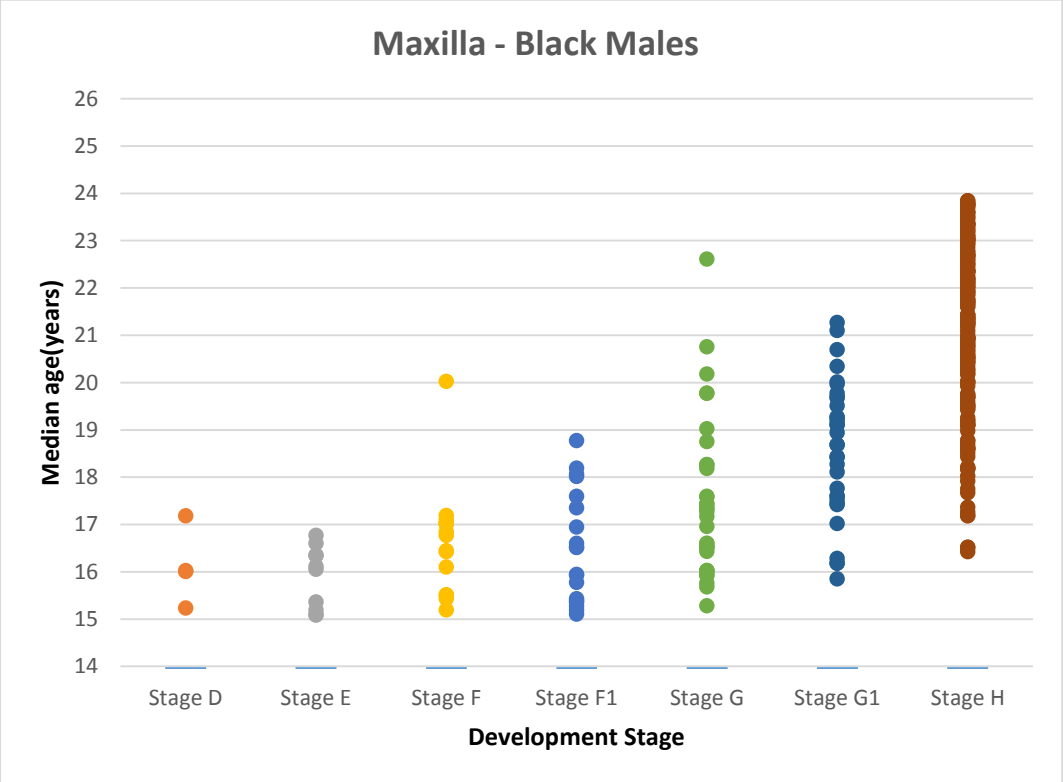


Figure 3.5.5: Dot plot of the median ages at which developmental stages D through to H were attained in the maxilla for black South African males.

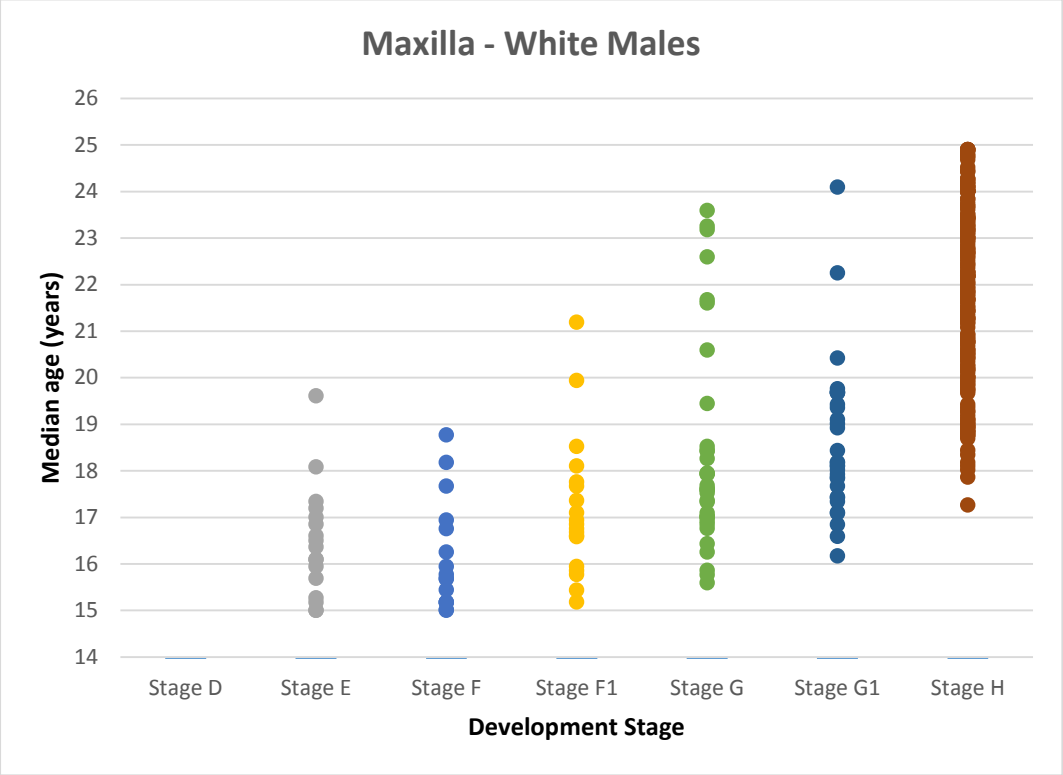


Figure 3.5.6: Dot plot of the median ages at which developmental stages D through to H were attained in the maxilla for white South African males.

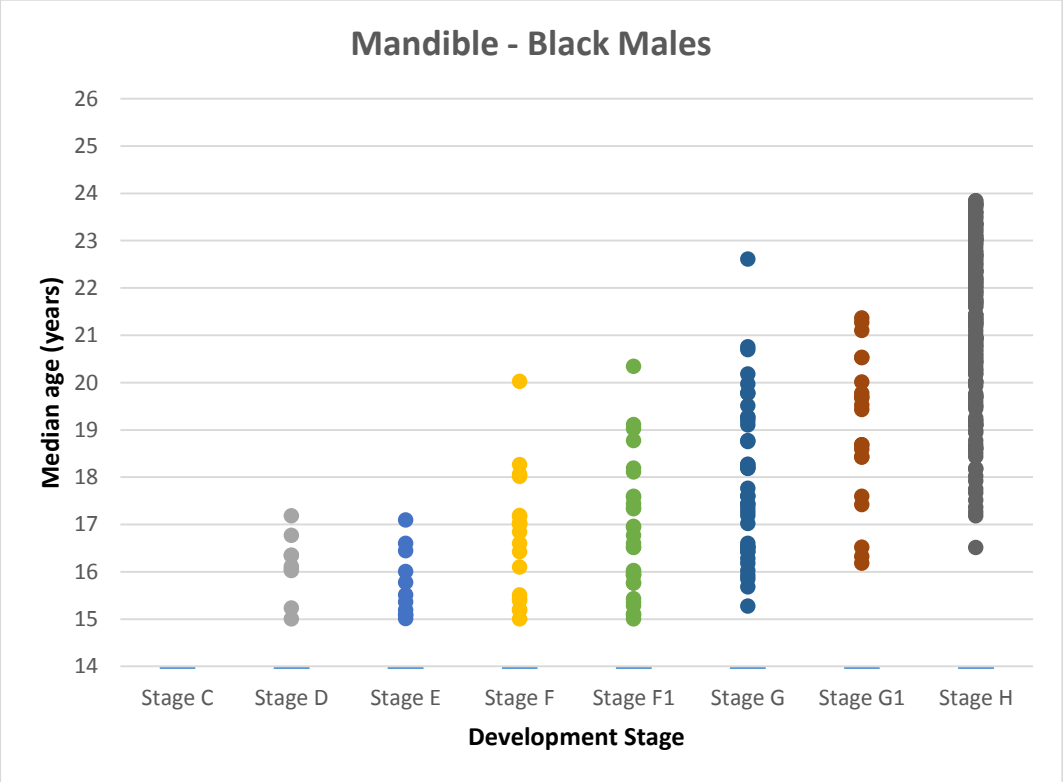


Figure 3.5.7: Dot plot of the median ages at which developmental stages D through to H were attained in the mandible for black South African males.

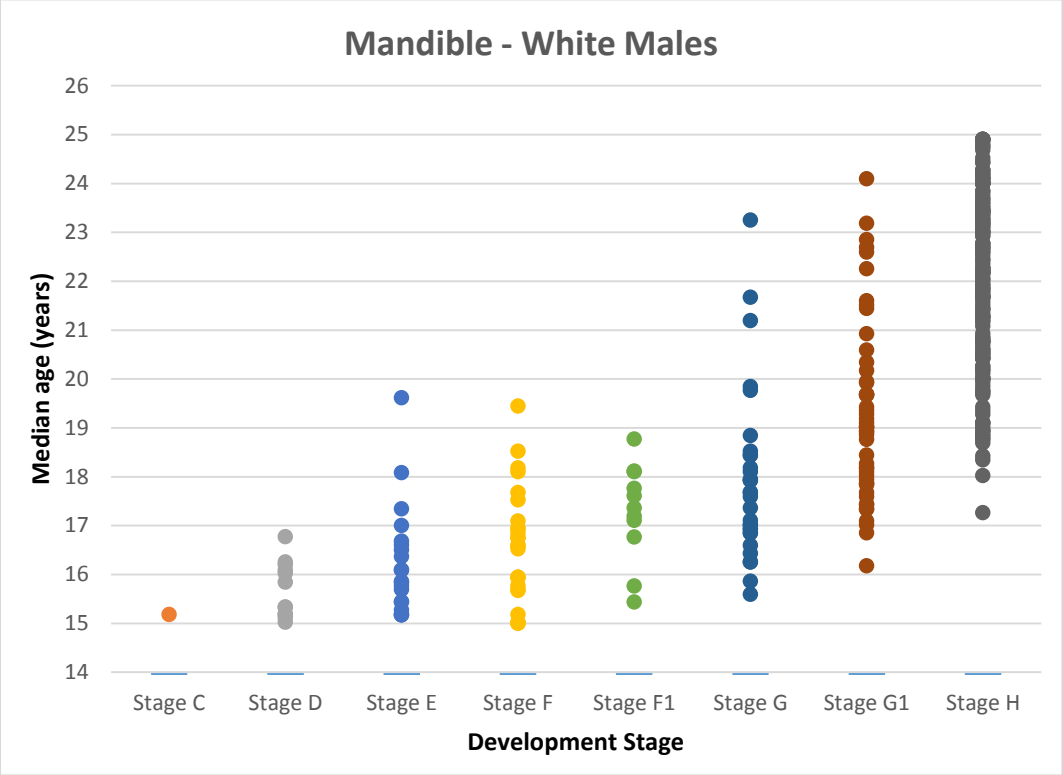


Figure 3.5.8: Dot plot of the median ages at which developmental stages D through to H were attained in the mandible for white South African males.

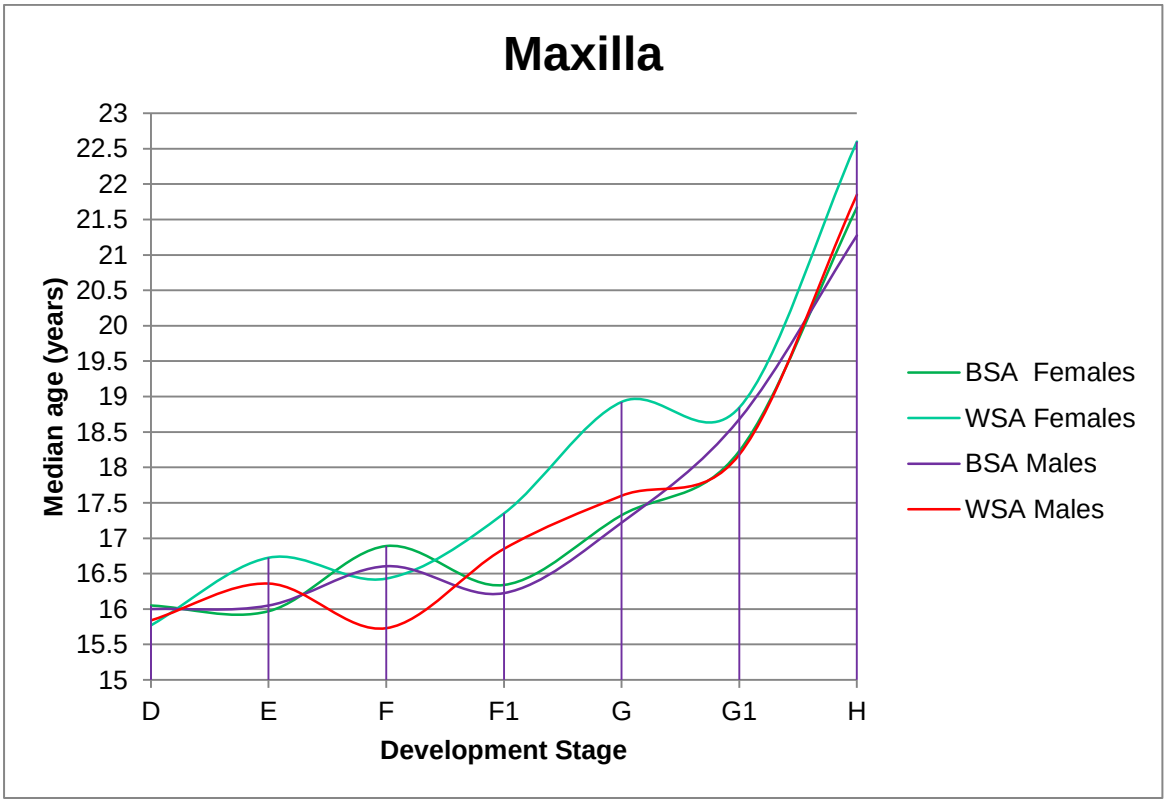


Figure 3.6.1: Median ages at which developmental stages D through to H were attained in the maxilla for BSA and WSA.

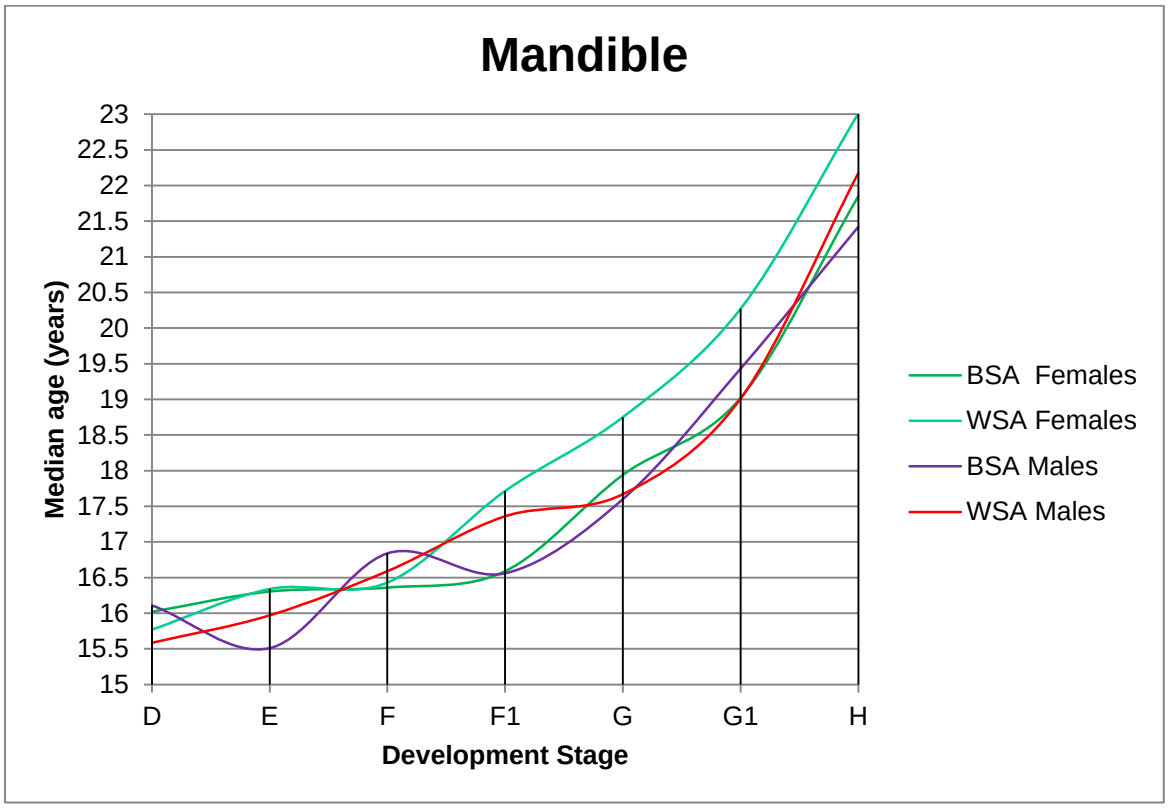


Figure 3.6.2: Median ages at which developmental stages D through to H were attained in the mandible for BSA and WSA.

Table 3.4 demonstrates the likelihood of an individual being 18 years of age based on the left third molar development stage for the maxilla and mandible separately. When a 95% probability is considered for stage H, only the BSA males for the maxilla and mandible respectively were below the level. All the other ancestry and sex groups had values above 0.95. A combined likelihood for the maxilla and mandible for each ancestry group for stage H increased the likelihood to above 95% for all the groups (Table 3.5).

Table 3.4: Likelihood of an individual being 18 years of age based on the third molar development stage for the maxilla and mandible.

	Stage						
Ancestry, Sex, Location	D	E	F	F_1	G	G_1	H
BSA Female Maxillary	0.125	0.071	0.083	0.167	0.438	0.649	0.951
BSA Female Mandibular	0.077	0.100	0.087	0.303	0.475	0.839	0.976
BSA Male Maxillary	0.000	0.000	0.063	0.200	0.294	0.647	0.934

BSA Male Mandibular	0.000	0.000	0.211	0.200	0.463	0.762	0.948
WSA Female Maxillary	0.174	0.071	0.207	0.353	0.711	0.708	0.963
WSA Female Mandibular	0.095	0.304	0.138	0.400	0.684	0.872	0.982
WSA Male Maxillary	0.059	0.118	0.125	0.211	0.353	0.613	0.989
WSA Male Mandibular	0.000	0.100	0.154	0.273	0.355	0.725	0.994

Black South African (BSA) and White South African (WSA) individuals.

The greatest likelihood to be older than 18 years of age for stage H was for WSA males for the mandibular third molar with a value of 0.994. This means that a WSA with a mandibular third molar in stage H will 99.4% of the time be older than age 18 years of age. In BSA males, for the maxillary and mandibular third molars with stages D and E, the values were 0.000. In WSA males for the mandibular third molars for stage D, the probability of being 18 was 0.000. This suggests that if a WSA male present with a stage D third mandibular molar development the likelihood is 100% that the individual will be younger than age 18 years.

Table 3.5: Combined likelihood for the maxilla and mandible for stage H.

	BSA Female	BSA Male	WSA Female	WSA Male
Combined likelihood - Stage H	0.9839	0.9542	0.9876	0.9938

The greatest likelihood to be older than 18 years of age for the combined maxilla and mandible for stage H was for WSA males with a value of 0.9938.

