# A cross-sectional radiographic study of permanent teeth development in Botswana's children

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DOCTORAL DISSERTATION

Zagreb, 2020

Sveučilište u Zagrebu

Stomatološki fakultet

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DOKTORSKI RAD

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Assistant Professor Ivan Galić DDM, PhD

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DOKTORSKI RAD

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Rad je ostvaren u Zavodu za Dentalnu antropologiju Stomatološkog fakulteta Sveučilišta u Zagrebu.

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**Summary**

**A CROSS-SECTIONAL RADIOGRAPHIC STUDY OF PERMANENT TEETH DEVELOPMENT IN BOTSWANA'S CHILDREN**

**Participants and methods:** Samples of panoramic radiographs (OPTs) of black African children and adolescents from the city of Gaborone, Botswana were used to form a suitable sample to study the development of teeth in this socio-geographic background. The final sample consisted of 1760 OPTs (807 males and 953 females) of individuals aged 6–23 years. a) The developmental stages of all permanent teeth on the left side of the maxilla and the mandible were evaluated. b) The dental age (DA) was calculated according to the Demirjian, Willems and Cameriere methods and were then compared to chronological age (CA) and the difference between DA and CA or (DA-CA) and mean absolute difference between DA and CA (MAE) was compared on a subsample of 616 OPTs where at least one mandibular tooth was found with incomplete mineralization, not including third molars. c) The subsample of third molars was randomly divided into a training dataset (900 OPTs) and test dataset (394 OPTs), taking into account similar distribution across age groups. The training dataset was used to generate the best linear regression model for age estimation, while the test dataset was used to study the performance of the model. d) The same subsample of third molars was tested according to the Demirjian and Köhler stages and Cameriere $I_{3M}$ to discriminate adults from minors.

**Results and Conclusions:** Females are slightly faster in developing permanent teeth, but without statistical significance, for most of the developmental stages. The Willems and Cameriere methods were the most accurate for estimating dental age in children. However, in older age groups over the age of 14, all methods underestimate dental age – the Cameriere method the most – therefore, they should not be used in children of 14 years of age and older. The linear regression formulas using Demirjian's staging method were the most accurate for estimating dental age in the sample of 13 to 23 years of age, followed by Köhler's staging method, while the Cameriere$I_{3M}$ was the least accurate. The best performance to discriminate adults from minors was seen with the cut-off value of $I_{3M}$<0.08, followed by the Köhler stage A½ in both sexes and the Demirjian stage H in males and stage G in females, a useful indicator to discriminate individuals of black African origin who are around the legal adult age of 18 years in Botswana.

**Keywords:** Botswana, permanent teeth, 3rd molar, dental mineralization, age estimation methods, age of majority.

**Sažetak**

**PRESJEČNO RADIOLOŠKO ISTRAŽIVANJE RAZVOJ TRAJNIH ZUBA DJECE U BOCVANI**

**Sudionici i metode:** Upotrijebljeni su uzorci panoramskih radiograma (OPT) crne afričke djece i adolescenata iz grada Gaboronea u Bocvani radi formiranja odgovarajućeg uzorka za procjenu razvoja trajnih zuba u ovom sociogeografskom okruženju. Konačni uzorak sastojao se od 1760 OPT-ova (807 muškaraca i 953 žena) pojedinaca u dobi od 6 do 23 godine. a) Analizirani su mineraliazacijiski razvojni stadiji svih trajnih zuba na lijevoj strani maksile i mandibule metodom prema Demirjianu; b) Dentalna dob (DA), razlika dentalne od kronološke dobi (DA-CA) i absolutna razlika dentalne od kronološke dobi (MAE) procijenjena je metodama prema Demirjianu, Willemsu i Camerieru uspoređena je na poduzorku od 616 OPT-ova gdje je pronađen najmanje jedan mandibularni zub s nedovršenom mineralizacijom, ne uključujući treće kutnjake; c) Poduzorak od 1294 OPT-a sudionika u dobi od 13 do 23 godine (582 muškaraca i 712 žena) analiziran je prema Demirjianovim i Köhlerovim stadijima i Camerierovom indeksu razvoja trećih kutnjaka (CameriereI$_{3M}$) kako bi se istražila mogućnost primjene svake metode za procjenu dobi. Poduzorak trećih kutnjaka nasumično je podijeljen na skup podataka za obuku (900 OPT-ova) i skup podataka za testiranje (394 OPT-a), uzimajući u obzir ravnomjernu raspodjelu po dobnim skupinama. Skup podataka za obuku korištena je za postavljanje linearnog modela za svaku metodu dok je uspješnost linaranih modela prema Demirjianu (Demirjian3M), Köhleru (Köhler3M) i Camerieru (CameriereI$_{3M}$) provjerena na skupu podataka za testiranje; d) Na istom poduzorku OTP-a trećih kutnjaka testirane su Demirjianovi i Köhlerovi stadiji te Camerierov I$_{3M}$ u odvajanju punoljetnih (≥18 godina) od maloljetnih (<18 godina) ispitanika. Učinkovitost pojedinih razvojnih stadija pojedinih metoda provjerena je s točnošću, osjetljivošću, specifičnošću, omjerima vjerojatnosti (LR+, LR-), Youdenovim indeksom, vrijednostima pozitivnosti (PPT) i negativnosti (NPT) testa i Bayesovom vjerojatnošću nakon ispitivanja.