In table 3.6 studies using the mean age are compared for each of the development stages according to Demirjian *et al.* [5] and Solari *et al*. [11]. These studies used a similar methodology and valuable comparisons can be made by comparing the mean ages between the different studies and population groups.

Table 3.6: Studies that provide data on mean ages and standard deviations according to Demirjian *et al.* [5] and Solari *et al*. [11] development stages for left maxillary and mandibular third molars.*this study.

Stages		D	E	F	F_1	G	G_1	H
Maxilla Males								
BSA*	Mean SD	15.89 0.85	15.81 0.67	16.54 1.20	16.45 1.19	17.43 1.69	18.53 1.49	21.06 1.87
WSA*	Mean SD	15.95 0.81	16.46 1.19	16.16 1.18	17.17 1.50	18.37 2.26	18.62 1.63	21.73 1.91
Hispanics[11]	Mean SD	15.3 1.4	16.0 1.4	16.1 1.5	16.6 1.4	16.7 1.4	18.0 1.9	20.1 2.6
American whites[82]	Mean SD	16.0 1.97	16.6 2.38	17.7 2.28		18.2 1.91		20.2 2.09
Japanese [127]	Mean SD	18.0 3.7	18.9 3.3	20.3 2.5		21.8 2.5		22.4 2.3
Japanese [149]	Mean	15.3	16.8	18.1		18.5		21.5
Turkish [150]	Mean SD	14.8 2.7	15.5 2.8	16.7 2.8		17.9 2.4		20.1 1.8
Chinese [141]	Mean SD	13.58 1.62	15.64 1.80	17.47 1.66		19.93 2.01		22.81 2.26
Korean [143]	Mean SD	14.0 1.6	15.9 1.7	16.8 1.4		18.3 1.5		20.9 1.3
Botswana [152]	Mean SD	12.75 1.56	14.74 1.62	16.37 1.28		18.40 1.59		
BSA [153]	Mean SD	13.4 1.5	16.1 2.9	17.9 2.4		20.6 2.5		22.7 2.5
Females								
BSA*	Mean SD	16.14 0.99	16.29 1.20	16.82 1.18	16.78 1.31	17.81 1.94	18.45 1.48	21.29 1.76
WSA*	Mean SD	16.25 1.20	17.07 2.03	17.26 2.29	17.65 2.14	19.37 2.24	18.94 1.59	22.45 2.22
Hispanics[11]	Mean SD	15.7 1.4	16.2 1.7	16.7 1.8	17.6 1.9	18.4 2.2	18.6 2.2	20.8 2.2
American whites[82]	Mean SD	16.0 1.55	16.9 1.85	18.0 1.95		18.8 2.27		20.6 2.09
Japanese [127]	Mean SD	19.3 2.5	19.5 3.2	19.9 2.3		21.4 2.0		22.3 2.1
Japanese [149]	Mean	16.1	17.4	18.6		19.5		21.7
Turkish [150]	Mean SD	15.4 2.6	16.4 2.3	17.4 2.5		17.9 2.2		20.0 1.9
Chinese [141]	Mean SD	13.82 1.65	16.15 1.95	18.23 1.91		20.85 2.37		23.30 1.98
Korean [143]	Mean SD	14.3 1.7	15.9 1.6	17.5 1.9		19.2 1.9		22.3 1.7
Botswana [152]	Mean SD	12.24 1.78	14.65 1.92	16.91 1.54		18.71 1.85		

BSA [153]	Mean SD	14.1 3.4	15.2 1.6	17.0 1.4		19.6 2.4		22.1 2.6
Mandible Males								
BSA*	Mean SD	16.12 0.68	15.74 0.71	16.71 1.30	16.79 1.36	17.94 1.65	19.03 1.57	21.23 1.76
WSA*	Mean SD	15.69 0.57	16.26 1.11	16.56 1.18	17.27 1.00	17.93 1.72	19.30 1.83	21.97 1.82
Hispanics [11]	Mean SD	15.5 1.5	15.8 1.2	16.3 1.3	16.7 0.77	17.1 1.7	18.4 2.2	20.6 2.3
American whites [82]	Mean SD	15.5 1.59	17.3 2.47	17.5 2.14		18.3 1.93		20.5 1.97
Japanese [127]	Mean SD	18.2 3.3	18.5 2.7	20.4 2.4		21.8 2.5		22.7 2.0
Japanese [149]	Mean	15.4	16.4	17.4		18.6		21.6
Turkish [150]	Mean SD	14.5 2.7	15.6 2.8	16.9 2.7		17.9 2.2		20.1 2.0
Spanish [147]	Mean SD	15.08 1.04	15.22 1.03	16.42 1.34		17.92 1.50		19.74 1.09
Finnish [139]	Mean	13.56	15.05	16.73		18.03		20.31
Chinese [141]	Mean SD	13.47 1.48	15.31 1.73	17.06 1.62		19.32 1.79		22.72 2.27
Korean [143]	Mean SD	14.6 1.5	16.2 1.7	16.7 1.4		18.6 1.6		21.1 1.2
Botswana [152]	Mean SD	12.69 1.44	15.03 1.48	16.60 1.56		18.30 1.57		
Italian [142]	Mean SD	15.82 1.35	16.12 1.44	17.31 1.72		18.62 1.49		20.02 1.46
German [145]	Mean SD	14.30 1.28	15.69 1.79	17.25 1.45		20.86 2.24		22.51 1.66
BSA [135]	Mean SD	13.9 1.3	15.2 2.4	18.7 2.3		20.8 2.2		22.6 1.9
BSA [153]	Mean SD	13.4 1.6	15.4 2.6	18.6 2.5		21.1 2.2		22.9 2.4
Females								
BSA*	Mean SD	16.05 0.86	16.43 1.39	16.71 1.26	17.18 1.73	18.19 1.70	19.12 1.22	21.55 1.64
WSA*	Mean SD	16.21 1.02	17.04 1.96	17.05 2.07	18.29 2.12	18.81 1.56	20.30 1.84	22.77 2.07
Hispanics [11]	Mean SD	15.6 1.4	16.1 1.4	17.3 2.6	18.0 1.4	18.5 2.1	19.3 2.0	21.7 1.8
American whites [82]	Mean SD	16.0 1.64	16.9 1.75	17.7 1.80		19.1 2.18		20.9 2.01
Japanese [127]	Mean SD	18.0 2.8	18.6 2.3	20.5 2.2		21.8 2.0		22.4 2.1

Japanese [149]	Mean	16.0	17.3	18.3		19.4		21.8
Turkish [150]	Mean SD	15.2 2.7	16.1 2.4	17.0 2.5		17.9 2.3		20.0 1.9
Spanish [147]	Mean SD	15.11 1.00	16.00 1.43	16.83 1.56		18.41 1.44		19.66 0.98
Finnish [139]	Mean	13.26	15.06	16.51		18.84		21.50
Chinese [141]	Mean SD	13.73 1.73	15.87 1.95	17.97 2.10		20.61 2.25		23.42 2.02
Korean [143]	Mean SD	15.0 1.6	16.4 1.7	17.6 1.9		19.5 1.9		22.4 1.7
Botswana [152]	Mean SD	12.41 1.73	14.79 1.69	16.89 1.55		18.45 1.53		
Italian [142]	Mean SD	16.25 1.7	16.57 1.61	17.76 1.79		18.64 1.67		20.34 1.37
German [145]	Mean SD	15.22 2.05	16.74 2.10	18.45 2.52		21.53 1.83		22.91 1.59
BSA [135]	Mean SD	14.5 2.3	15.9 2.3	17.4 2.5		19.8 2.3		22.4 1.9
BSA [153]	Mean SD	13.6 2.5	15.7 1.8	17.1 2.5		19.6 2.2		22.5 2.3

In table 3.7 the mean age difference was calculated between WSA and BSA as well as between WSA and German individuals.

Table 3.7: Comparison between the mean age differences of WSA individuals with BSA- and German individuals.

Stages		**D**	**E**	**F**	**F_1**	**G**	**G_1**	**H**
Mandible Males								
WSA	Mean SD	15.69 0.57	16.26 1.11	16.56 1.18	17.27 1.00	17.93 1.72	19.30 1.83	21.97 1.82
BSA	Mean SD	16.12 0.68	15.74 0.71	16.71 1.30	16.79 1.36	17.94 1.65	19.03 1.57	21.23 1.76
WSA-BSA	Mean age difference	-0.43	0.52	-0.15	0.48	-0.01	0.27	0.74
WSA	Mean SD	15.69 0.57	16.26 1.11	16.56 1.18	17.27 1.00	17.93 1.72	19.30 1.83	21.97 1.82

German [145]	Mean SD	14.30 1.28	15.69 1.79	17.25 1.45		20.86 2.24		22.51 1.66
WSA-German	Mean age difference	1.39	0.57	-0.69		-2.93		-0.54
Females								
WSA	Mean SD	16.21 1.02	17.04 1.96	17.05 2.07	18.29 2.12	18.81 1.56	20.30 1.84	22.77 2.07
BSA	Mean SD	16.05 0.86	16.43 1.39	16.71 1.26	17.18 1.73	18.19 1.70	19.12 1.22	21.55 1.64
WSA-BSA	Mean age difference	0.16	0.61	0.34	1.11	0.62	1.18	1.22
WSA	Mean SD	16.21 1.02	17.04 1.96	17.05 2.07	18.29 2.12	18.81 1.56	20.30 1.84	22.77 2.07
German [145]	Mean SD	15.22 2.05	16.74 2.10	18.45 2.52		21.53 1.83		22.91 1.59
WSA-German	Mean age difference	0.99	0.3	-1.4		-2.72		-0.14

Chapter 4: Age estimation from anterior cervical vertebral ring apophysis ossification in South Africans

4.1 Introduction

The aim of this part of the study is to establish the relationship between the chronological age of South Africans and the timing of ossification and fusion of the anterior inferior vertebral ring apophysis of cervical vertebrae C2, C3, and C4. The likelihood of being 18 years of age at a specific stage of development was assessed and differences between populations and sexes were determined. Multiple regression equations to estimate age were also established for each ancestry and sex group.

4.2 Materials and methods

Cephalometric radiographs of 974 individuals with known age and sex were retrospectively selected using a quota sampling method. Cephalometric radiographs were obtained from the School of Dentistry, University of Pretoria, Sefako Makgatho Health Sciences University (Pretoria) and two private orthodontic practices situated in Pretoria, South Africa. All radiographs were exposed between 2013 and 2018. The sample comprised of 496 White individuals (WSA) (235 males and 261 females) living in South Africa and 478 Black South African (BSA) (226 males and 252 females) individuals aged between 15 and 22 years (Table 4.1). The allocation of ancestry was made according to self-classification information present in the patient's hospital or practice records. All cephalometric radiographs were part of the patient's routine dental treatment and no radiographs were taken primarily for this research project.

Table 4.1: Age, ancestry and sex distribution. Age 15 indicates all individuals aged 15.00-15.99 years, etc.

	BSA		WSA	
Age (last birthday)	Males	Females	Males	Females
15	35	34	34	31
16	31	33	32	36
17	33	32	33	33
18	30	34	33	34
19	30	31	30	32
20	30	31	30	35
21	20	30	26	30
22	17	27	17	30
Total	226	252	235	261
Total	478		496	

4.2.1 Exclusion criteria

Exclusion criteria included the following: the presence of systemic diseases; presence of congenital anomalies; unclear cephalometric radiographs and cephalometric radiographs that did not include C2, C3 and C4.

4.2.2 Assessment of cervical vertebral ring apophysis ossification

All cephalometric radiographs were taken in a digital format using Instrumentarium, ORTHOPANTOMOGRAPH™ OP200 D/ OC200 D units and Sidexis, Orthophos XG5 units. Care was taken so that the individuals were positioned correctly during cephalographic radiography and that all radiographs were of good image quality.

The different categories (sex and ancestry) were divided into groups according to their chronological age and each age was calculated to two decimals points. The 15 year-olds included individuals of ages ranging from 15.0 to 15.99, 16-year olds from 16.0 to 16.99, etc.

The anterior inferior vertebral ring apophysis development of cervical vertebrae (C2, C3, and C4) was evaluated on cephalometric radiographs and scored according to a self-developed scoring system from stage “0” to “4”. Scoring stages for the

apophysis ossification and progress towards union were scored as follows, as also demonstrated in Fig 4.1. Each individual vertebra was assessed according to the scoring system.

Scoring:

Stage 0- No ossification of the apophysis visible. A cervical vertebral maturation stage (CVMS) of I to IV is present [201].

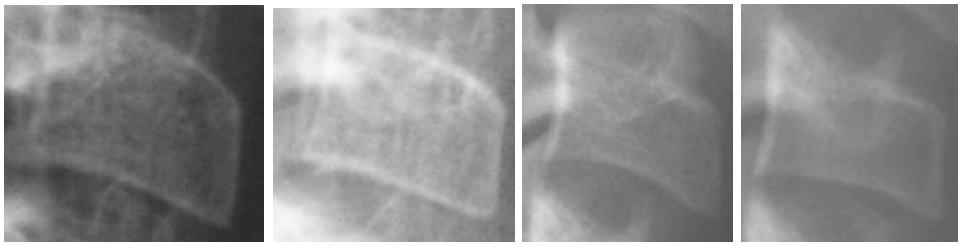
The following features characterized stage 0: The inferior borders of all the cervical vertebrae are flat or a slight concavity may be present at the inferior borders of C2 and C3. The superior borders are tapered from posterior to anterior.

Stage 1- Ossification of the apophysis. No union between the ossification center and the inferior border of the vertebral body has taken place, but the apophysis is visible as a small radiodense area at the anterior border of the vertebral body.

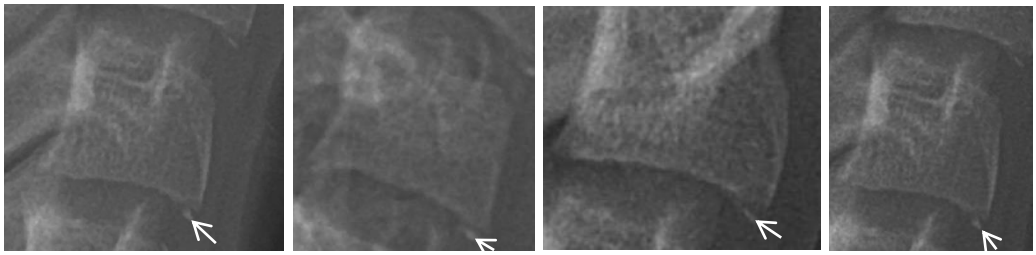
Stage 2- The apophysis has begun to unite/fuse with the inferior vertebral body at the posterior end of the ossification center. A radiolucent opening/line is present between the ossification center and inferior vertebral body anteriorly.

Stage 3- Union has taken place, but a notch is still present between the apophysis and the inferior vertebral body.

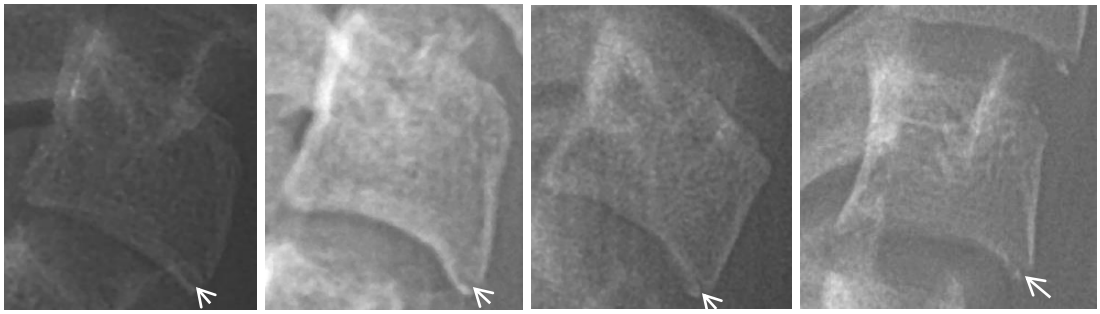
Stage 4- Complete union with an intact and smooth cortical margin.



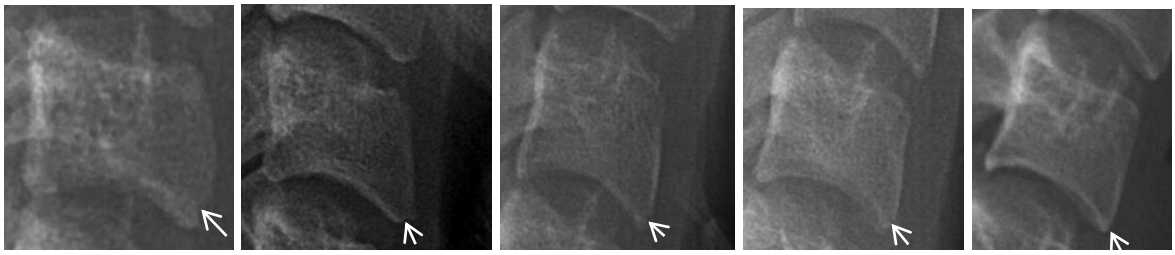
Stage 0- No ossification of the anterior inferior apophysis visible. The inferior surface of C2, C3 and C4 are flat or somewhat concave.



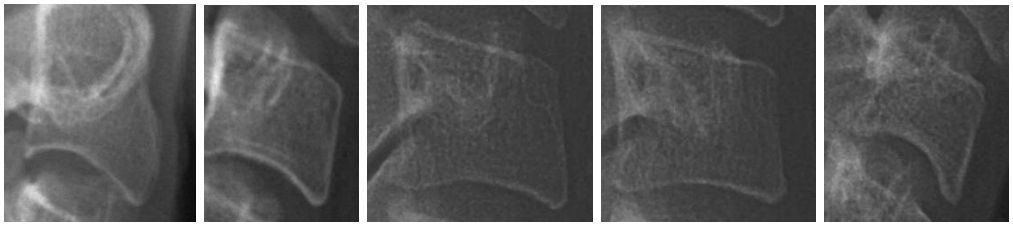
Stage 1- Ossification of the apophysis.No union between the ossification center and the inferior border of the vertebral body has taken place (indicated by the arrows).



Stage 2- The apophysis has begun to unite/fuse with the inferior vertebral body at the posterior end of the ossification center. A radiolucent opening/line is present between the ossification center and inferior vertebral body anterior(indicated by the arrows).



Stage 3- The apophysial ring has united with the vertebral body,but a notch is still present between the apophysis and the inferior vertebral body(indicated by the arrows).



Stage 4- Complete union with an intact and smooth cortical margin.

Figure 4.1: Radiographic stages, progress, and description of the anterior inferior ring apophysis ossification of cervical vertebrae C2, C3 and C4.