**Rezultati:** Uspoređujući maksilu i mandibulu, utvrđen je sličan razvoj u različitim stadijima mineralizacije za većinu zuba. U usporedbi s prosječnom dobi u svakom stadiju razvoja, uključujući treći kutnjak između muškaraca i žena, vidljivo je da se u žena nešto brže razvijaju trajni zubi, ali bez statističkog značaja u većini razvojnih stadija. Za procjenu dentalne dobi uspoređene su Demirjianova, Willemsova i Camerieeova metoda na OPT-ovima 299 muškaraca i

317 žena, raspona od 6,08 do 16,80 godina starosti. U muškaraca najmanju razliku DA-CA pokazala je Cameriereova metoda (-0,11 ± 01,16 godina), potom slijede Willemsova metoda (0,58 ± 1,00 godina) i Demirjianova metoda (1,26 ± 1,10 godina). U žena najmanja se DA-CA pokazala primjenom Willemsove metode (-0,10 ± 1,02 godine), nakon čega slijede Cameriereova (-0,33 ± 1,14 godina) i Demirjianova metoda (0,72 ± 1,02 godine). Najmanja prosječna apsolutna pogreška ili MAE utvrđena je Willemsovom metodom, 0,91 ± 0,71 godina kod muškaraca i 0,81 ± 0,62 godine kod žena. Najveći MAE bio je za Demirjianovu metodu, 1,36 ± 0,96 godina kod muškaraca i 0,96 ± 0,80 godina kod žena. Usporedba točnosti zubne dobi izračunata linearnim regresijskim formulama prikazana je u muškaraca, najmanja DA-CA metodom Köhler3M (-0,05 ± 1,81 godina), nakon čega slijedi metoda Cameriere$I_{3M}$ (0,05 ± 2,11 godina), dok je metoda Demirjian3M bila najmanje točna (-0,20 ± 1,58 godina). U žena najmanja se DA-CA pokazala kod metode Köhler3M (-0,07 ± 1,89 godina), potom kod metode Cameriere$I_{3M}$ (0,17 ± 2,17 godina) te metode Demirjian3M (-0,24 ± 1,74 godine). Najmanja prosječna apsolutna pogreška ili MAE utvrđena je za metodu Demirjian3M, 1,32 ± 0,89 godina kod muškaraca i 1,40 ± 1,03 godine kod žena. Najveći MAE iznosio je za metodu Cameriere$I_{3M}$, 1,74 ± 1,18 godina kod muškaraca i 1,81 ± 1,17 godina kod žena. Najbolji učinak diskriminacije odraslih od maloljetnika pokazao je graničnu vrijednost $I_{3M}$ <0,08 u oba spola. Točno je klasificirano 529 od 582 (91%) muškarca te 654 od 712 žena (92%). Ova vrijednost $I_{3M}$ pokazuje najbolje učinke među ostalim graničnim vrijednostima $I_{3M}$ i ostalim fazama Demirjian i Köhler. To upućuje na blisku povezanost između punoljetnosti i činjenice da je taj test bio pozitivan, tj. $I_{3M}$ <0.08. Kod muškaraca je osjetljivost ili omjer ispravno klasificiranih sudionika starih 18 i više godina bio 0,88 (95% CI, 0,86 do 0,90), dok je specifičnost ili udio ispravno klasificiranih sudionika mlađih od 18 godina 0,94 (95% CI, 0,91 do 0,96). PPV testa, koji označuje kod sudionika čiji je $I_{3M}$ <0,08 da je osoba odrasla, bio je 0,94 (95% CI. 0,91 do 0,96), dok je NPV testa, koji označuje da je sudionik čiji je $I_{3M}$ ≥0,08 maloljetnik, bio je 0,88 (95% CI, 0,85 do 0,90). Najviša vrijednost J-indeksa iznosila je 0,83 (95% CI, 0,81 do 0,87) za graničnu vrijednost $I_{3M}$ <0,10. Omjer pozitivne vjerojatnosti (LR +) bio je 13,67 (95% CI. 9,21 do 21,02), dok je omjer negativne vjerojatnosti (LR-) 0,12 (95% CI, 0,10 do 0,16). Bayesova vjerojatnost nakon ispitivanja *p* iznosila je 0,94 (95% CI, 0,90 do 0,98) kod muškaraca. Točnost za žene bila je 0,92 (95% CI, 0,90 do 0,93), a osjetljivost i specifičnost 0,88 (95% IZ 0,86 do 0,89) i 0,96 (95% CI, 0,94 do 0,98). Vrijednosti PPV i NPV bile su 0,97 (95% CI, 0,94 do 0,98) i 0,87 (95% CI, 0,85 do 0,89), dok je najveća vrijednost J-indeksa iznosila 0,85 (95% CI. 0,80 do 0,88)