4.2.3 Data analysis

All examinations were carried out by the researcher who scored each vertebra separately and entered each value into an Excel spreadsheet. Fifty randomly selected cases were also re-examined by one of the co–supervisors to determine the level of inter-examiner reliability. The main researcher “calibrated” the co-supervisor by presenting examples of each cervival verebrae stage until proficiency in identifying the apophysis stage was reached. The researcher then independently scored the 50 assigned cases. Fleiss’s kappa coefficient was determined to assess inter-observer repeatability. The kappa values gave a measure of how consistent the ratings were and the scoring range was between 0 and 1. A score closer to 1 represented a higher level of agreement. The *p*-values were determined for the kappa tests to assess whether the agreement was not due to chance and if some correlation existed for the intraclass correlation coefficient (ICC) tests (measurement data). The *p*-values in these cases tested whether the estimated kappa was not due to chance and did not test the strength of agreement. *P*-values and confidence intervals are sensitive to sample size, and with a large enough sample size, any kappa above 0 will become statistically significant. Low levels of agreement as demonstrated here are usually down to the design of the classification system. The percentage of disagreement between the raters was very low and most disagreement was only by one stage.

Median, minimum and maximum values, together with means and standard deviations, were calculated for ages at each stage of development for the different sex-ancestry groups. Since the residuals were not found to follow a normal distribution, a parametric one-way ANOVA was not suitable as indicated by the results of the Shapiro-Wilk test. Therefore non-parametric tests were used. The Kruskal-Wallis rank sum test was used to determine if there were any statistically significant differences between the groups delineated by ancestry and sex. Dunn’s test was then conducted to establish where the differences, if any, arose.

The likelihood of an individual being at least 18 years at a specific apophysis stage was calculated. A similar method to Liversidge *et al.* [156] was used where the

number of observed individuals, per category, older than age 18 were divided by the total number of observations in that category to establish probability estimates. The combined probability was estimated by the number of individuals who were classified jointly in both stages. The number of individuals older than 18 in that stage combination was then divided by the total number of individuals in that stage combination.

Multiple linear regression analysis was considered for each population and sex group to estimate age by using the stage classifications as independent variables. Straightforward linear regressions fitted, assumed that the stages are equally spaced and directly correlated to the magnitude of the numerical value associated with them. From each model, determination coefficients (R^2) and root mean square errors (RMSE) were analysed. Alternatively, it was also assumed that the ordinal classification categories are independent of their numerical value and should therefore be coded. By creating dichotomous variables for each level of categorical variable as contrasted to the reference level (in this case determined as the combined lowest levels for each category as observed or C2 Stage = 1, C3 Stage = 1 and C4 Stage = 1) we derived alternative regression formulae for this dataset. Note that for males, the four observations with category zero classifications (2 for white males and 2 for black South African males) were excluded in this case. In the case of zero observations in practice, it should be assumed that the age is less than the intercept implied by the regression formula.

4.3 Results

The results for inter-observer repeatability indicated that the agreement among raters exceeded what would be expected if all raters made their ratings completely random. The Fleiss's kappa values for the stage classifications were 0.3730, 0.4090, and 0.5700 for C2,C3 and C4 respectively. The *p*-value tested whether the estimated kappa was not due to chance and did not indicate the strength of the agreement. The data were separated into population and sex groups and further subdivided into each vertebra. For Stage C2, raters agreed in 60% of cases and differed by 1 stage in the remaining 40% of cases. For Stage C3, raters agreed in 58% of cases,

differed by 1 stage in 40% of cases and by more than 1 stage in 2% of cases. For Stage 4, raters agreed in 70% of cases, differed by 1 stage in 26% of cases and by more than 1 stage in 4% of cases.

Tabel 4.2 displays the mean and median ages of attainment for each stage of anterior inferior apophysis ossification for vertebrae C2, C3 and C4. The analysis began at stage 0. Stage 0 was only present in black and white South African males, demonstrating that the ossification of the apophysis has started in all females by age 15. Two black South African males, aged 15 and 16 years, were still in stage 0 for C2, C3 and C4. In white South African males, aged 15 years, stage 0 was achieved in one individual only for C2, and in two individuals for C3 and C4. The median ages of attainment for stage 1 for BSA males were lower compared with WSA males. For stages 2, 3 and 4, the average ages were lower for WSA males relative to those of BSA males, suggesting that WSA males mature earlier than their black South African counterparts. All stages, except stage 2 for C2, were reached earlier in WSA females compared with BSA females. The largest difference between BSA and WSA males was for stage 1 for C2 with a 0.81 year difference and stage 3 for C4 with a 0.65 year difference. The largest difference between BSA and WSA females was for stage 3 for C3, with a 0.8 year difference and stage 3 for C4 with a 0.96 year difference. Anterior inferior apophysis development of C2, C3 and C4 did not exceed a one year difference for any developmental stage between BSA and WSA males, or for BSA and WSA females.

The median ages for attainment of stages 0, 1, and 2 were below the 18 year threshold for all ancestry and sex groups. Additionally, WSA males and BSA females attained stage 3 for C2, and WSA females attained stage 3 for C2,C3 and C4 below the 18 year threshold. The maximum ages for attainment of stage 0 and stage 1 were below the 18 year threshold for BSA males for all vertebrae. White South African females also had a maximum age of attainment below 18 years for stage 1 for C2, C3 and C4. This suggests that the ossification of the apophysis can provide valuable information on ageing around the age of 18 years.

Table 4.2: Median, mean, minimum, and maximum ages and standard deviations (SD) of anterior inferior apophysis development at the given stages for Black South African (BSA) and White South African (WSA) individuals.

Stages		**0**			**1**			**2**			**3**			**4**		
Vertebra		C2	C3	C4	C2	C3	C4	C2	C3	C4	C2	C3	C4	C2	C3	C4
Males																
BSA	Mean	15.59	15.59	15.59	15.53	15.68	15.80	16.74	16.85	17.07	18.08	19.20	19.57	20.25	20.64	20.62
	Median	15.59	15.59	15.59	15.28	15.45	15.73	16.60	16.61	17.10	18.17	19.22	19.67	20.44	20.77	20.77
	SD	0.72	0.72	0.72	0.72	0.76	0.74	1.08	1.16	1.24	1.66	1.42	1.60	1.78	1.59	1.53
	LCI 95%	-6.02	-6.02	-6.02	7.8	9.22	9.75	12.27	13.12	13.45	13.52	14.17	13.82	16.18	15.84	15.76
	UCI 95%	37.2	37.2	37.2	23.26	22.13	21.84	21.21	20.59	20.68	22.64	24.23	25.32	24.32	25.44	25.48
	Min	15.08	15.08	15.08	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.27	15.00	15.27	16.32	16.59
	Max	16.10	16.10	16.10	17.76	17.76	17.76	19.84	19.85	19.85	21.94	22.43	22.82	22.96	22.96	22.96
WSA	Mean	15.18	15.39	15.39	16.31	16.01	16.14	16.50	16.89	17.02	17.89	18.42	18.98	20.24	20.70	20.79
	Median	15.18	15.39	15.39	16.09	15.81	15.98	16.42	16.95	16.97	17.76	18.84	19.02	20.56	20.92	21.01
	SD	0	0.29	0.29	1.05	0.98	0.93	1.17	1.08	1.22	1.50	1.56	1.39	1.68	1.39	1.42
	LCI 95%	-14.57	-5.94	-5.94	10.82	10.54	11.31	10.81	12.19	12.49	13.24	13.76	14.13	16.47	16.35	16.16
	UCI 95%	44.93	36.71	36.71	21.8	21.49	20.98	22.19	21.59	21.55	22.54	23.07	23.83	24.01	25.05	25.42
	Min	15.18	15.18	15.18	15.00	15.00	15.00	15.00	15.02	15.00	15.17	15.17	15.17	16.01	16.51	16.27
	Max	15.18	15.59	15.59	18.43	18.43	18.43	19.18	19.77	19.92	20.85	21.76	21.76	22.92	22.92	22.92
Median difference in months (years)					-9.72 (-0.81*)	-4.32 (-0.36)	-3.00 (-0.25)	2.16 (0.18)	-4.08 (-0.34)	1.56 (0.13)	4.92 (0.41)	4.56 (0.38*)	7.80 (0.65)	1.44 (-0.12)	1.8 (-0.15)	2.88 (-0.24)
Females																
BSA	Mean				16.61	16.40	16.49	16.47	16.73	16.81	18.05	18.17	18.74	19.75	20.05	20.12
	Median				16.51	16.36	16.17	16.03	16.44	16.59	17.76	18.31	18.48	19.89	20.29	20.33
	SD				0.77	0.97	1.5	1.42	1.31	1.35	1.73	1.82	1.98	2.05	1.95	1.88
	LCI 95%				6.85	8.12	9.03	11.5	12.12	12.46	12.47	12.99	13.82	16.69	16.69	16.54
	UCI 95%				26.37	24.68	23.96	21.43	21.33	21.15	23.62	23.35	23.67	22.81	23.41	23.70
	Min				15.58	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.02	15.00
	Max				18.51	18.51	21.6	21.6	21.6	20.36	21.58	21.61	22.59	22.93	22.93	22.93
WSA	Mean				16.06	16.08	16.33	16.63	16.4	16.39	17.52	17.79	18.04	19.49	19.9	20.04
	Median				16.17	15.94	16.14	16.52	16.18	16.23	17.33	17.51	17.52	19.77	20.06	20.14
	SD				0.81	0.72	0.95	1.12	1.19	1.07	1.72	1.78	1.82	2.2	1.94	1.88
	LCI 95%				6.5	9.26	9.87	9.56	10.88	11.36	10.73	11.19	11.43	16.78	16.97	17.01
	UCI 95%				25.61	22.89	22.78	23.7	21.92	21.42	24.32	24.4	24.65	22.21	22.83	23.06
	Min				15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.67	15.00	15.43	15.00
	Max				17.18	17.76	18.00	19.36	19.68	19.68	21.76	21.92	21.92	22.92	22.92	22.92
Median difference in months (years)					4.08 (0.34)	5.04 (0.42)	0.36 (0.03)	5.88 (-0.49)	3.12 (0.26)	4.32 (0.36)	5.16 (0.43)	9.6 (0.8)	11.52 (0.96)	1.44 (0.12)	2.76 (0.23)	2.28 (0.19)

*statistically significant at $p < 0.05$

Black South African (BSA) males achieved stage 1 earlier for C2, C3 and C4 compared with BSA females (Table 4.3). However, after that, BSA females achieved stages 2, 3 and 4 earlier for all vertebrae compared with BSA males. Similarly, white

South African (WSA) males achieved stage 1 earlier for C2, C3, and C4 and stage 2 for C2 compared with females. Thereafter, stage 2 for C3 and C4 and stages 3 and 4 were achieved earlier for C2, C3 and C4 by females compared with males (Table 4.3). Median differences exceeding one year between BSA males and females were noted for stage 1 for C2 and stage 3 for C4. Stage 3 for C3 and C4 exceeded a one year median difference between WSA males and females.

Table 4.3: Median ages and median difference comparison of anterior inferior apophysis ossification at the given stages of development for males and females.

Stages		0			1			2			3			4		
Vertebra		C2	C3	C4	C2	C3	C4	C2	C3	C4	C2	C3	C4	C2	C3	C4
BSA																
	Males	15.59	15.59	15.59	15.28	15.45	15.73	16.60	16.61	17.10	18.17	19.22	19.67	20.44	20.77	20.77
	Females				16.51	16.36	16.17	16.03	16.44	16.59	17.76	18.31	18.48	19.89	20.29	20.33
	Median difference in months(years)				-14.76 (-1.23*)	-10.92 (-0.91)	-5.28 (-0.44)	6.84 (0.57)	2.04 (0.17)	6.12 (0.51)	4.92 (0.41)	10.92 (0.91*)	14.28 (1.19*)	6.60 (0.55)	5.76 (0.48)	5.28 (0.44)
WSA																
	Males	15.18	15.39	15.39	16.09	15.81	15.98	16.42	16.95	16.97	17.76	18.84	19.02	20.56	20.92	21.01
	Females				16.17	15.94	16.14	16.52	16.18	16.23	17.33	17.51	17.52	19.77	20.06	20.14
	Median difference in months (years)				-0.96 (-0.08)	-1.56 (-0.13)	-1.92 (-0.16)	-1.2 (-0.1)	9.24 (0.77)	8.88 (0.74)	5.16 (0.43)	15.96 (1.33)	18.00 (1.5)	9.84 (0.79*)	10.32 (0.86*)	10.44 (0.87*)

Black South African (BSA) and White South African (WSA) individuals. *statistically significant at $p < 0.05$

Table 4.4 demonstrates the likelihood of an individual being 18 years of age or older based on the anterior inferior ossification of the apophysis for C2, C3 and C4 respectively. When a 95% probabilty is considered for an individual to be younger than 18 years the following stages are below that level for stage 1 : black South African males for C2, C3, and C4, white South African females for C2 and C3, and white South African males for C4. When a 95% probability is considered for an individual to be older than 18 years the following stages are above that level for stage 4: white males for C3 and C4. Figures 4.2 and 4.3 show the age dispersion for each ossification stage. Tables 4.5 – 4.10 show the results of the multiple regression analysis for each population and sex group, if the data are used to actually give a point estimate of the age of an unknown individual. From table 4.5, it can be seen that the R^2 values ranged between 0.49 and 0.70, with standard errors from 1.22 years in white males to 1.65 years in black South African females. The practical use

of these formulae, with examples, are shown in Fig. 4.4. By age 22.96 years, all apophyses are completely ossified, and the formulae can obviously not be used beyond this age. Table 4.6 demonstrates the results of the regression formulae using dichotomous indicator variables. The adjusted R^2 values ranged between 0.5119 and 0.7171, with standard errors from 1.19 to 1.60, and are thus similar to those shown in Table 4.5. The dichotomomous regressions are good for use for categorical variables, and allows for an easy calculation. In order to use these, one simply has to select the appropriate stage for the vertebra in question, while the other values are excluded from the formula. For example, for black South African males, if C2 is in Stage 2, C3 in stage 3, and C4 in stage 2, the formula becomes:

Age = 15.2034 + 0.6460 I(C2 Stage 2) + 1.6965 I(C3 Stage 3) + 0.1834 I(C4 Stage 2) ; with age thus calculated as

Age = 15.2034 + 0.6460 + 1.6965 + 0.1834

= 17.7293 ± 1.27 years.

More examples are shown in Fig. 4.4.

Table 4.4: Likelihood of a South African individual being at least 18 years of age based on the anterior inferior apophysis ossification stage for C2, C3 and C4.

Probability of being 18 years of age at last birthday					
	Stage				
Ancestry, Sex, Vertebra	**0**	**1**	**2**	**3**	**4**
BSA, Female, C2		0.091	0.125	0.462	0.796
BSA, Female, C3		0.0667	0.1429	0.5833	0.8357
BSA, Female, C4		0.0556	0.2143	0.6296	0.8548
BSA, Male, C2	0.000	0.000	0.132	0.557	0.887
BSA, Male, C3	0.000	0.000	0.158	0.875	0.917
BSA, Male, C4	0.000	0.000	0.244	0.889	0.943
WSA, Female, C2		0.000	0.190	0.360	0.725
WSA, Female, C3		0.000	0.121	0.333	0.822
WSA, Female, C4		0.083	0.100	0.444	0.841
WSA, Male, C2	0.000	0.091	0.156	0.446	0.904
WSA, Male, C3	0.000	0.094	0.120	0.635	0.978
WSA, Male, C4	0.000	0.049	0.204	0.780	0.975

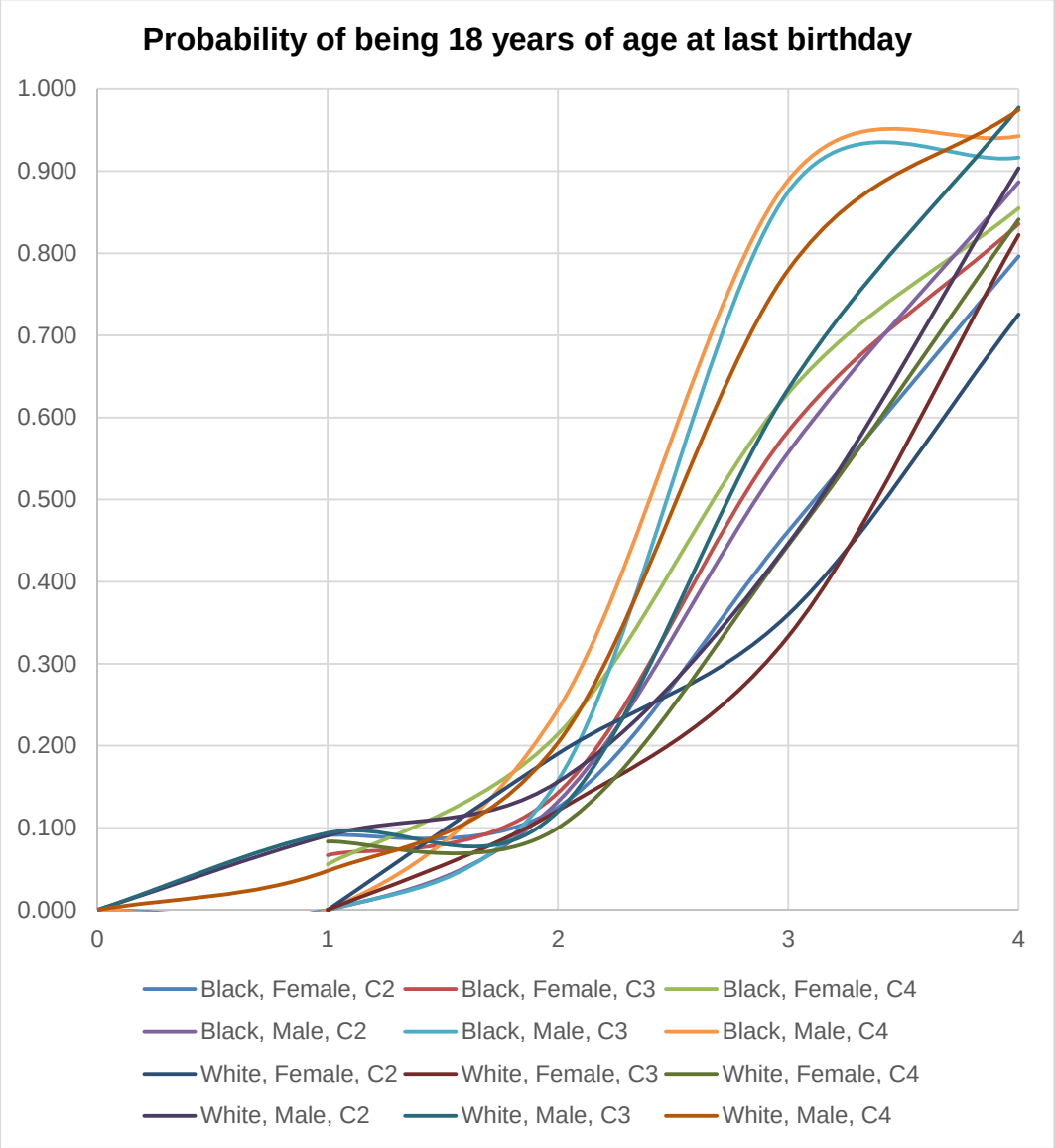
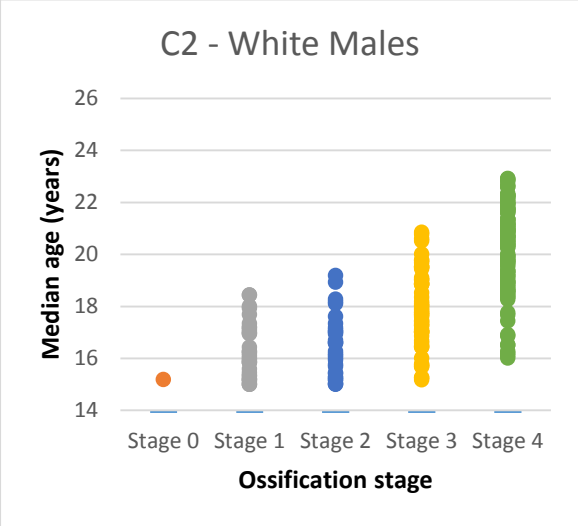
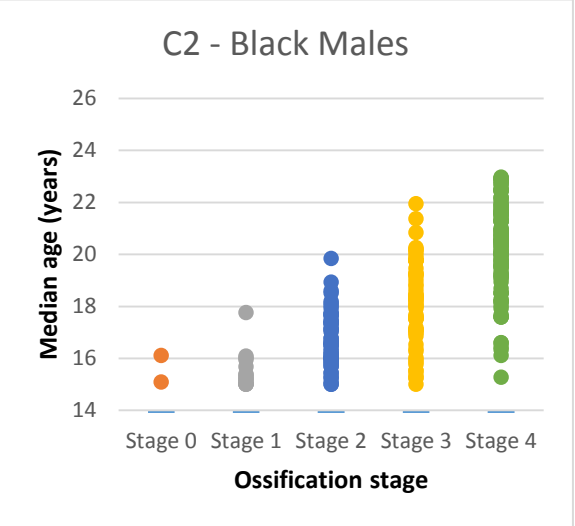
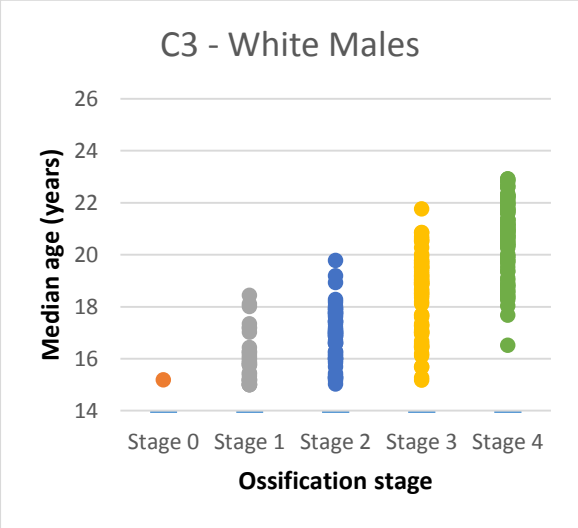
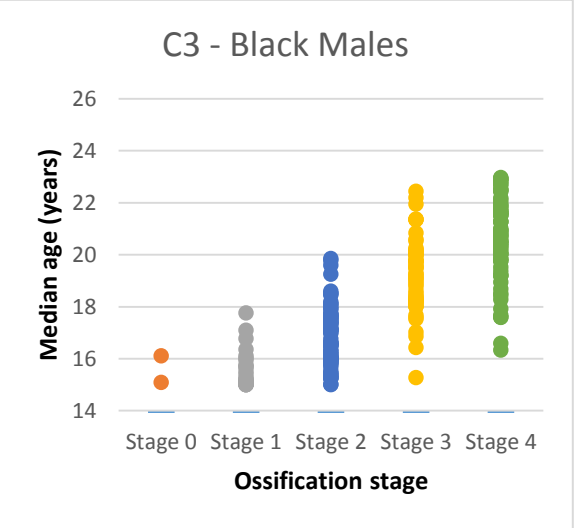


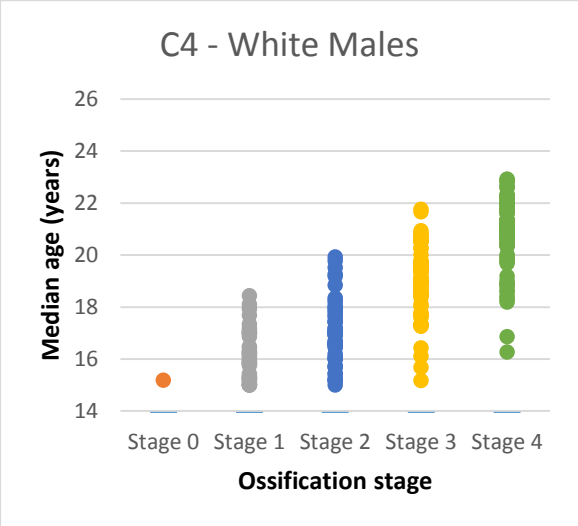
Figure 4.2: Graph displaying the likelihood of a South African individual being 18 years of age based on the anterior inferior apophysis ossification stage for C2, C3 and C4.

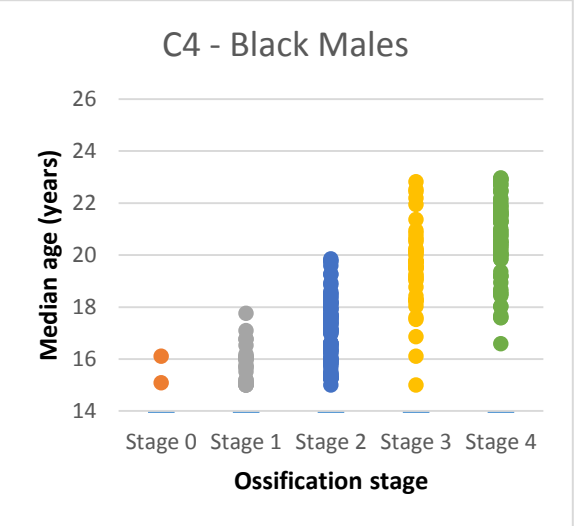

C2 - White Males
Median age (years)
26
24
22
20
18
16
14
Stage 0
Stage 1
Stage 2
Stage 3
Stage 4
Ossification stage


C2 - Black Males
Median age (years)
26
24
22
20
18
16
14
Stage 0
Stage 1
Stage 2
Stage 3
Stage 4
Ossification stage


C3 - White Males
Median age (years)
26
24
22
20
18
16
14
Stage 0
Stage 1
Stage 2
Stage 3
Stage 4
Ossification stage


C3 - Black Males
Median age (years)
26
24
22
20
18
16
14
Stage 0
Stage 1
Stage 2
Stage 3
Stage 4
Ossification stage


C4 - White Males
Median age (years)
26
24
22
20
18
16
14
Stage 0
Stage 1
Stage 2
Stage 3
Stage 4
Ossification stage


C4 - Black Males
Median age (years)
26
24
22
20
18
16
14
Stage 0
Stage 1
Stage 2
Stage 3
Stage 4
Ossification stage

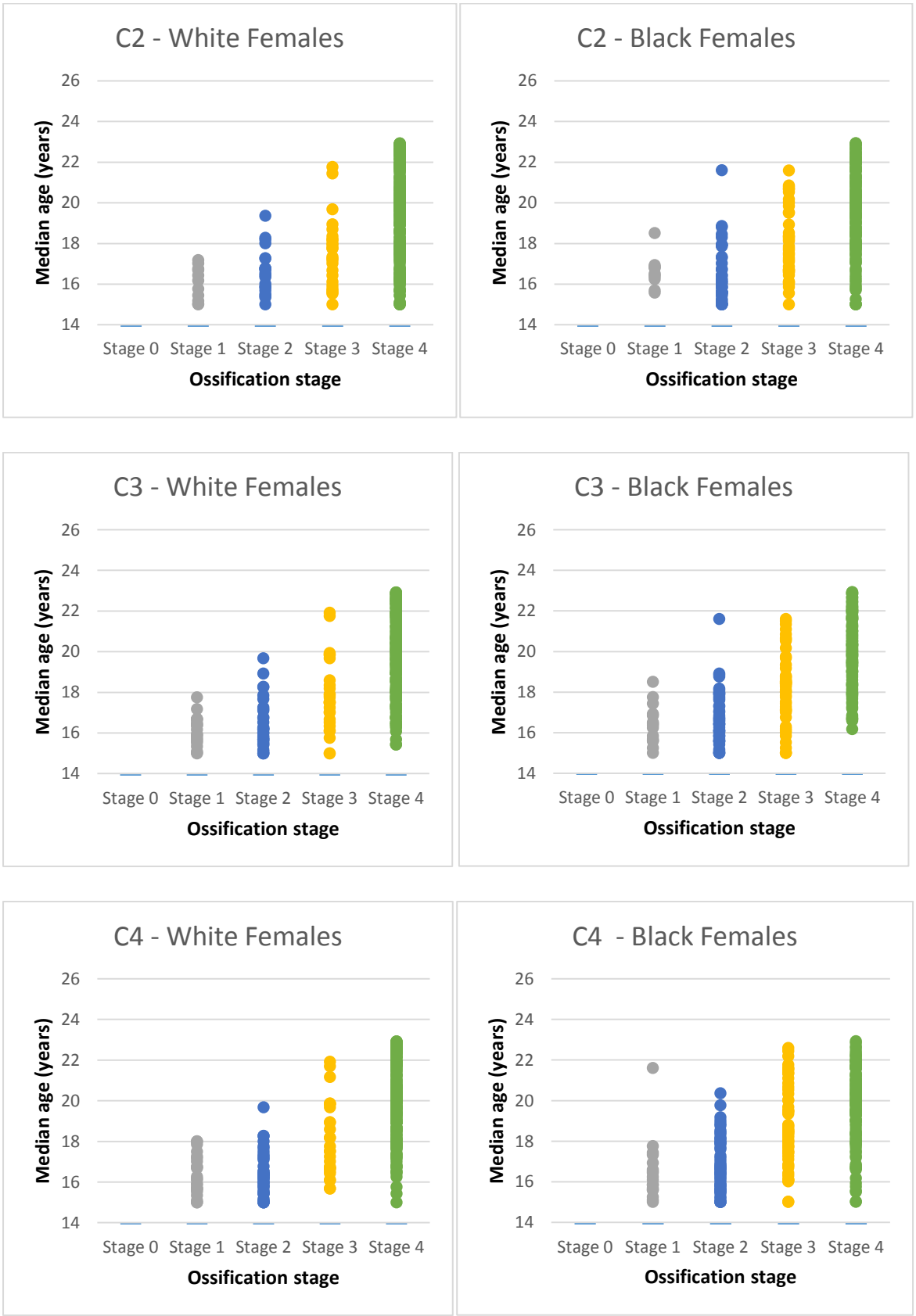


Figure 4.3: Dot plots of the median ages at which ossification stages 0 through 4 were attained for the anterior inferior apophysis for C2, C3 and C4.

Table 4.5: Regression equations, correlation coefficients (*R*), coefficients of determination (R^2) and standard errors of estimate of multiple regression analyses with age as the dependent variable and apophysis stage development changes as independent variables for vertebrae C2, C3 and C4.

Ancestry, Sex	Formula	*R*	R^2	Standard error
BSA,males	Age =13.1512+0.3558 (C2 stage) + 0.8201(C3 stage) + 0.7593 (C4 stage)	0.82	0.68	1.32
WSA,males	Age =13.6065+0.1376 (C2 stage) + 0.7078 (C3 stage) + 0.9344 (C4 stage)	0.84	0.70	1.22
BSA,females	Age = 12.6790 + 0.5171(C2 stage) + 0.6442(C3 stage) + 0.7371(C4 stage)	0.70	0.49	1.65
WSA,females	Age =12.3657 + 0.3717 (C2 stage) + 0.6602(C3 stage) + 0.8972(C4 stage)	0.71	0.51	1.64

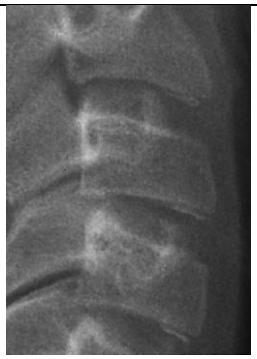
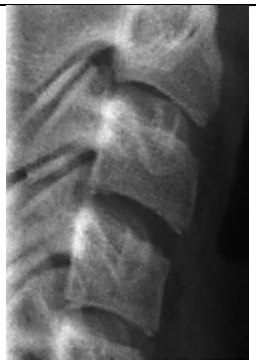
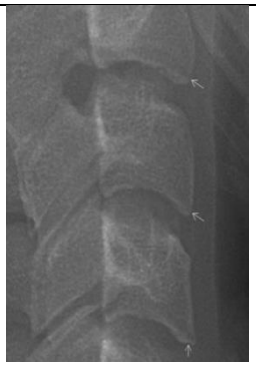
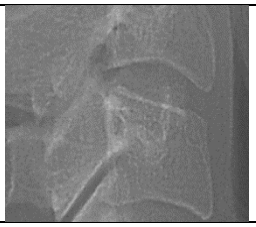
Table 4.6: Regression equations using dichotomous indicator variables for different stage classifications.

Ancestry, Sex	Formula	*Adjusted* R^2	Standard Error
BSA,males	Age = 15.2034 + 0.6460 I(C2 Stage 2) + 0.8826 I(C2 Stage 3) + 1.3062 I(C2 Stage 4) + 0.7031 I(C3 Stage 2) + 1.6965 I(C3 Stage 3) + 2.3734 I(C3 Stage 4) + 0.1834 I(C4 Stage 2) + 1.5664 I(C4 Stage 3) + 1.8673 I(C4 Stage 4)	0.6819	1.27
WSA,males	Age = 15.9375 – 0.1695 I(C2 Stage 2) - 0.1004 I(C2 Stage 3) + 0.5747 I(C2 Stage 4) + 0.4142 I(C3 Stage 2) + 1.0593 I(C3 Stage 3) + 1.9955 I(C3 Stage 4) + 0.4828 I(C4 Stage 2) + 1.5515 I(C4 Stage 3) + 2.4435 I(C4 Stage 4)	0.7171	1.19
BSA,females	Age = 16.1144 – 0.0304 I(C2 Stage 2) + 0.6903 I(C2 Stage 3) + 1.4348 I(C2 Stage 4) – 0.0065 I(C3 Stage 2) + 0.0485 I(C3 Stage 3) + 1.4075 I(C3 Stage 4) – 0.22591 I(C4 Stage 2) + 1.0089 I(C4 Stage 3) + 1.4919 I(C4 Stage 4)	0.5119	1.60
WSA,females	Age = 15.6341 – 0.5256 I(C2 Stage 2) - 0.2254 I(C2 Stage 3) + 0.5555 I(C2 Stage 4) + 0.1953 I(C3 Stage 2) + 0.9296 I(C3 Stage 3) + 1.614 I(C3 Stage 4) + 0.1195 I(C4 Stage 2) + 1.2230 I(C4 Stage 3) + 2.3748 I(C4 Stage 4)	0.5119	1.62

Key: I(Cy(Cervical verebrae C2,C3 or C4) Stage x(2, 3 or 4) = 1 if Cy Stage is present. If the stage is abscent a zero (0) gets allocated to the stage.

Figure 4.4 includes four examples of cropped cephalometric radiographs showing the different stages of apophyses development for C2, C3 and C4 in different ancestry and sex groups. The examples included in the figure were specifically selected for illustration purposes and include examples with the same apophysis development stage for each cervical vertebra. The anterior cervical vertebral ring apophysis ossification development does not necessarily develop at the same rate for each cervical vertebra (Fig. 4.5). Two regression formulae were developed for

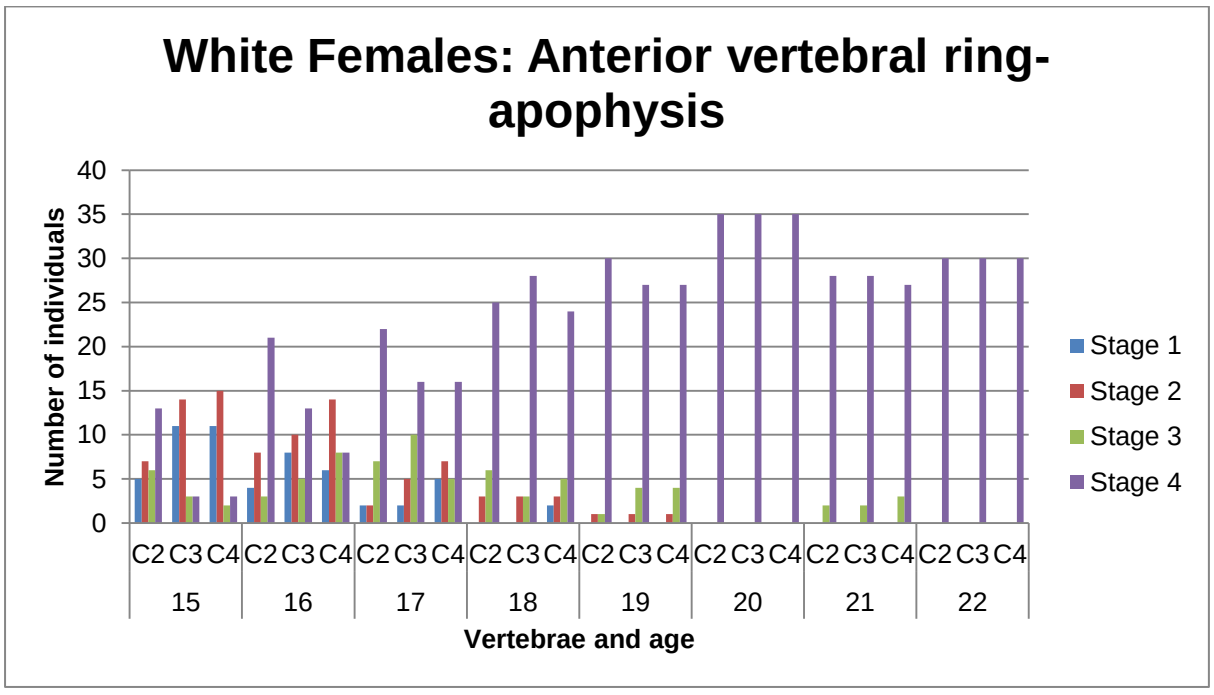
each example. The first model represents a multivariate regression formula and the second model represents a dichotomous (categorical) regression formula. The chronological age can be compared with the two regression formulae.