za graničnu vrijednost $I_{3M}$ = 0,10 (tablica 35), kao kod muškaraca. Vrijednosti (LR +) i (LR-) bile su 23,73 (95% CI, 14,20 do 42,28) i 0,12 (95% CI, 0,11 do 0,15), dok je Bayesova vjerojatnost nakon ispitivanja je bila 0,97 (95% CI, 0,93 do 1,00). Od razvojnih stadija trećih kutnjaka, Demirjian stadij H (točnost, 0,87) kod muškaraca i stadij G (točnost, 0,88) kod žena te Köhlerov stadij A½ u oba spola (točnost u oba spola, 0,88) pokazali su najbolji rezultat u odvajanju odraslih od maloljetnih ispitanika.

**Zaključci**: Kod žena se trajni zubi razvijaju nešto brže, ali bez statističkog značaja za većinu razvojnih stadija u odnosu na muškarce. Willemsova i Cameriereova metoda bile su najtočnije u procjeni dentalne dobi djece. Međutim, u starijim dobnim skupinama, iznad dobi od 14 godina, sve metode podcjenjuju dentalnu dob, najviše Cameriereova metoda, i ne smiju se primjenjivati kod djece starije od 14 godina. Demirjianova metoda iz 1973. godine nije prikladna za rutinsku uporabu i za procjenu dentalne dobi te je potrebno je uspostaviti posebne Demirjianove standarde za crnu djecu u Bocvani. Linearne regresijske formule u oba spola primjenom Demirjianovih razvojnih stadija bile su najtočnije u procjeni dentalne dobi u uzorku od 13 do 23 godine, nakon čega slijede Köhlerovi razvojni stadiji, dok je Cameriere $I_{3M}$ najmanje točan. Sve metode za procjenu detalne dobi primjenom trećih kutnjaka podcjenjuju dentalnu dob u dobi od 21 godinu i starije kod oba spola. Najbolji učinak u odvajanju odraslih od maloljetnika pokazao je granična vrijednost $I_{3M}$ <0,08, nakon čega slijede stadij A½ prema Köhleru u oba spola te stadij H prema Demirjianu u muškaraca i stupanj G užena. Ovi rezultati pokazuju da $I_{3M}$ može biti korisna dodatna metoda s velikom točnošću u pravnoj i forenzičkoj praksi za odvajanje punoljetnih od maloljetnih osoba, crnaca afričkog podrijetla iz Bocvane.

**Ključne rječi:** Bocvana, trajni zubi, umnjak, dentalna mineralizacija, metode procjene dentalne dobi, dob punoljetnosti.

# LIST OF KEY WORDS, ACRONYMS AND ABBREVIATIONS

**OPT** – orthopantomogram

**N** – number of OPTs

**Mean** – mean value

**SD** – standard deviation

**Min** – minimal value

**Max** – maximal value

**Median** – most common value

**MAE** – mean absolute error

**IR** – intra-rater confidence

**IRR** – inter-rater confidence

**CA** – chronological age

**DA** – dental age

**SA** – skeletal age

**PPV** – positive predictive value

**NPV** – negative predictive value

**LR+** – positive likelihood ratio

**LR -** – negative likelihood ratio

**Demirjian3M** – Demirjian's 3rd molar analysis

**Köhler3M** – Köhler's 3rd molar analysis

**CameriereI$_{3M}$** – Cameriere's 3rd molar maturity index

## TABLE OF CONTENTS

# 1. INTRODUCTION

The study of one's lifetime and the various processes the human body undergoes has been researched for centuries (1). It is a sequence that is continuous from the moment of creation, until death, and one that no one can avoid. By definition, human life begins from the moment of conception, when a spermatozoon fertilizes an oocyte. Growth and development commence in-utero and continue after birth, during infancy, childhood, adolescence, until adulthood, and finally end with the process of aging, which ultimately ends in death (2). The human body undergoes many changes in the life cycle, not just physical changes but developmental and psychological. These changes are beginning in the neonatal and infancy period, wherein a healthy newborns' cognitive and motor abilities are minimal but with time gradually developing. During adolescence, sexual maturation occurs, along with anatomical, physiological, and behavioral changes. Adolescence continues until full adult height and size is attained, and then the teenager can be considered an adult (1). However, there are few body biomarkers or evidence during late adolescence differentiating a subject from being a minor or an adult, which will be another topic of discussion in this project. We begin aging the day we are born and throughout our lifetime. The process is cumulative and has a biological, physical, and psychological effect on humans. Every cell in the human body undergoes changes throughout a lifetime, and this complex and multi-component process has intrigued researchers for many years (3). The concept of time and age as with most things in the universe must be quantified, thus determining the age of a person is considered fundamental in the identification and characterization of the subject in question. In the past, various growth parameters have been used to establish an accurate definition of biological and physiological age (4).

Time and age quantification dates back to the start of anthropology studies themselves. In the case of archeological remains, the establishment of a time frame from when remains existed can give a clue to past populations (5). In the medical field, it is fundamental to know the age of a patient, as a physician would have a very different treatment plan for a geriatric patient compared to a child. In pediatrics, its significance lies in following a child's progress to ensure they are developing in the adequate growth curves for their age and that they fit into the established growth percentiles. Previous studies set out to establish growth patterns and to this day, we track a child's progress based on standard development studies that evaluated radiographs of the developing skeleton in children to assess skeletal maturity, by incorporating size, shape and mineralization attained, as well as predicting a child's maximum growth achievable (6). If a child's development differs significantly from the growth percentiles, it could alert the clinician to possible metabolic or endocrine abnormalities or pediatric syndromes (7). Similarly, in pediatric dentistry and orthodontics, assessment of dental development compared to a child's age can also help determine whether a child's development complies with the norms and allows clinicians to identify anomalies ranging from simple tooth agenesis to syndromes involving the craniofacial system.

In the forensic science field, age establishment is fundamental for identification. It may be required in cases where victims wish to hide their identity due to fears for their safety or in the case of asylum seekers whose documents have been lost or repossessed during the war (8). In sub-Saharan Africa, where the population sample for this study was obtained, only 43% of the births are recorded in the first five years of the child's life, and only 26.9% of children are issued a birth certificate (9). This stresses the importance of establishing other methods to provide a clue of the age since there are still parts of the world where birth certificates and adequate documentation are not available. Establishing age according to the evaluation of dentition has been performed by many researchers who wish to provide those in need of an estimated age and has been used as an alternative method to provide evidence of age when there isn't any. This study aims to do just that by assessing dental development based on radiographs and providing age estimation tables, which are population-specific for the Botswana population.