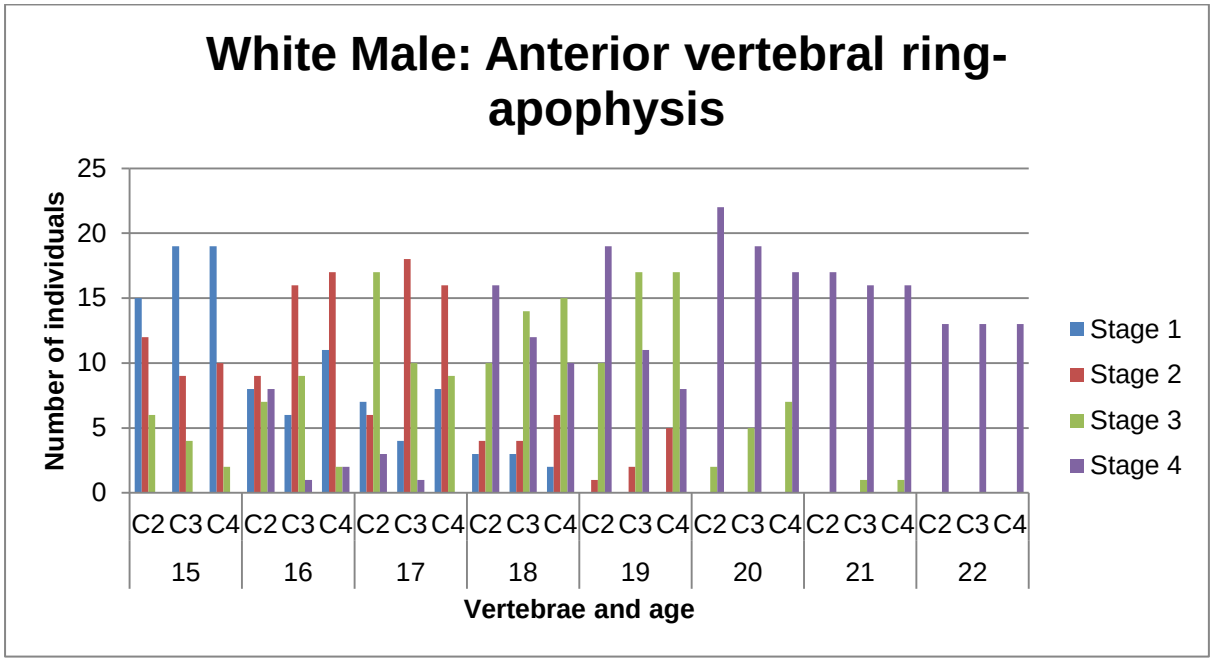
Cropped lateral cephalometric radiograph of C2, C3 and C4	**Stage**	**Ancestry, sex**	**Chronological age**	**Age determined with regression equations:** **1= Multiple linear regression analysis** ***2= Dichotomous indicator variable analysis***
	C2 stage = 1 C3 stage = 1 C4 stage = 1	WSA, male	15.59 years	1: Age =13.6065+0.1376 (C2 stage) + 0.7078 (C3 stage) + 0 .9344 (C4 stage) =13.6065+0.1376 (1) + 0.7078 (1) + 0.9344 (1) =15.35 ± 1.22 years *2: Age = 15.9375 – 0.1695 I(C2 Stage 2) - 0.1004 I(C2 Stage 3) + 0.5747 I(C2 Stage 4) + 0.4142 I(C3 Stage 2) + 1.0593 I(C3 Stage 3) + 1.9955 I(C3 Stage 4) + 0.4828 I(C4 Stage 2) + 1.5515 I(C4 Stage 3) + 2.4435 I(C4 Stage 4)* *= 15.9375 ± 1.19 years*
	C2 stage = 2 C3 stage = 2 C4 stage = 2	BSA, female	17.02 years	1: Age = 12.6790 + 0.5171(C2 stage) + 0.6442(C3 stage) + 0.7371(C4 stage) = 12.6790 + 1.0342 + 1.2884 + 1.4742 = 16.48 ± 1.65 years *2: Age = 16.1144 – 0.0304 I(C2 Stage 2) + 0.6903 I(C2 Stage 3) + 1.4348 I(C2 Stage 4) – 0.0065 I(C3 Stage 2) + 0.0485 I(C3 Stage 3) + 1.4075 I(C3 Stage 4) – 0.22591 I(C4 Stage 2) + 1.0089 I(C4 Stage 3) + 1.4919 I(C4 Stage 4)* *= 16.1144 – 0.0304(1) - 0.0065(1) – 0.22591(1)* *= 15.8516 ± 1.6 years*
	C2 stage = 3 C3 stage = 3 C4 stage = 3	BSA, male	19.45 years	1: Age = 13.2284+0.3617 (C2 stage) + 0.8018(C3 stage) + 0.7300 (C4 stage) = 13.2284+ 0.3617 (3) + 0.8018 (3) + 0.7300 (3) = 18.91± 1.30 years *2: Age = 15.2034 + 0.6460 I(C2 Stage 2) + 0.8826 I(C2 Stage 3) + 1.3062 I(C2 Stage 4) + 0.7031 I(C3 Stage 2) + 1.6965 I(C3 Stage 3) + 2.3734 I(C3 Stage 4) +0.1834 I(C4 Stage 2) + 1.5664 I(C4 Stage 3) + 1.8673 I(C4 Stage 4)* *= 15.2034 + 0.8826 (1) + 1.6965 (1) + 1.5664 (1)* *= 19.3489 ± 1.27 years*
	C2 stage = 4 C3 stage = 4	WSA, female	21.25 years	1: Age =12.3657 + 0.3717 (C2 stage) + 0.6602(C3 stage) + 0.8972(C4 stage) = 12.3657 + 0.3717 (4) + 0.6602(4) + 0.8972(4) = 20.08± 1.64 years *2: Age = 15.6341 – 0.5256 I(C2 Stage 2) - 0.2254 I(C2 Stage 3) +*

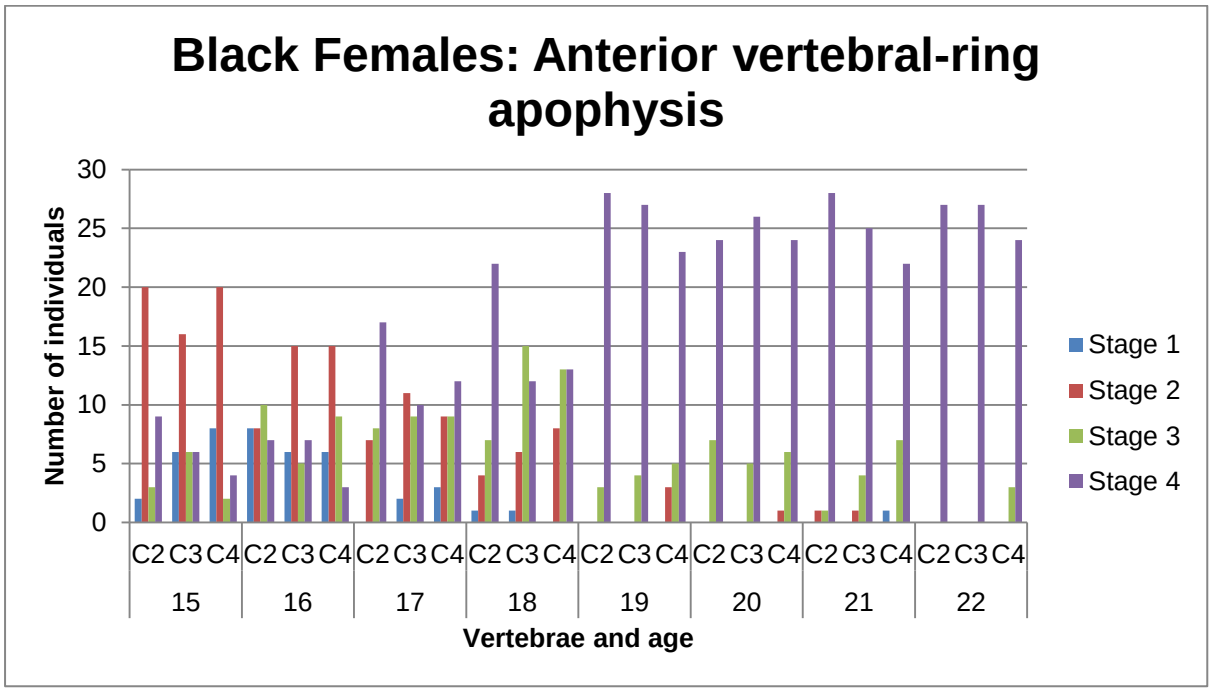
	C4 stage = 4			*0.5555 I(C2 Stage 4)+ 0.1953 I(C3 Stage 2) + 0.9296 I(C3 Stage 3) + 1.614 I(C3 Stage 4)+ 0.1195 I(C4 Stage 2) + 1.2230 I(C4 Stage 3) + 2.3748 I(C4 Stage 4)* *=15.6341+ 0.5555 (1) + 1.614 (1)+ 2.3748(1)* *=20.1784 ± 1.62 years*

Figure 4.4: Cropped lateral cephalometric radiographs of four individuals to illustrate the practical use of the regression equations with one example of each population and sex group. (Please take note that the cases were specifically selected for illustration purposes and with the same apophysis development stage for each cervical vertebra. The four examples were taken from individuals outside of the sample The anterior cervical vertebral ring apophysis ossification development is not necessarily at the same stage for each cervical vertebra.)

Figure 4.5 illustrates the development pattern for each vertebral ring apophysis stage according to age. The graphs provide insight into the relationship of development among the vertebrae. In general the apohysis development stages related to C2 seems to be reached earlier compare to C3 and C4. From the graphs it is evident that stages 3 and 4 are more commonly observed after the age of 18 years.




White Male: Anterior vertebral ring-apophysis
Number of individuals
25
20
15
10
5
0
C2 C3 C4 C2 C3 C4 C2 C3 C4 C2 C3 C4 C2 C3 C4 C2 C3 C4 C2 C3 C4 C2 C3 C4
15 16 17 18 19 20 21 22
Vertebrae and age
Stage 1
Stage 2
Stage 3
Stage 4


Black Females: Anterior vertebral-ring apophysis
Number of individuals
30
25
20
15
10
5
0
C2 C3 C4 C2 C3 C4 C2 C3 C4 C2 C3 C4 C2 C3 C4 C2 C3 C4 C2 C3 C4 C2 C3 C4
15 16 17 18 19 20 21 22
Vertebrae and age
Stage 1
Stage 2
Stage 3
Stage 4

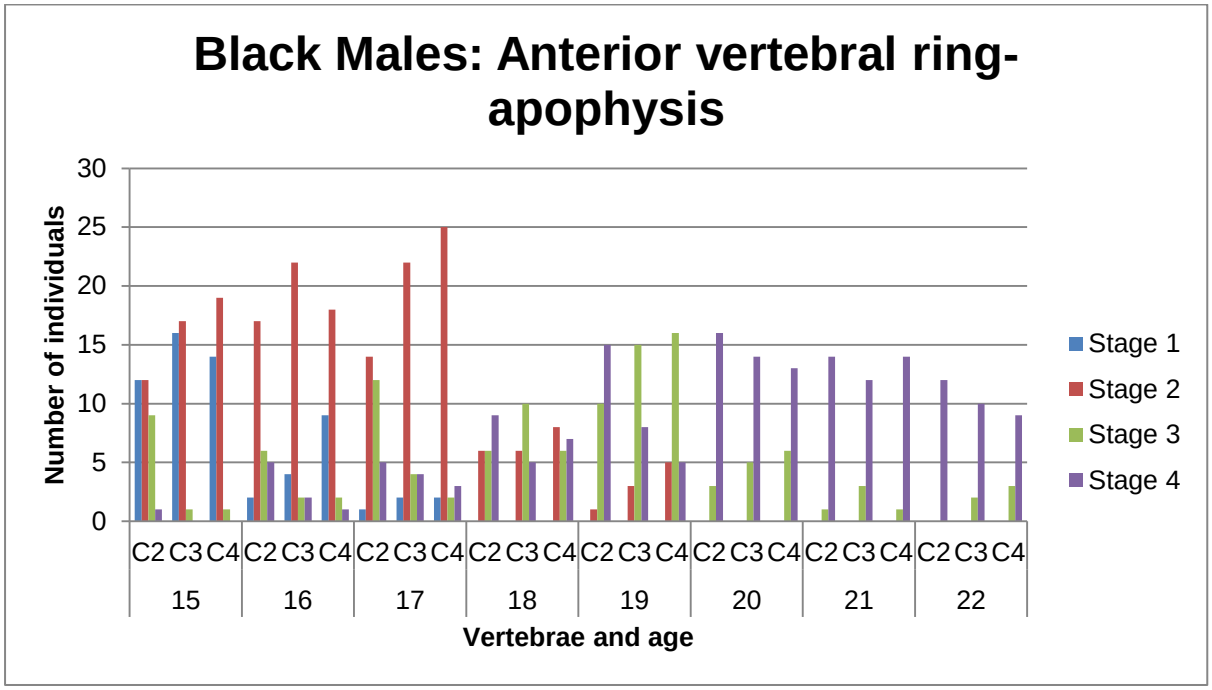


Figure 4.5: Graphs display the development of the anterior inferior apophysis for C2,C3 and C4 and the relationship of development according to age for each ancestry and sex group.

Chapter 5: Multifactorial model: combining third molar and cervical vertebral ring apophysis development

5.1 Introduction

Third molar and cervical ring apophysis development follow clear and distinct sequences and can be used to estimate chronological age, as demonstrated in the previous chapters [37, 231]. Most European Union countries make use of two or more age indicators [52], and therefore in this section the two techniques developed in this study will be combined. The advantage of this approach is that it gives information on both skeletal and dental maturation, which are influenced to different degrees by environmental and genetic factors.

Few studies have attempted to combine dental and skeletal indicators into a single formulae [20, 232, 233]. Third molar and cervical vertebrae information were combined by Thevissen *et al.* [20], while Cameriere *et al.* [232] combined dental and hand-wrist development. Linear regression was used in these cases [142]. Shi *et al.* [214] combined DNA methylation markers in blood with dental and hand-wrist development by using a multivariate linear stepwise regression model. The outcomes of numerous studies proved that combing developmental features enhanced age estimation [19–21, 214, 232, 233]. The studies found that a combined method lead to overall improvement in the accuracy of age assessment.

According to the literature, different statistical approaches towards age estimation can be followed. In the first instance, a regression model is applied with age being the dependant and the age indicators being the independent variables [234]. Alternatively, multivariate distributions for the age indicators can be considered. Bayes' rule can be applied to these models to estimate age irrespective of the type of distribution used [224]. In Bayesian statistics the Bayes' theorem is repeatedly applied to conditions with observed data and unknown quantities. The unknown parameter in forensics is the chronological age. The posterior probability distribution is the result from a normalized combination of a prior probability distribution of the likelihood function and the unobserved variable [220].

The use of multiple age indicators may improve accuracy of age estimation methods [222, 235], but there are many debates as to the best possible method to use to combine age indicators [19]. A solution to combine data from multiple methods would be to take the average of the different multiple age estimates. To improve accuracy a weighted average can be considered. The challenge arises in the construction of the prediction intervals due to the correlation between the multiple age indicators. If the correlation is disregarded the result will be prediction intervals which are too small [19].

Methods managing correlations between multiple indicators by incorporating them in the age estimation process are discussed extensively in the literature [19, 21, 71, 236]. Multiple linear regression models are an example where the conditional distribution of age given the age indicator is predicted. A multivariate normal distribution is seldom found because of the different types of age indicators and when the number of age indicators increase. A multivariate distribution model is based on an assumption of conditional independence. If conditional independence is assumed inappropriately the prediction intervals will be too small. This will lead to a biased degree of certainty when estimating age. The multivariate distribution can be replaced by a set of univariate distributions to overcome the problem [19].

Boldsen *et al*. [222] and others introduced a transitional analysis model to combine different age indicators in order to attempt to improve age estimates. Bayes' rule was used to estimate age-at-death from skeletons. The maximum likelihood estimates serve as the point estimates for age. The posterior distributions of age are used to calculate the prediction intervals [222]. Fieuws *et al*. [19] confirmed that the procedure gave appropriate prediction intervals in a practical setting and Kumagai *et al.* [21] performed age estimation by making use of dental and skeletal age parameters and applying the Bayes' rule. The procedure was based on the application of Bayes' rule to a multivariate continuation ratio model and additionally the calculation of corrections was performed using an *ad hoc* procedure [19, 222].

Chapter 5 explores the theory that a multi-factorial approach to age estimation will provide age estimations with higher precision compared to stand alone age markers. The literature suggests that age indicators should be combined to enhance age estimation. Although many methods are available and suggested e.g. transitional

analysis, we decided to test our multiple age indicators with a multivariate model against the individual indicators. A previous study demonstrated equal accuracy when a classical regression model was compared to a Bayesian approach [224]. The decision was made to first test our multiple age indicators with a multivariate model. Future research will include testing by using a complicated Bayesian approach.

5.2 Materials and methods

The digital panoramic radiographs of 1268 individuals were used for third molar assessment (Chapter 3), combined with the cephalometric radiographs of 974 individuals in which the anterior inferior apophysis was assessed (Chapter 4). These were scored according to a newly developed scoring system from stages “0” to “4” (Chapter 4). Third molar and vertebral data were not always collected from the same individuals due to practical constraints, and therefore the sample was divided into five data sets: both age indicators from the same individual available (group 1), individuals with only third molar development stages (group 2), individuals with only cervical ring apophysis ossification stage information (group 3), individuals with third molar development stages from group 1 and 2 (group 4), and individuals with cervical ring apophysis ossification stages from group 1 and 3 (group 5) (Table 5.1).

The data were imported and filtered to include individuals between 15 to 18 years with both panoramic (third molar stages) and cephalometric (cervical vertebral ring apophysis ossification stages) radiographs obtained on the same day (group 1). Only individuals between 15 and 18 years were included because after 18 years of age it became challenging to find individuals with both cephalometric and panoramic radiographs taken on the same day. The age group between 15 and 18 years consisted of a large enough sample size to test our approaches and most of these individuals received orthodontic treatment and this justified the exposure of both radiographs.

Table 5.1: Sample size for each ancestry and sex group.

	Black South Africans		**White South Africans**		
Groups:	Females	Males	Females	Males	Total
Group 1	87	51	78	71	287
Group 2	47	77	66	70	260

Group 3	55	80	67	68	270
Group 4	134	128	144	141	547
Group 5	142	131	145	139	557

As discussed in the Literature Review, there is currently no consensus as to which statistical approach to use in cases of multivariate age assessment. Here we opted for regression as it allows one to easily compare accuracies achieved by different combinations of variables as well as single age indicators. The aim here was not so much to create formulae to estimate age in living individuals, as this is not appropriate, but rather to evaluate whether the inclusion of different types of data (in this case third molar and vertebral apophyseal data) achieves better results than using single age indicators. Firstly, the data set with individuals having combined age indicators were subjected to a generalised linear model (GLM) analysis (Table 5.2). The model included age (continuous), ancestry (black and white South Africans), sex (males, females), apophysis stages for C2, C3 and C4 and third molar stages for the mandibular and maxillary third molar teeth. These data were used to test the model and to make adjustments to obtain a model with better fit for our set of observations.

Table 5.2: Generalised linear model (GLM) for data set containing combined age indicators.

$Y = ß + F_1 + F_2 + ß_1X_1 + ß_2X_2 + ß_3X_3 + ß_4X_4 + ß_5X_5$

Where: ß is the intercept value, F_1 is the factor ancestry, F_2 is the factor sex, X_1 is the variable stage C2, X_2 is the variable stage C3, X_3 is the variable stage C4, X_4 is the variable for the maxillary third molar stage, X_5 is the variable for the mandibular third molar stage.