## 1.1 The rationale behind the study

Assessing age using radiographs of dentition is what this study focuses on. It has proved to be an essential part of age indication (10). Radiographic evaluation of dentition involves identifying the extent of calcification of the enamel, determining root and crown development, and further assessing the eruption sequence. This is especially true in a developing dentition – assessing their development, maturation, and tooth eruption provide a relatively accurate age estimation. It has been found to be more accurate then assessing bone development as in contrast, odontogenesis seems to be a very useful indicator of maturity and hence of biological age since tooth development is less influenced by external factors compared to osteogenesis (11). Age estimation methods used in this study are non-invasive and harmless to the subjects studied. All that was required for the analysis was an evaluation of OPTs that were previously taken for orthodontic or other diagnostic clinical purposes, not for the sole purpose of age estimation. There have been many studies on age estimation in children of various ethnic descents, but few have studied African children. Some studies have found that one of the first age estimation methods by Demirijan et al. may not be reliable for populations of different ethnic descent as they were based on Caucasian children of French-Canadian heritage, but they have shown some success in children of European descent, with some modifications. However, with children of African descent, particularly studies on black South African children, the method has been shown to be unreliable, but other methods of age estimation such as the Phillips method has shown to be more reliable (12, 13). The consecutive studies on black South African children were in concordance with the Phillips 2009 study, finding that the original and the modified Demirjian method greatly overestimated age (14). Also, studies in North and East Africa, more specifically Egypt and Kenya, found the Willems method (a method based on Demirjian's with modifications) more suitable for age estimation for their sample populations (15, 16). This will be the first study done on the Batswana population using dental development by assessing radiographs for evaluating the age of children and young adults, and the goal was to produce population-specific tables for better reference in establishing dental age in this population. Few studies have been conducted on children in Southern Africa and age estimation tables specific for this population are necessary to aid in a variety of fields, including forensics, pediatrics, and orthodontics.

## 1.2 Determining chronological, dental and skeletal age

If a person's birth date is known and can be determined by legal documents stating so, we are able to calculate what is known as the chronological age by subtracting the birth date from the date the subject is examined. However, chronological age will not necessarily be the same as the physiological age of the body. Such discrepancies are explained by the fact that some children develop faster and may in fact, appear older than their chronological age when systems such as skeletal development are assessed. It is necessary to evaluate several systems to make an accurate age assessment. This is evident in the physiological growth difference seen in males and females, where a 12-year old girl may have reached puberty, but her male counterpart has not (Figure 1) (17).

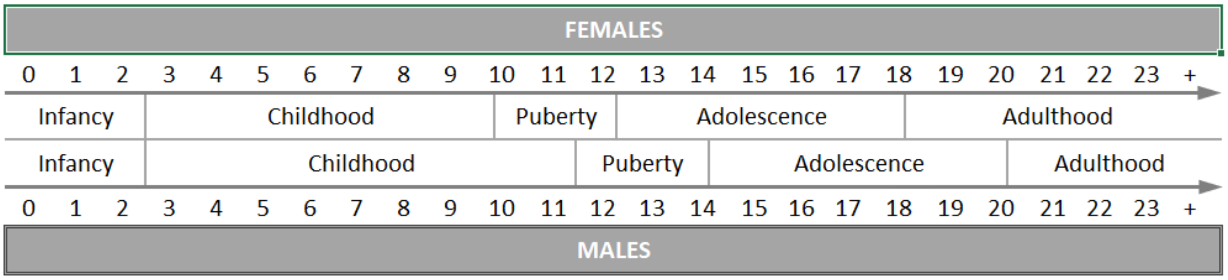



**Figure 1.** Stages in growth and development from infancy up to adulthood, shown for males and females. Chart interpreted from IAAF Introduction to coaching, published June 20, 2017. Peter JL Thompson.

It is also evident when females who mature earlier also reach their full growth capacity earlier. In turn, females reach their full growth capacity in their mid-teens, but males can continue growing into their 20s. A radiographic study on Scottish males and females from birth to 20 years reported that no maturational changes were visible at the knee after 19 years in males and 16 years in females (18).

**Physiological age** is the degree of maturity of different tissues in the body. It can be determined when several biological ages have been assessed and compared, such as skeletal age, morphological age, secondary sexual characteristic age and dental age (19, 20). Normal standards of growth are required to perform a clinical assessment to establish physiological age and compare it to a known or proposed chronological age.

**Chronological age (CA)** is the fundamental value we can use as a reference when comparing how quickly different systems (skeletal, dental) develop and it allows us to quantify to what extent of development of a certain system has reached that age.

**Skeletal age** refers to the analysis of bone and its mineralization to give an age value according to development. It is determined by analyzing radiographs of various areas of the body including the wrist, skull and knee (21-25).

**Sexual maturation age** refers to the age where the development of secondary sexual characteristics occurs, and the ability of the reproductive organs begins to function. We must keep in mind that there is an increase in the prevalence of obesity in developed countries amongst adults and children;

thus children are reaching puberty earlier. This again emphasizes that a child's chronological age may not match its physical appearance (26).

**Dental age** refers to an assessment of dentition and its developmental stages, providing an age value according to dental maturation.

Children of a specific CA may develop faster, and the estimated skeletal age (SA) or dental age (DA) may show a higher or lower age to the CA. This cautions us to use SA, DA, or sexual maturity age exclusively, as they are estimates only, and if any legal or forensic purposes are in question and adequate documents are present, CA is still the main value to focus on. However, in cases of unknown or withheld dates of birth (for the purpose of estimating CA), evaluating sexual maturity, SA and DA have all been found to be easy and quite reliable methods for estimating age (27). Furthermore, DA has proved to be more reliable than SA since there is evidence that systemic factors less influence it (19, 28-30). As previously mentioned, the onset of puberty is occurring at a younger age, and according to WHO (World Health Organization), which now defines adolescence as being aged 10–19, DA can be considered more accurate than assessing sexual maturity too. In children of African descent, skeletal and dental maturity occurs earlier, and both skeletal and dental standard age evaluation methods need modification when applied to African samples (31).

In the past, the eruption sequence has been shown to provide a decent estimate of age due to dentition erupting in a specific sequence that can estimate age, particularly during infancy and childhood (32). However, the eruption sequence can be disrupted by merely the premature extraction of a carious deciduous tooth, and this affects succedaneous tooth eruption. This is why age assessment using dental x-rays can be a better option since a better picture is represented, showing not only the emerging and erupted teeth but also the stage of development of teeth still submerged in the bone.

### 1.3 Skeletal development in African children

Some differences in skeletal development have been noted in different races. Bone mineral density (BMD) is used in adults as a marker of osteoporosis, and in children, it is used to assess bone health (33, 34). It has been found that black children of African ancestry have higher BMD than white children. In developed countries such as the USA, African-American children have been showing consistently to be more advanced in skeletal development than children of European origin as measured by the appearance of ossification centers in the early years of life (35). Also, a study comparing the whole-body bone mass of American and South African black and white children found that irrespective of geographic region, bone mass is lower in children of European ancestry than African ancestry (36). However, in some studies on South African children, a slower development has been found compared to CA, but this is primarily explained by a lower socio-economic status resulting in a reduced dietary intake. In the sample studied in this research, the socio-economic status was not recorded, but it does represent children of various socio-economic standings. They live in a specific geographic region, different from the pilot study. Generally, it is considered that ethnicity does not affect skeletal development while socioeconomic status does (37, 38). With the difference in skeletal development in various ethnicities, researchers have been prompted to identify whether there is a difference in dental development as well.

## 1.4 In utero face, oral cavity and dentition development

### *1.4.1* Brief general embryology

Human growth and development begins when the spermatocyte from the father fertilizes the oocyte of the mother (2). After fertilization, the zygote (cell created during fertilization) divides to form a ball of 8 cells called the Morula during day 3-4 and then a hollow cavity, the blastocyst, during day 4-5 (39). After the blastocyst has divided to form a 16-cell mass, it can now be considered an embryo, at the end of week 1. By the end of week 2, the blastocyst (now embryo) has implanted into the uterine endometrium (2). The embryo continues to divide, and by week three, it splits into primitive tissues made up of ectoderm, endoderm, mesoderm and neural crest cells (arising from the ectoderm) that commence development, folding and beginning differentiation into various bodily tissues. The ectoderm will form the skin, the endoderm the digestive tract, the mesoderm will be responsible for much of the muscle formation in the body, and the neural crest cells (undifferentiated mesenchymal cells) make bone, cartilage and various neural tissue. What is important, the migrated neural crest cells will form the bones and cartilages of the face and neck from the pharyngeal arches. Muscles in the facial region originate from somites which migrate during development to the future head and neck region, pulling their innervation with them (2).