Age was considered the dependant variable for the model and sex and ancestry were coded as factors because of the different levels (males and females and black and white South African individuals). The stages for C2, C3 and C4, and third molar stages were coded as numeric (stages) because of the different ordinal development stages. The null deviance, residual deviance, Akaike Information Criterion (AIC) and the Fisher scoring iterations were calculated. The *p*-values for ancestry, C3 apophysis development, and the mandibular and maxillary third molar development did not significantly influence the model. A new GLM model (Table 5.3) was subsequently developed with a lower AIC value, after taking the significant values

and intercorrelation between third molar data into consideration for a better fit. The observations which had a significant influence on the model fit were sex and C2 and C4 apophysis stage development. The fit of the model was improved and assessed with a leave-one-out cross validation.

Table 5.3: Generalised linear model (GLM) with lower AIC for data set containing combined age indicators.

$Y = ß + F_1 + F_2 + ß_1X_1 + ß_2X_2 + ß_3X_3 + ß_4X_4 + ß_5X_5 + ß_6X_4X_5$

Where: ß is the intercept value, F_1 is the factor ancestry, F_2 is the factor sex, X_1 is the variable stage C2, X_2 is the variable stage C3, X_3 is the variable stage C4, X_4 is the variable for the maxillary third molar stage, X_5 is the variable for the mandibular third molar stage, X_4X_5 is the interaction between variables X_4 and X_5.

Sex and ancestry were included in the GLM because it was one of our differentiators and they influence the age estimation. The prediction indicator was calculated as a mean square error value. Cross validation (CV) was then performed by a leave-one out CV process. Models were separated by sex and ancestry and the mean square error value was calculated for each population and sex group.

GLM's were separately coded for the individuals with only third molar data and cervical apophysis development data respectively (Tables 5.4 and 5.5).

Table 5.4: Generalised linear model (GLM) for data set containing only third molar development stages.

$Y = ß + F_1 + F_2 + ß_4X_4 + ß_5X_5 + ß_6X_4X_5$

Where: ß is the intercept value, F_1 is the factor ancestry, F_2 is the factor sex, X_4 is the variable for the maxillary third molar stage, X_5 is the variable for the mandibular third molar stage, X_4X_5 is the interaction between variables X_4 and X_5.

Table 5.5: Generalised linear model (GLM) for data set containing only cervical vertebrae apophyses development stages.

$Y = ß + F_1 + F_2 + ß_1X_1 + ß_2X_2 + ß_3X_3$

Where: ß is the intercept value, F_1 is the factor ancestry, F_2 is the factor sex, X_1 is the variable stage C2, X_2 is the variable stage C3, X_3 is the variable stage C4.

The AIC and the prediction error as a mean square error value were determined for each data set. The models were separated by sex and ancestry and a GLM was determined for each group. The AIC and mean square error value were also determined for each group separately.

5.3 Results

The results for the first round GLM (Table 5.2) found that ancestry, C3 apophyses stages and both the maxillary and mandibular third molar development stages did not significantly influence the model (Table 5.6). The AIC value was 802.27 for the first round GLM.

Table 5.6: Intercept estimates, standard errors, t-values and *p*-values for the first round GLM model.

Coefficients:				
	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
Intercept (ß)	13.09776	0.40635	32.232	< 2e-16 ***
F_1 is the factor ancestry	-0.07728	0.11744	-0.658	0.511027
F_2 is the factor sex	0.34808	0.12589	2.765	0.006073 **
X_1 is the variable stage C2	0.24456	0.06705	3.648	0.000316 ***
X_2 is the variable stage C3	0.08676	0.08318	1.043	0.297799
X_3 is the variable stage C4	0.40242	0.07895	5.097	6.37e-07 ***
X_4 is the variable for the maxillary third molar stage	-0.2263	0.03869	-0.585	0.558979
X_5 is the variable for the mandibular third molar stage	0.05753	0.03823	1.505	0.133519

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

After the first round, a GLM was fitted by using the non significant values to obtain a new GLM with improved *p*-values (Table 5.7) by incorporating an interaction effect.

Table 5.7: Intercept estimates, standard errors, t-values and *p*-values for the selected GLM model.

Coefficients:				
	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**

Intercept (ß)	14.41379	0.60002	24.022	< 2e-16 ***
F_1 is the factor ancestry	-0.06043	0.11599	-0.521	0.602813
F_2 is the factor sex	0.29018	0.12573	2.308	0.021737 *
X_1 is the variable stage C2	0.23476	0.06622	3.545	0.000461 ***
X_2 is the variable stage C3	0.08344	0.08206	1.017	0.310121
X_3 is the variable stage C4	0.37486	0.07844	4.779	2.86e-06 ***
X_4 is the variable for the maxillary third molar stage	-0.26708	0.09129	-2.926	0.003720 **
X_5 is the variable for the mandibular third molar stage	-0.10766	0.06755	-1.594	0.112108
X_4X_5 is the interaction between variables X_4 and X_5	0.03376	0.01145	2.948	0.003473 **

Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

The maxillary third molar stage development *p*-value changed from 0.558979 to 0.003720, while that of the mandibular third molar stage development *p*-value changed from 0.133519 to 0.112108. The combination of the maxillary and mandibular stage development data was added to the selected GLM and was also found to be significant. The AIC value changed from 802.27 to a lower value of 795.44 and this indicates a model with a better fit for our observations. A prediction error was calculated as a mean square error value of 1.633136.

The prediction error through a cross-validation procedure was calculated for each of the five data set groups by separating the models by sex and ancestry (Table 5.8). The prediction error was expressed as a mean square error value with a smaller number indicating a better fit (i.e., higher accuracy). From Table 5.8, it can be seen that the mean square errors of the group (group 2) with only third molar development were the smallest, and ranged from 1.178625 to 1.677132. In group 3, consisting of individuals with only cervical ring apophysis ossification development, the values ranged between 1.772198 and 2.037545 indicating lesser accuracy.

Table 5.8: The prediction error expressed as a mean square error value for each data set group through a cross-validation procedure.

Data set	Combined sex and ancestry	Females	Males	BSA Females	WSA Females	BSA Males	WSA Males	WSA	BSA
Group1: Individuals with combined age indicators	1.633136	1.56104	1.793984	1.728426	1.496248	1.823611	1.900472	1.691555	1.703898
Group 2: Individuals with only third molar teeth development stages	1.36312	1.467256	1.301906	1.610587	1.677132	1.378902	1.178625	1.365946	1.396691
Group 3: Individuals with									

only cervical ring apophysis ossification stages	1.903976	NA	1.947672	NA	NA	1.967164	1.984327	2.037545	1.772198
Group 4: Individuals with third molar teeth development stages from groups 1 & 2	1.431504	1.424568	1.455238	1.429421	1.481639	1.424051	1.495598	1.461787	1.414983
Group 5: Individuals with cervical ring apophysis ossification stages from both groups1 & 3	1.791354	1.694354	1.919187	1.67339	1.753572	1.87938	1.964466	1.85046	1.747554

For each ancestry and sex group, regression formulae were calculated. Standard errors were calculated to practically use when estimating age as well as formulae for upper and lower age estimates (Table 5.9).

Table 5.9: Age estimate values and regression formulae for combined and separate sex and ancestry groups.

Age estimate values					
Coefficients	**Estimate**				
	Combined (sex and ancestry)	BSA females	WSA females	BSA males	WSA males
Intercept (ß) value	14.41379	13.792198	15.63836	14.43819	14.31183
Ancestry (F_1) factor estimate for white individuals	-0.06043	0	-0.06043	0	-0.06043
Ancestry (F_1) factor estimate for black South African individuals	0	0	0	0	0
Sex (F_2) factor estimate for male individuals	0.29018	0	0	0.29018	0.29018
Sex (F_2) factor estimate for female individuals	0	0	0	0	0
C2 ($ß_1$) apophysis stage numeric estimate	0.23476	0.397948	0.10246	0.06903	0.16628
C3 ($ß_2$) apophysis stage numeric estimate	0.08344	-0.238324	0.21662	0.50709	0.30671
C4 ($ß_3$) apophysis stage numeric estimate	0.37486	0.532897	0.24410	0.35629	0.26020
Maxillary third molar ($ß_4$) stage numeric estimate	-0.26708	-0.131652	-0.59136	-0.11952	-0.37451
Mandibular third molar ($ß_5$) stage numeric estimate	-0.10766	0.068256	-0.14716	-0.39473	-0.07590
Combined mandibular and maxillary third ($ß_6$) molar stage numeric estimate	0.03376	0.002982	0.06957	0.03901	0.04874
	Standard error values				
Intercept (ß) standard error value	0.60002	1.163177	1.30164	1.09488	1.32309
Ancestry (F_1) standard error factor estimate for white individuals	0.11599	0	0.11599	0	0.11599

Ancestry (F_1) standard error factor estimate for black South African individuals	0	0	0	0	0
Sex (F_2) standard error factor estimate for male individuals	0.12573	0	0	0.12573	0.12573
Sex (F_2) standard error factor estimate for female individuals	0	0	0	0	0
C2 ($ß_1$) standard error apophysis stage numeric estimate	0.06622	0.122117	0.11304	0.18884	0.17697
C3 ($ß_2$) standard error apophysis stage numeric estimate	0.08206	0.160999	0.11593	0.32245	0.18322
C4 ($ß_3$) standard error apophysis stage numeric estimate	0.07844	0.145852	0.11989	0.29615	0.20362
Maxillary third molar ($ß_4$) stage standard error numeric estimate	0.09129	0.156396	0.24832	0.14447	0.24259
Mandibular third molar ($ß_5$) stage standard error numeric estimate	0.06755	0.129440	0.14286	0.13678	0.15372
Combined mandibular and maxillary third ($ß_6$) molar stage standard error numeric estimate	0.01145	0.020499	0.02966	0.02060	0.02766

Regression formula:
$Age = ß + F_1 + F_2 + ß_1X_1 + ß_2X_2 + ß_3X_3 + ß_4X_4 + ß_5X_5 + ß_6X_4X_5$

Combined (sex and ancestry) regression model:
Age = 14.41379 – 0.06043(for a WSA individual, 0 for a BSA individual) + 0.29018 (for a male, 0 for a female) + 0.23476(X_1) + 0.08344(X_2) + 0.37486(X_3) + (-0.26708) (X_4) + (-0.10766) (X_5) + 0.03376(X_4) (X_5)

Upper age estimate:
Age = 14.41379 + 0.60002 + 0.11599 (for a WSA individual, 0 for a BSA individual) + 0.12573(for a male, 0 for a female) + 0.06622 (X_1) + 0.08206 (X_2) + 0.07844 (X_3) + 0.09129(X_4) + 0.06755(X_5) + 0.01145(X_4) (X_5)

Lower age estimate:
Age = 14.41379 - 0.60002 - 0.11599 (for a WSA individual, 0 for a BSA individual) - 0.12573(for a male, 0 for a female) - 0.06622 (X_1) - 0.08206 (X_2) - 0.07844 (X_3) - 0.09129(X_4) - 0.06755(X_5) - 0.01145(X_4) (X_5)

Black South African female regression model:

Age = 13.792198 + 0 + 0 + 0.397948 (X_1) + (-0.238324) (X_2) + 0.532897 (X_3) + (-0.131652) (X_4) + (0.068256) (X_5) + 0.002982 (X_4) (X_5)

Upper age estimate:
Age = 13.792198 + 1.163177 + 0.122117(X_1) + 0.160999 (X_2) + 0.145852(X_3) + 0.156396 (X_4) + 0.129440 (X_5) + 0.020499 (X_4) (X_5)

Lower age estimate:
Age = 13.792198 - 1.163177 - 0.122117 (X_1) - 0.160999 (X_2) - 0.145852 (X_3) - 0.156396 (X_4) - 0.129440 (X_5) - 0.020499 (X_4) (X_5)

White South African female regression model:

Age = 15.63836 – 0.06043 + 0 + 0.10246 (X_1) + 0.21662 (X_2) + 0.24410 (X_3) + (-0.59136) (X_4) + (-0.14716) (X_5) + 0.06957 (X_4) (X_5)

Upper age estimate:
Age = 15.63836 + 1.30164 + 0.11599 + 0.11304 (X_1) + 0.11593 (X_2) + 0.11989 (X_3) + 0.24832 (X_4) +

0.14286 (X_5) + 0.02966 (X_4) (X_5)

Lower age estimate:
Age = 15.63836 - 1.30164 - 0.11599 - 0.11304 (X_1) - 0.11593 (X_2) - 0.11989 (X_3) - 0.24832 (X_4) - 0.14286 (X_5) - 0.02966 (X_4) (X_5)

Black South African male regression model:

Age = 14.43819 + 0 + 0.29018 + 0.06903 (X_1) + 0.50709 (X_2) + 0.35629 (X_3) + (-0.11952) (X_4) + (-0.39473) (X_5) + 0.03901 (X_4) (X_5)

Upper age estimate:
Age = 14.43819 + 1.09488 + 0.12573 + 0.18884 (X_1) + 0.32245 (X_2) + 0.29615 (X_3) + 0.14447 (X_4) + 0.13678 (X_5) + 0.02060 (X_4) (X_5)

Lower age estimate:
Age = 14.43819 - 1.09488 - 0.12573 - 0.18884 (X_1) - 0.32245 (X_2) - 0.29615 (X_3) - 0.14447 (X_4) - 0.13678 (X_5) - 0.02060 (X_4) (X_5)

White South African male regression model:

Age = 14.31183 – 0.06043 + 0.29018 + 0.16628 (X_1) + 0.30671 (X_2) + 0.26020 (X_3) + (-0.37451) (X_4) + (-0.07590) (X_5) + 0.04874 (X_4) (X_5)

Upper age estimate:
Age = 14.31183 + 1.32309 + 0.11599 + 0.12573 + 0.17697 (X_1) + 0.18322 (X_2) + 0.20362 (X_3) + 0.24259 (X_4) + 0.15372 (X_5) + 0.02766 (X_4) (X_5)

Lower age estimate:
Age = 14.31183 - 1.32309 - 0.11599 - 0.12573 - 0.17697(X_1) - 0.18322(X_2) - 0.20362(X_3) - 0.24259(X_4) - 0.15372(X_5) - 0.02766(X_4) (X_5)

X_1 = C2 cervical vertebral ring apophysis stage development, X_2 = C3 cervical vertebral ring apophysis stage development, X_3 = C4 cervical vertebral ring apophysis stage development, X_4 = maxillary third molar development stage, X_5 = mandibular third molar development stage.

In Table 5.10 the corresponding numerical value for each third molar stage according to Solari *et al.* [11] criteria is indicated. The corresponding numerical value should be used in the formulae when estimating age.

Table 5.10: Third molar development stage and assigned corresponding value.

Third molar development stage	Value assigned to each stage
A	1
B	2
C	3
D	4
E	5
F	6
F_1	7
G	8

G_1	9
H	10

In Table 5.11 the corresponding numerical value for each cervical vertebral ring apophysis stage according to the criteria described in Fig. 4.1 is indicated. The corresponding numerical value should be used in the formulae when estimating age.

Table 5.11: Cervical vertebral ring apophysis stage and assigned corresponding value.

Cervical vertebral ring apophysis Stage	Value assigned to each stage
Stage 0	0
Stage 1	1
Stage 2	2
Stage 3	3
Stage 4	4

Figure 5.1 and all the subsequent calculations illustrate an example of how to practically use the regression formulae to estimate age from the dental development combined with cervical vertebral ring apophysis stage development. We recommend that both methods (third molars and vertebral changes) be used to determine a range of possible age estimates. The results obtained from the different models are similar and can be used to construct an argument combined with other considerations such as probability values to provide an expert opinion. This case was not included in the sample population, and is included here to demonstrate the practical application of the method to a real live situation**.** This was a black South African female with a known age of 15.41years.

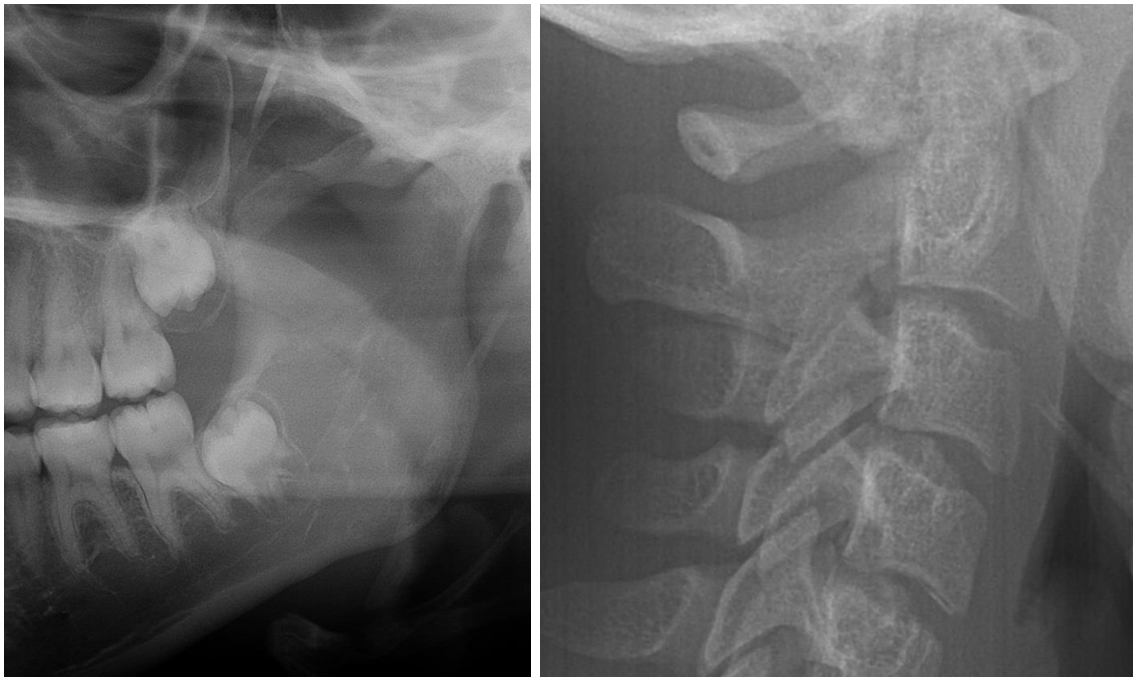


Figure 5.1: Cropped panoramic and cephalometric radiographs of a BSA female.

Third molar stage classification:
Maxillary third molar stage = E = 5
Mandibular third molar stage = F = 6

Cervical vertebral ring apophysis stage:
C2 apophysis stage 1 = 1
C3 apophysis stage 1 = 1
C4 apophysis stage 2 = 2

Combined (sex and ancestry) regression model:

Age = 14.41379 + 0 + 0 + 0.29018(X_1) + 0.08344(X_2) + 0.37486(X_3) + (-0.26708) (X_4) + (-0.10766) (X_5) + 0.03376(X_4) (X_5)
= 14.41379 + 0 + 0 + 0.29018(1) + 0.08344(1) + 0.37486(2) + (-0.26708) (5) + (-0.10766) (6) + 0.03376(5) (6)
= 14.41379 + 0 + 0 + 0.29018(1) + 0.08344(1) + 0.74972 + (- 1.3354) + (- 0.64596) + 1.0128
= 14.56 years

Upper age estimate = 14.41379 + 0.60002 + 0 + 0 + 0.06622(1) + 0.08206(1) + 0.07844(2) + 0.09129(5) + 0.06755(6) + 0.01145(5) (6)
= 14.41379 +0.60002 + 0 + 0 + 0.06622 + 0.08206 + 0.15688 + 0.45645 + 0.4053 + 0.3435
= 16.52 years

Lower age estimate = 14.41379 – 0.60002 + 0 + 0 - 0.06622 - 0.08206 - 0.15688 – 0.45645 – 0.4053 - 0.3435
= 12.30 years

Black South African female regression model:

Age = 13.792198 + 0 + 0 + 0.397948 (X_1) + (-0.238324) (X_2) + 0.532897 (X_3) + (-0.131652) (X_4) + (0.068256) (X_5) + 0.002982 (X_4) (X_5)
= 13.792198 + 0 + 0 + 0.397948 (1) + (-0.238324) (1) + 0.532897 (2) + (-0.131652) (5) + (0.068256) (6) + 0.002982 (5) (6)
= 13.792198 + 0 + 0 + 0.397948 - 0.238324 + 1.065794 - 0.65826 + 0.409536 + 0.08946
= 14.85 years

Upper age estimate = 13.792198 + 1.163177 + 0 + 0 + 0.122117 (1) + 0.160999 (1) + 0.145852 (2) + 0.156396 (5) + 0.129440 (6) + 0.020499 (5)(6)
= 13.792198 + 1.163177 + 0 + 0 + 0.122117 + 0.160999 + 0.291704 + 0.78198 + 0.77664 + 0.61497
= 17.70 years

Lower age estimate = 13.792198 - 1.163177 + 0 + 0 - 0.122117 (1) - 0.160999 (1) - 0.145852 (2) - 0.156396 (5) - 0.129440 (6) - 0.020499 (5) (6)
= 13.792198 - 1.163177 + 0 + 0 - 0.122117 - 0.160999 - 0.291704 - 0.78198 - 0.77664 - 0.61497
= 9.88 years

Chapter 6: Discussion

6.1 Introduction

The ability to ascertain whether a person has reached 18 years of age is critical in cases of unaccompanied minors, criminal investigations, illegal immigration, asylum seekers and children involved in child labour. Estimating a living individual's age after 14 years of age is challenging as only a few age indicators are still available. Accurate and reliable methods are therefore required as the outcomes of such estimations can have far-reaching consequences. The methods used should be practical, accurate and easy to use. The aim of this study was to address the shortcomings with regard to age estimation methods in living individuals in South Africa and to develop accurate, workable and preferably multifactorial age estimation methods.

For this purpose, representative population data needed to be collected. The data used for age estimation in South Africa are mostly unrepresentative of the majority of the population as European reference samples are used. The data and methods from this study will provide the legal system with accurate outcomes and quality standards, although no clear guidelines exist in South Africa regarding which techniques should be used to estimate age. The Child Justice act, 75 of 2008 (CJA) does, however, protect children under the age of 18 years and the ages of criminal capacity are clearly defined. This study will help to reduce doubt when the decision making body (court) must assess age for a credible legal outcome.

This study focused on three key areas namely, dental development (third molars), skeletal development (anterior inferior vertebral ring apophysis of cervical vertebrae) and a combination of the features into a multifactorial age estimation model for living individuals. The present study offers complete data regarding these aspects and therefore fulfils the needs for age estimation in living individuals in a South African population and these methods now need to be verified using independent samples. Age estimation procedures make use of radiographs and the advantage for the individual from the information obtained from the radiographs should always

outweigh the ionizing radiation exposure risk [61]. Cephalometric radiographs with an effective dose of less than half of a panoramic radiograph is used for the evaluation of the cervical vertebral apophysis [63]. This is an added advantage of the cervical vertebrae apophysis development method as age estimation is performed on young individuals which are more sensitive to the effects of radiation. This can therefore be used as a method / indicator on its own, should exposure to radiation be an issue.

Reproducibility is a fundamental requirement for any method used in age estimation. Therefore, the reproducibility of the third molar stage classification and the newly developed cervical vertebrae apophysis development classification was subjected to intra- and inter-observer reliability assessment. The repeatability was found to be satisfactory, giving validity to the outcomes.

6.2 The probability of being at least 18 years using third molars

Limitations to the accuracy of age estimation include individual variation of tooth development between individuals from birth to adulthood. After the age of 14 years, age estimation becomes difficult due to the fact that most teeth are already fully developed [237]. Third molar development is quite variable and non-linear with less variation among ancestry groups when approaching the 18 year age threshold [11, 128]. Third molars are generally the only teeth still developing and useful to determine the probability of an individual being 18 years of age. The staging used by Demirjian *et al.* [5] proved to be accurate for the use in forensic age estimation by other researchers [82, 109, 149, 170], although limitations exist [9, 219]. Having clearly defined stages increased the intra- and inter-examiner agreement [238]. A ten stage scoring system was used to achieve higher accuracy in separating the stages of development towards apex closure [11]. Demirjian *et al*. [5] did not describe F1 and G1 as permanent molar stages but the additional stages have been found to be of particular value for third molar development evaluation by other researchers [11]. The two additional stages, F1 and G1, proved to be valuable in this study in determining the likelihood of being 18. The increase in likelihood between stages F, F1, G, G1 and H is shown in Tables 3.4 and 3.5.

To the authors' knowledge, the present study is the first to give reference data with a large, representative sample on the development of third molars in South African subjects of the relevant ancestry, sex and age groups. The data presented in Tables 3.2, 3.3, 3.4 and 3.5 can be used for forensic age diagnostics of living subjects, unidentified corpses and skeletons.

In this study it was found that third molar development terminates earlier in BSA females than in WSA females and stage H is reached, on average, 11.16 months earlier in the maxilla and 13.92 months earlier in the mandible in BSA. BSA females reached 11 out of the 14 evaluated stages before the WSA females with 6 stages being statistically significant. BSA males reached stage H an average of 6.90 months earlier in the maxilla and 9.06 months earlier in the mandible when compared with WSA males. BSA males reached 8 out of the 14 evaluated stages before WSA males with 5 stages being statistically significant. For both the maxillary and mandibular third molars, the pattern of stage development was similar. Stages F1, G and H were reached earlier by BSA males compared with WSA males with only stage G1 reached earlier by WSA males. The faster tempos of development in black individuals compared with white individuals have been widely researched [135, 153, 163, 164, 168] and it has generally been found that black South African children compared with French-Canadian children showed an average advancement of 0.8 years for boys and 0.5 years for girls when the dental age was compared for individuals between 6 to 16.9 years [13]. The same pattern was found in north Americans [108, 115, 163, 168]. Although we found the same general trend, our results demonstrated that the difference between BSA and WSA males for the maxilla compared with the mandible was not that pronounced. However, the females demonstrated a larger absolute difference for mandibular third molars compared with the maxillary third molars. Some authors suggest that ancestry differences are greater during the earlier crown formation stages [115], and becomes less during the more advanced stages. The greatest difference between BSA and WSA males was 0.88 years for stage F in the maxilla. Stage D and E had the smallest difference between BSA and WSA males indicating that in this sample early root formation stages in the maxilla did not display great differences between ancestry groups for

males. The greatest difference between BSA and WSA males was 0.8 years for stage F1 in the mandible, while the greatest difference between BSA and WSA females was 1.6 years for stage G and 1.01 years for stage F1 in the maxilla. Stage D had the smallest difference between BSA and WSA females for the maxilla indicating small differences during early root formation stages. The greatest difference between BSA and WSA females was 1.25 years for stage G1 and 1.16 years for stage F1 in the maxilla. Stages E and F had the smallest difference with 0.04 and 0.07 years respectively, indicating that earlier root development had the smallest differences. This also suggests that an earlier or later initiation of root formation between ancestral groups may be present. Completion of certain stages may take longer followed by periods of growth acceleration. Liversidge [157] proposed that the population differences are the result of both earlier initiation and completion of third molar development in black South African individuals. The mean age difference between black individuals and other groups including a white group living in London was significant in 44 out of 45 comparisons. Black individuals in a combined sex group reached stage Ac (apex closed) at an earlier age compared with the white group. [157]. The observed differences between the current study sample and the black South African sample used by Liversidge [157] with regard to the mean age could be due to the small sample size used by Liversidge [157]. The median age for reaching stage H also differs between black South African males and females in this study compared with black South African individuals used in previous studies [135, 153]. These differences could also possibly be explained by the small sample sizes used for some of the groups in previous studies and by only making use of conventional radiographs. The development of a tooth is a complex process controlled by a specific sequence of cellular and molecular networks acting at specific times [93].

Olze *et al*. [239] compared a German sample with a black African sample and also found a clear tendency for the black African sample to reach mineralisation at an earlier stage. The median age for reaching stage H in the current study is about a year higher compared with a German study including all third molars present [171]. These results also indicate more than a year difference between the black female groups from neighbouring countries [152] . These differences could be due to using

an eight stage classification system compared with a ten stage classification system, the inclusion of all third molars compared with just the left third molars, and the use of conventional and digital radiographs compared with using only digital radiographs. The median ages for reaching stage H for WSA males and females in the current study were very similar to the results found for German and Japanese individuals [135] (Table 3.6). Reference data should be adjusted to accommodate different ancestry groups because of the variation within the chronology of tooth formation. The differences between ancestries (Table 3.6) should be noted especially when it is applied to practice. The consensus seems to be that persons of African origins tend to mature earlier compared to other ancestry groups, and this should be taken into account when an age assessment is attempted.

Larger mean age differences (Table 3.7) are noted between WSA- and German individuals as compared with WSA and BSA individuals, especially for stage G. The difference for stage H is however, larger for WSA and BSA individuals compared with WSA and German individuals. This suggests that different populations from the same geographical area display smaller differences compared with populations from a different area.

Studies have suggested that skeletal maturation does not depend on ethnicity, but rather optimum environmental conditions (i.e. high socioeconomic status). Lower socioeconomic status may lead to retardation of skeletal maturation [41]. South Africa is considered a developing country (third world country), as opposed to a developed country such as Germany. Dental maturation falls under strict genetic control, although it may be somewhat influenced by environmental factors. Similar environmental influences on dental maturation may be shared among different ancestry groups of the same demographic area [10], thus leading to similar developmental rates.

Males tend to reach third molar mineralisation stages at earlier chronological ages compared with females [148, 161]. Steroid –mediated adolescent growth phases can be a possible explanation for the unique pattern of earlier third molar development in males [123]. Two other postulations are that factors modulated by the X

chromosome slow down third molar development in females or that the factors modulated by the Y chromosomes enhance the rate of third molar mineralisation in males [123]. This study shows that third molar development terminates on average earlier in males compared with females in both populations included in this study. However, only one stage for BSA individuals reached statistical significant difference (stage G1 in the mandible). Stages G1 and H in the maxilla and stages G and H in the mandible were statistically different in WSA individuals. These findings regarding sexual dimorphism correspond to the findings in other populations where third molar maturity appears to be more advanced in boys [11, 82, 122, 133, 144]. In contrast, Liversidge [157] found that the mean age was reached earlier for almost all stages in black African girls compared with boys and the sex difference was significantly different for only the crown complete stage where boys were 0.73 years later than girls. These conflicting results could be due to the small number of individuals used by Liversidge [157] in some of the age categories, but may also reflect variations between populations. In the Liversidge study, for age 16 years, 18 males and 19 females were used, and age for a large number of the black individuals were assumed to be on the half year. A different staging system, namely Moorrees *et al*. [99], was used in the Liversidge study with additional descriptive criteria [157] which may have also influenced the results. Olze *et al*. [153] also found that, on average, female teeth developed 1.5 years earlier than those of males in a black African population. The sex distribution of the sample was skewed with the males making up most of the sample. For example, age 17 years only comprised of 15 males and 7 females [153]. Mean ages were found not to be statistically significant between males and females across all the third molar maturity index ranges in a black African population in Botswana [151]. These discrepancies between different studies need further investigation and may suggest that there are other factors that play a role. Sexual dimorphism as far as dental development is concerned appears to be greater in the WSA population than in the BSA. Because of the observed sexual dimorphism in various groups, it is advised that age estimation standards must be sex specific [82] until more clarity on the matter is obtained.

The results for the inter-observer repeatability indicated substantial agreement between observers for the evaluation of the maxillary third molars and only a

moderate agreement for the mandibular third molars which are usually easier to rate. The mandibular third molars are generally easier to rate due to the fact that there is no anatomical structures superimposed over the area of evaluation on a panoramic radiograph, and therefore this result is somewhat unexpected. The quality of the panoramic radiographs used was very high. Only digital panoramic radiographs were used with the added advantage of using the software tools to create clear images and optimal viewing conditions making evaluation of the upper maxillary third molars easier. Stages F and F1 had a high rate of disagreement between the observers for both the maxillary and mandibular third molars. This could be due to the difficulty in exactly determining the length of the root in relation with the crown to assign a specific stage, especially in cases where the mesial and distal root lengths are different. Stages G and G1 in the mandible also had a high rate of disagreement. Most disagreement between observers was to decide if the apical foramen was closed enough to categorize the tooth as a stage G1 instead of a stage G. The transition from stage G to G1 created difficulty in accurately staging some teeth.

In this study the likelihood of an individual being 18 years of age based on the third molar development stage for the maxilla and mandible was determined (Table 3.4). The likelihood for an individual being 18 years of age increased considerably from stage F to stage G and from stage G to H for all ancestry and sex groups (Table 3.4). The additional stages F1 and G1 make the transition of likelihood ratios between stages more gradual. The likelihood to be younger than age 18 years were 100% for BSA males for stages D and E for the maxillary and mandibular third molars as well as for WSA males for stage D for the mandibular third molars. The likelihood ratios between ancestry and sex groups were different for all development stages. These results also differ from those of other population groups [143]. With a combined likelihood for the maxilla and mandible for stage H, BSA females and males had likelihood values of 0.9839 and 0.9542, respectively. According to Liversidge [157] Black South Africans displayed values of 0.901 for females and 0.992 for males. When a 95% confidence is considered for the maxilla and mandible respectively for stage H, the majority of the females and WSA males are above, or very close to, age 18 with correspondingly high likelihoods. Combined results for the maxilla and mandible for stage H increased the likelihood of being 18 years to above

95% for all ancestry and sex groups (Table 3.5). All ancestry and sex groups were older than 16 years when stage H was reached (Figure 3.4). Therefore, a combined likelihood of the maxillary and mandibular third molar is considered to be a reliable indicator to determine if an individual is older than 18 years of age. When estimating the age of a South African individual from BSA or WSA ancestry, the combined likelihood for the left maxillary and mandibular third molar must be considered for stage H to increase the likelihood of being at least 18 years of age to above 95%. However, the study data cannot serve as the only criterion to estimate age and additional methods must be applied to estimate age more accurately. Ancestry and sex differences should be taken into consideration when estimating age.

6.3 Age estimation from anterior cervical vertebral ring apophysis ossification

Cervical vertebral growth increments of C1, C2, C3 and C4 have been studied before and the characteristic morphological changes of the six stages of cervical vertebral maturation (CVM) related to growth changes have been suggested as an alternative method to determine skeletal maturation [14, 198, 199, 240]. To our knowledge, however, the present study is the first to investigate the question of whether it is possible to estimate the age and probability of an individual being younger/older than 18 years based on cervical vertebral ring apophysis ossification.

In this study, a new approach was proposed, in which the ossifications of the anterior inferior vertebral ring apophysis of cervical vertebrae C2, C3 and C4 are categorized into stages 0 through 4. The regions of interest are clearly visible on cephalometric radiographs used in routine orthodontic evaluations, and it is thus not necessary to expose a patient to additional radiation. Another advantage is that the radiation dose needed for a cephalometric radiograph varies between 2 to 3 microsievert (µSv) compared with panoramic radiographs with effective radiation doses of between 3.85 to 30 µSv [193]. The classification system proved to be easy to use and is usable to discern between stages. The simple classification system reduces doubt as to which stage an individual belongs to. This was also reflected in the acceptable inter-observer repeatability rates.

The Fleiss's kappa values for the stage classifications represented the statistical measure for assessing the reliability of agreement between a fixed number of raters when the categorical ratings were assigned. The levels of agreement were relatively low, but the percentage of disagreement between the raters was only by one stage.

The median ages for attainment of stages 0, 1, and 2 were below the 18 year threshold for all ancestry and sex groups. Additionally, WSA males and BSA females attained stage 3 for C2, and WSA females attained stage 3 for C2,C3 and C4 below the 18 year threshold. The maximum age of stage 0 and stage 1 for C2, C3, and C4 in BSA males, stage 0 in WSA males, and stage 1 in WSA females were also below the 18 year threshold. These stages are important to determine whether an individual is below 18 years of age, demonstrating that they can add valuable information around the age period of interest.

Black South African males achieved stage 1 for C2, C3, C4 and stage 2 for C3 earlier than WSA males. All the other stages were achieved earlier by WSA males, suggesting that they have faster bone maturation. This is in contrast to the development of third molars where BSA males reached stage H earlier by 6.90 months in the maxilla and 9.06 months in the mandible compared with WSA males [241]. All stages, except for stage 2 for C2, were achieved earlier by WSA females compared with BSA females, again suggesting earlier skeletal maturation in white as opposed to black individuals. BSA females reached 11 out of 14 evaluated third molar development stages before WSA females [241]. These results indicate that ancestry and sex differences are present and that skeletal development takes place at different rates in the same population. A possible explanation for this can be earlier sexual maturation by the white individuals. Earlier sexual maturation is found in industrialised populations [242, 243]. Sexual maturation is closely linked to skeletal maturation and pubertal onset may play an important role in skeletal maturation [244]. It has been suggested that skeletal maturation does not depend on ancestry, but rather optimum environmental conditions (i.e. high socio-economic status). Conversely, lower socio-economic status may lead to retardation of skeletal maturation [41]. However, there are many contributing factors to the differences observed in skeletal and dental maturation and the exact cause is difficult to pinpoint.

Franchi *et al*. [245] found that attainment of CMV stage 3 ranged between 8 years 6 months to 11 years 5 months for girls, and for boys it ranged from 10 years to 14 years. However, it must be emphasized that the CMV method is not sensitive for detecting growth maturity and cannot be used to estimate age.

Dunn's test was performed to investigate ancestry differences. Statistically significant differences were found between BSA and WSA males for stage 1 for C2, and stage 3 for C3. No statistically significant differences were found when BSA females were compared with WSA females. Larger median age differences were noted between males and females from the same ancestry compared with sexes from different ancestries, suggesting that ancestry is important but sex not. The significant differences found between ancestry groups would suggest that ancestry specific data must be used to estimate age.

Several factors have been suggested to explain accelerated maturation in individuals which include a stable calorie intake or a reduced calorie expenditure, an increase in calcium intake, an increase in the uptake of processed sugars and fats, reduced physical activity, and improved socioeconomic and health status [246]. Socioeconomic factors are often indicated as the most important variable in maturation differences [242, 246]. The individuals from the School of Dentistry, University of Pretoria and Sefako Makgatho Health Sciences University comprise of different social groupings and include individuals living in both the city and the surrounding rural areas. The sample from the private practices mainly comprises of individuals living in the city. The sample as a whole includes the entire socio-economic spectrum, and therefore it is not possible to attribute observed differences to varying socio-economic circumstances. These differences are more likely to be attributable to genetic factors [247].