### *1.4.2* The basic facial form

During week 4, the median nasal process and maxillary facial process fuse to form the maxilla and lip, and the non-union of these two processes would result in cleft lips of various forms. The lateral nasal process forms the lateral nose, nasal ala, and creates the nasal seal. The primary palate is formed during weeks 7–8, and the secondary palate develops caudally during the 8$^{th}$ week. In order for the palatal shelves to descend and fuse in the mid palatal suture, the tongue should descend to allow for the shelves to meet. In cases where there is a micrognathia of the mandible, such as in Treacher Collins syndrome, there is no space for the tongue descent, and the palatal shelves do not meet fully, resulting in a palatal cleft and a narrow, arched palate form (2, 40).

### *1.4.3* The pharyngeal arches

The pharyngeal arches play a critical role in facial development, and they are swellings composed of the primitive tissues that will give rise to the majority of facial features. Tooth development will occur in the alveolar process of the maxilla and mandible, which will arise from the first pharyngeal arch (2).

### *1.4.4* Tooth development

Dental development commences in week 6 in utero for primary dentition by the thickening of oral epithelium into the U shaped dental laminae (41). Permanent tooth development starts at approximately four months in utero. Dentition is formed from the ectoderm, mesenchyme and underlying layer of neural crest cells. Only the enamel is formed from the ectoderm, the rest of the tissue – dentine, cementum, pulpal tissue and periodontium – from the mesenchyme and neural crest cells. Expression of Dlx, MSX and BMP genes in the neural crest, mesenchyme and ectoderm initiate tooth development; in particular, the neural crest mesenchyme's influence over the overlying ectoderm induces odontogenesis (2).

### *1.4.5* Induction stage of tooth development

In the 6$^{th}$ week of fetal development, the neural crest induced mesenchyme influences over the overlying ectoderm lining the stomodeum to thicken and give rise to the oral epithelium and then the dental lamina as seen in Figure 2, image A & B (2).

### *1.4.6* Bud stage of tooth development

In the 8th week of fetal development, the dental lamina, a U-shaped band that follows the structural outline of the primitive jaw and projects from the oral epithelium, has ten proliferation centers in which tooth buds develop into the underlying mesenchyme. These buds will make the upper or lower primary dentition and deeper to the primary buds. At 10 weeks in utero, posterior, deeper extensions of dental laminae will present and form the permanent tooth buds (2). The estimated times of calcification for primary and permanent dentition are given in the table below, Figure 2, image C.

### *1.4.7* Cap stage of tooth development

During weeks 8–9 of fetal development, each tooth bud of the primary dentition is further invaginated by the mesenchyme, creating a cap shape over the dental papilla and follicle. The ectodermal portion, i.e. the "cap", is the enamel organ and will begin producing enamel. The enamel organ is now made up of an inner and outer enamel epithelium on the inner and outer sides of the cap, along with a core of loosely connected cells known as the stellate reticulum. In the inner portion of the tooth bud, there is a dental papilla, the primordium of the dental pulp. The enamel organ and papillae make up the tooth germ. Figure 2, image D. The mesenchyme surrounding the tooth germ thickens and forms the dental sac, which is the precursor to the cementum and periodontal ligament of the tooth. Image C. (2).