Black South African males achieved stage 1 for C2, C3 and C4 earlier compared with BSA females. The BSA females achieved all the other stages earlier. This is in contrast to the results for third molar development where development terminated earlier in BSA males compared with BSA females [241]. Dunn's test indicted that statistically significant differences were present between BSA males and females for

stage 1 for C2, as well as stage 3 for C3 and C4. White South African males achieved stage 1 for C2, C3, C4 and stage 2 for C2 earlier compared with WSA females. Statistically significant differences were found between WSA males and females for stage 4 for C2, C3 and C4. WSA males reached, on average, each third molar development stage earlier than WSA females [241]. The final stage (stage 4) for anterior inferior apophysis development was reached 9.84, 10.32, and 10.44 months later for C2, C3 and C4 respectively in WSA males compared with WSA females. Compared with third molar development, WSA males reached the final stage (stage H) an average of 9.0 months earlier in the maxilla and 10.02 months earlier in the mandible [241]. In both ancestry groups, the patterns of development between the males and females were similar. These results indicate that sexual differences for both ancestry groups are present. The difference in development between skeletal and dental indicators between ancestry and sex groups emphasizes the need to use population specific data. The combination of age indicators might improve age estimation compared to only using dental parameters.

In this study, the likelihood of an individual being older or younger based on the ossification of the anterior inferior apophysis was determined. When a 95% probability is considered for an individual to be younger than 18 years a few stages can be considered to be a reliable indicator. These stages include stage 0 for black and white South African males, stage 1 for black South African males for C2, C3, and C4, stage 1 for white South African females for C2 and C3, and stage 1 for white South African males for C4. When a 95% probability is considered for an individual to be older than 18 years, stage 4 for white South African males for C3 and C4 can be included. The cervical vertebral ring apophysis likelihood data strengthen age estimations methods. The use of many skeletal markers for age estimation analysis is thought to provide the most accurate age estimation [190].

Stepwise multiple regression analysis and a dichotomous indicator regression formulae were used to develop a model based on the anterior inferior ossification of the apophysis to actually estimate age.The progressive apophysis changes which correlate significantly with age were used to determine the corresponding correlation coefficients. The regression equations calculated in the present study can be

recommended for the estimation of age in a South African population. Regression models is an easy and useful method to practically estimate age for a specific individual. However, such an approach should be used with caution in a legal setting involving living individuals where it may be more prudent to provide probabilities rather than actual estimates which may be misinterpreted by uninformed individuals.

6.4 Multifactorial model: combining third molar and cervical vertebral ring apophysis development

A generalised linear model (GLM) was used to compare the data obtained from third molar development and cervical vertebrae apophysis development and to investigate if the combination of multiple age indicators will result in improved age estimation. A GLM strictly assumes that the residuals will follow a normal distribution.

In general, several biological indicators need to be assessed when estimating age and the combination of the different age predictors may result in improved age estimates [21, 227, 248]. Mineralisation of teeth and skeletal development show large biological variability and individuals with the same age may show different developmental stages; conversely, individuals with the same development stage may differ in age by 4 years or more [248]. In this study similar age ranges for the third molar mineralisation and cervical apophysis development were found. For example, white South African males showed a difference of 8 years between the minimum and maximum values for individuals in stage G for the maxillary third molar development. The overall difference between ages was not as large for cervical vertebrae development but some stages did show a difference of nearly 7 years. Overall, though, there seemed to be more variability in vertebral apophysis development than in third molar development.

The concept that tooth development is under significant genetic control is widely accepted [10, 123, 249]. Monozygotic twins showed a concordance rate of 0.9 for tooth emergence and certain genetic disorders affect tooth eruption [250]. Tooth development is also minimally influenced by exogenic factors such as disease or malnutrition, usually making it a better measure of chronological age than skeletal

development [229]. The rates of growth and skeletal maturation between populations vary and is affected by the socio-economic status of a population or an individual [180, 251]. This creates the question if skeletal maturation can be used for age estimation. Schmeling *et al.* [180] pointed out that a lower socio-economic status will lead to underestimation of the person's age which in terms of criminal responsibility has no adverse consequences.

The data used in this study were filtered to only include individuals between the ages of 15 and 18 years of age. When combining data the statistical analysis can only be optimal in data sets containing both panoramic and cephalometric radiographs taken on the same day. In our study population the largest sample of individuals who fitted these criteria was between the ages of 15 and 18 years. This problem was also pointed out by Thevissen *et al.* [20] when third molar and skeletal development was combined. In the South African context obtaining both panoramic and cephalometric radiographs from the same individual on the same day is problematic especially after 18 years of age as very few individuals seek orthodontic treatment after this age. All the radiographs used for this study were retrospectively obtained and were taken for a specific clinical indication at the time of exposure. The sample used for the combined data set with both panoramic and cephalometric radiographs was mostly from orthodontic patients. Best practice suggests that no radiograph should be taken on a patient without a clinical evaluation and that all exposures should be clinically justified [252]. This was also evident in the sample used in the study by Thevissen *et al.* [20] with some age categories only comprising of three individuals. Despite these challenges, we managed to put together a data set containing 287 individuals, but future research should ideally also include individuals older than 18 years. A small sample size will have an influence when determining the root mean squared error for a data set. The sample sizes used for the five types of data groups identified in this study were equally distributed between sex and ancestry.

The AIC value was determined for the first round GLM. The AIC is an estimator of the relative quality of the statistical model for the data set and provides a means for model selection. A model with a lower AIC value is preferred. The quality of the model was then assessed by the comparison with related models identified by the

independent variables with a not-significant *p*-value (Table 5.6). A new GLM was then determined with an improved AIC value. The mean square error value from a cross-validation procedure was calculated as 1.633136 and used to cross-validate the model by comparing the value with the mean square error values from the separated models (Table 5.8). This process is a statistical method to improve the age estimation model and to compare the influence of the variables with the chosen model. The biological indicators can be assessed separately and in combination and the prediction error can be used to compare the different indicators statistically to produce a more favourable outcome. The mean square error measures the average squared difference between the estimated values and the actual values which in this case is age. A lower mean square error value is preferred as it shows that the data values are dispersed closely to the mean. The values for all five groups combined and separated by sex and ancestry produced mean square error values between 1.178625 and 2.037545 (Table 5.8). In the combined GLM, sex and ancestry are included as differentiators because of their importance in age assessment. The mean square error values from the groups only including third molar development (groups 2 and 4) were smaller compared to the values from the individuals with combined age indicators (group 1). This indicates that third molar development between the ages of 15 and 18 years is better correlated to actual age than the apophysis development. The mean square error values for the groups only including the cervical vertebral ring ossification development (groups 3 and 5) were all larger compared to the values from the individuals with combined age indicators. Factors influencing skeletal growth such as socio-economic status, diet, growth- and thyroid hormone, sex steroids and ancestry differences may contribute to the larger values [180, 251, 253]. Larger mean square error values can be expected when skeletal markers are used in age estimation as skeletal maturation is subject to numerous external influences. Recent studies have suggested that dental maturation is not hindered by malnutrition [254, 255], but a delay in skeletal maturation is observed in malnourished adolescents [256]. Puberty has varying effects on skeletal mineralisation at different skeletal sites with trabecular bone seemingly being very sensitive to hormone concentrations [257]. Vertebral apophysis development corresponds well with our investigated age range with many developmental changes taking place around the critical age of 18 years, but the observed mean square error

values can be expected for the skeletal indicators because of the development changes still taking place and the influence of external factors.

Age estimation methods are based on the biology of the individual where variability is the rule and ageing patterns differ. The results from this study are in contrast to the results from the study by Kumagai *et al*. [21] which demonstrated an improved root mean square error rate when more skeletal indicators were added to the model. The authors concluded that combining specific age estimation indicators will improve age prediction, which was not the case in the current study. The conflicting results may be due to the relatively small and heterogeneous sample that was used in their study compared to our representative equally distributed sample. Similar to what was found by Jooste *et al.* [258] when assessing accuracy of age estimates using transition analysis, the addition of a relatively low information trait will decrease rather than increase the accuracy in a multifactorial model.

The larger data sets used for groups 4 and 5 compared to groups 2 and 3 respectively reduced the mean squared values for the combined age indicator models. The larger data sets therefore improved the models. The values obtained from studies combining age indicators with small unrepresented data sets over a large age range should be assessed with caution.

The GLM model was successfully used to create a model that can be used to assess age for multiple age indicators. The model demonstrates the variables with statistically significance. The important factors are those which do not significantly influence the model such as the C3 apophysis development stages and the third molar development stages. All the other age indicators contributed significantly to the model. The factors influencing the model significantly such as sex, and C2 and C4 apophysis development were used to improve the new GLM model fit. Age indicators cannot be omitted as a data set with missing age indicators will create intervals which are to small and not comparable to a data set from individuals without omitted age indicators [19]. When combining age indicators the significant indicators should be clearly presented along with the mean squared error values. In the study by Thevissen *et al.* [20], the root mean square error values were only presented for the skeletal component according to all the different cervical vertebrae registration

techniques. A comparison with the third molar values to evaluate if a combined method improves age estimation is therefore not possible.

Numerous studies advocate the use of a Bayesian approach to evaluate age [19, 219, 259, 260]. Most of these recent studies, however, only include one biological marker and don't combine multiple age indicators. The Bayesian approach is usually seen as only one of the statistical alternatives [224, 261]. Valsecchi *et al.* [218] stated that there is no consensus currently to express, calculate and interpret the error when validating methods. One of the aims of this study was to develop an accurate, workable multifactorial age estimation method for living South African individuals between 15 and 25 years of age. The data will be used in legal and juridical settings to obtain an estimation of the age of an individual. To obtain a specific point estimate a logistic regression model should be considered [70, 140, 142]. A Bayesian approach has been suggested by some authors combining age indicators. Fieuws *et al.* [19] used the approach of Boldsen *et al.* [222] to obtain appropriate interval estimates for age when multiple indicators are used. The authors concluded that the method is suggested to overcome the drawbacks of the classical regression models but becomes less significant when the number of age indicators increase. The Bayesian approach is highly complex to compute and was found not to strongly outperform a classical approach [224]. The statistical method used to combine multiple age indicators in this study is similar to a recent study combining DNA methylation markers with skeletal and dental ages [214].

The findings of this study produced results that did not improve the outcome when multiple age indicators where combined. The mean square error values were the lowest for third molar tooth development and become higher when the skeletal features of cervical vertebrae apophysis development were added. Our results are in contrast to numerous other studies that suggested that combing developmental features enhanced age estimation [19–21, 214, 232, 233]. Comparison with other studies are difficult as the study by Thevissen *et al.* [20] and the study by Chen *et al.* [262] compared third molar development with cervical vertebral growth. Tooth calcification stage was found to be significantly correlated with the cervical maturation stage [262]. Even though the addition of vertebrae data in this study did

not improve the accuracy, it may better reflect reality as the inclusion of different indicators will give a better comprehensive age of the individual.

The claims made by studies that the combination of age indicators will improve age estimation compared to stand alone indicators should be interpreted with caution. A large, diverse sample is necessary to recognise all biological variation within the sample and to include skeletal indicators which substantially contribute to the model. Analysis of the sample size and the raw data used in the construction of these models should provide sufficient information for future comparison with similar studies. The age range of the sample and type of skeletal marker will also have an influence on the results [21, 215]. Cardoso *et al.* [215] assessed eight males between the ages of 13 and 19 years. Skeletal maturity was assessed for the radius and the ulna, and dental maturity was assessed for the second and third molar according to Demirjian. Logistic regression was used to determine the probability of an individual being 16 years of age or older, by either combining dental and skeletal maturity scores and by using them separately. The conclusion was that dental development is less reliable than skeletal maturity for age estimation [215]. The sample size should, however, be questioned as eight individuals were used over a six year interval. Kumagai *et al.* [21] combined two dental and four skeletal age predictors in a sample of 256 individuals aged between 4 and 20 years using Bayes' rule with a multivariate continuation ratio model. The authors concluded that the combination of teeth and hand and wrist bones information is recommended but pointed out that a limitation was the relatively small and heterogeneous sample.

6.5 Practical application of the findings of this study

Third molar maturation, despite their shortcomings, remains the main and most important method in assessing skeletal age in living individuals around the age of 18 years. However, all other evidence should also be considered. In this study, using a probabilistic (Bayesian) approach, likelihoods were calculated for an individual being 18 years of age given a specific developmental stage. We advise the use of sex and age specific likelihoods, and the inclusion of both upper and lower molars, as these

would provide the most reliable results. These likelihood curves can be used in current cases of disputed legal age.

Similarly, likelihoods of being 18 years were developed using vertebral apophyseal development. Assessment of the accuracy of these two methods (through regression analysis) suggest that third molar development on its own may be more accurate than using vertebral development on its own or a combination of the two methods. However, vertebral development may provide additional valuable information and can also be used on their own especially if for some reason dental data are not available. Currently no likelihood / probability data are available for a combination of the two methods, but can be developed in future studies. This, however, implies some computation complexity and a computer programme, as the background data are needed to achieve this.

Multiple regression formulae to actually estimate age were developed. These were used in this study to compare accuracies of using third molars on their own, cervical vertebrae on their own and a combination of the two methods to estimate age. The use of these formulae are not advised in cases of living individuals, as a probabilistic approach are more suited in high stakes cases of living individuals, where a final decision as to age of majority is made in court. However, they can be used in cases of unknown deceased individuals, where it may be prudent to actually provide an age estimate to create a biological profile to assist in a positive identification.

6.6 Future directions and research needs

Studies using the methods described in the thesis should be extended to include all South African population groups and should include Indian and Coloured individuals as well as individuals from other regions of the country. The new data provided here on both the third molar and cervical ring apophysis development should be tested and validated using other independent samples, also including other local and international European and African population samples. Future collection of data will allow expansion of the current combined regression model to include more individuals with a wider age range with both panoramic and cephalometric radiographs taken on the same day.

A Bayesian approach should be applied to the combined data and the performance of this likelihood data should be evaluated in a practical setting. This fell outside of the scope of the current study, but is a valuable line of enquiry that should be followed up in future studies.

Actual age estimates using regression methods from this study can also be compared to estimates using a Bayesian approach to determine which approach provides a more accurate estimate. This is a topic that is hotly debated in current literature [19, 218] and there is no current consensus.

Efforts should be made to establish and standardise age estimation protocols for living South African individuals, which are used throughout the country. A dedicated working group may be helpful in this regard. The minimum criteria for acceptable radiographs used in age estimation techniques should be established and included in age estimation protocols. The methods described in the thesis should be used to construct population specific data for other African populations, and to establish standardized protocols. Currently no data are available for individuals from neighbouring countries such as Namibia, Zimbabwe and Mozambique. Future research should aim at using our South African population data on third molar development to compare to other African populations. Accurate and reliable data is necessary to accurately estimate age and therefore country specific data is needed.

Chapter 7: Conclusions

In conclusion, third molar development of 1268 South African individuals with known age, sex and ancestral origin was studied to determine if the rate of development differed between ancestry and sex groups. It also aimed to present the likelihood of being 18 at a given developmental stage. Results of the study indicated that third molar development of BSA individuals is completed at earlier chronological ages compared with that of WSA individuals. The individual median ages at which BSA individuals achieve stage H development were significantly different to the WSA individuals, indicating that ancestry-specific data should be used. The use of ancestry-specific data is necessary to prevent the overestimation of the age of BSA individuals. The third molars of males from BSA and WSA individuals matured earlier than those of females and these results are conflicting to previous studies done on similar population groups. The individual median ages at which WSA males achieved development stages were statistically significantly different to WSA females for certain stages, indicating that sex-specific data should ideally be used.

Third molar development can be used as a reliable method to determine the likelihood of being 18 years of age if the probability and 95% confidence interval is considered for stage H. Considering that no accurate, non-invasive method is currently available to estimate age in living individuals the development of the third molars is a useful and reliable method of age estimation.

Cephalometric radiographs of a large sample of individuals with known age, sex and ancestral origin were studied to determine the ossification rates in sex and ancestral groups of South Africa. The likelihood of being younger or older than 18 years, at a given ossification stage was determined using these stages.

Data suggest that anterior inferior apophysis ossification stages of C2, C3, and C4 can be used as a reliable indicator to determine the likelihood of being 18 years of age at a 95% confidence index level. Ancestral and sex differences were found which emphasize the need to use ancestry and sex specific data. Currently no completely accurate non-invasive method to discern whether an individual is younger

or older than 18 years of age is available, and is unlikely to exist due to inherent individual biological variation. To achieve the most accurate age estimation it is recommended that the results from this study are combined with other age estimation indicators. Evaluation of the anterior inferior apophysis could prove to be a valuable additional tool to discriminate the age of a living individual. Therefore, further studies are needed to support our results.

A GLM model was used to combine multiple age indicators from South African individuals into a statistical model. The method used in our study to combine multiple age indicators proved valuable to identify the factors which significantly influenced the model. The identification of these factors made it possible to improve the overall fit of the GLM. The influence of the various age indicators was clearly demonstrated by the prediction errors during cross-validation. We suggest that the addition of more variables in a multifactorial model should be carefully weighed, as additional indicators are not always better. The research needs to be followed up with a larger sample size and from different regions of the world. The influence of the different age indicators in a combined model also needs to be studied as additional age indicators may better reflect reality than using just one age indictor to estimate age, even though the accuracy may be perceived to decrease.

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Chapter 8: Addenda

Appendix A: Faculty of Health Sciences PhD Committee Protocol Approval

UNIVERSITEIT VAN PRETORIA
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KANTOOR VAN DIE SKOOLVOORSITTER
SKOOL VIR GENEESKUNDE
FAKULTEIT GESONDHEIDSWETENSKAPPE

OFFICE OF THE CHAIR
SCHOOL OF MEDICINE
FACULTY OF HEALTH SCIENCES

20 July 2014

Prof M Steyn
Department of Anatomy
University of Witwatersrand

Dear Prof Steyn

STUDENT: UYS A (PhD ANATOMY)
"Age estimation of living South African individuals: A multifactorial model."

Mentioned student's protocol has been approved by the Committee meeting held on the 23rd of June 2015.

Kind regards

PROF BG LINDEQUE
CHAIR: PhD COMMITTEE

CC. Ms A Strauss

Appendix B: Ethics Approval

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 20 Oct 2016.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 22/04/2017.

UNIVERSITEIT VAN PRETORIA
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Faculty of Health Sciences Research Ethics Committee

27/08/2015

Approval Certificate
New Application

Ethics Reference No.: 263/2015

Title: Age estimation of living South African individuals: A multifactorial model

Dear Andre Uys

The **New Application** as supported by documents specified in your cover letter dated 14/08/2015 for your research received on the 21/08/2015, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 26/08/2015.

Please note the following about your ethics approval:
- Ethics Approval is valid for 2 years
- Please remember to use your protocol number (**263/2015**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

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

We wish you the best with your research.

Yours sincerely

*** Kindly collect your original signed approval certificate from our offices, Faculty of Health Sciences, Research Ethics Committee, H W Snyman South Building, Room 2.33 / 2.34.*

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

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

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