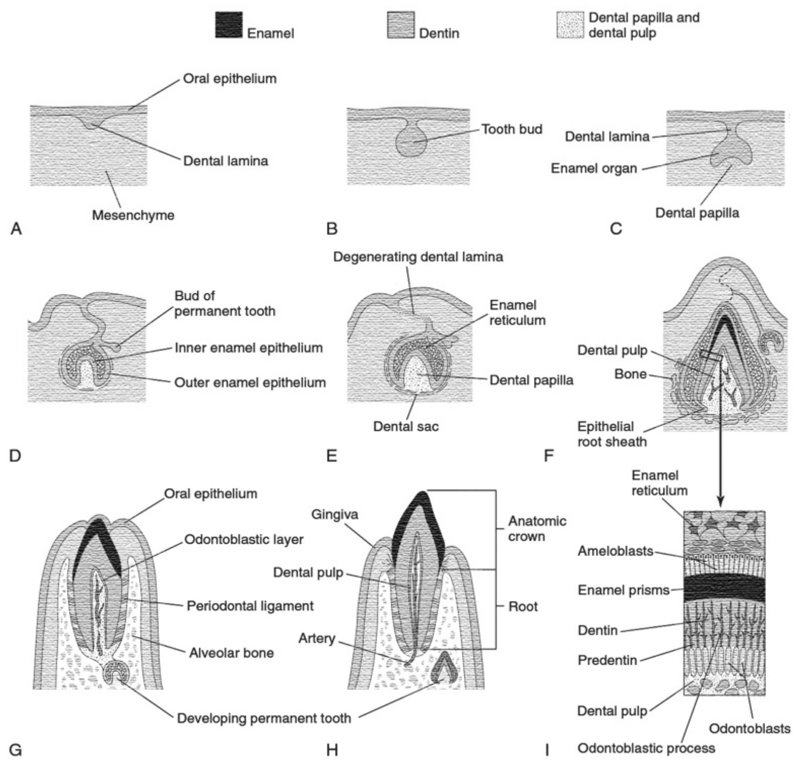



**Figure 2.** Sagittal sections, showing successive stages in the development and eruption of an incisor tooth. A – At 6 weeks, showing the dental lamina; B – At 7 weeks, showing the tooth bud developing from the dental lamina; C – At 8 weeks, showing the cap stage of tooth development; D – At 10 weeks, showing the early bell stage of a deciduous and the bud stage of a permanent tooth; E – At 14 weeks, showing the advanced bell stage of tooth development. Note that the connection (dental lamina) of the tooth to the oral epithelium is degenerating; F – At 28 weeks, showing the enamel and dentin layers; G – At 6 months postnatally, showing early tooth eruption; H – At 18 months postnatally, showing a fully erupted deciduous incisor tooth. The permanent incisor tooth now has a well-developed crown; I – Section through a developing tooth, showing ameloblasts (enamel producers) and odontoblasts (dentin producers). Image adapted and interpreted from Moore LM et al. Before We Are Born: Essentials of Embryology and Birth Defects, 9th Edition (2).

### *1.4.8* Bell stage of tooth development

During weeks 11–12 of fetal tooth development, the tooth germ begins resembling a "bell" in image D (Figure 2). The mesenchymes of the dental papillae next to the inner enamel epithelium differentiate into odontoblasts and begin producing predentine. Predentine mineralizes into dentine, and the odontoblasts begin regressing towards the pulp, leaving the odontoblastic processes behind.

The deposition of predentine is induced by cells of the inner enamel epithelium differentiating into ameloblasts. The deposition of predentine then, in turn, initiates ameloblasts to begin producing enamel matrix over the dentine in the DEJ direction. As the enamel is deposited, the ameloblasts regress in the opposite direction, towards the outer enamel epithelium (2).

### *1.4.9* Root development

Once the crown development is almost complete, root development is initiated at the cervical loop, which is made up of the most apical portion of the inner and outer enamel epithelium meeting at the apical tip. The inner and outer enamel epithelium continues apical descent into the mesenchyme of the dental sac and forms Hertwig's root sheath. Hertwig's sheath continues downward growth, shaping the root, and depositing root dentine towards the pulp chamber, in turn making the root canal narrower. The inner cell layer of the dental sac differentiates into cementoblasts, which begin producing cementum, which surrounds the root dentine, all the way up to the neck of the tooth where it meets the enamel. The outer cells of the dental sac will have a role in forming alveolar bone and periodontal ligament, which aids embed the tooth in the bone (2).

### *1.4.10* Apposition and calcification

The newly differentiated cells – odontoblasts, ameloblasts, cementoblasts – continue depositing their specific dental tissue. Tooth mineralization varies for each tooth, beginning in the 4$^{th}$ month in utero for deciduous mandibular central incisors and continuing into late childhood for permanent third molars (Figure 3). The 4$^{th}$ month in utero signals the first clinical evidence of tooth mineralization, and in the past, anatomical dissections have made prenatal tooth development studies of expelled fetuses to be able to identify on average when the calcification of different primary teeth commence. These have been used to follow the development and mineralization of primary teeth. Permanent teeth mineralization, however, occurs entirely postnatally, its full development takes 8–12 years (11).

### *1.4.11* Eruption and exfoliation

An eruption can vary in individuals, but commences typically 6–8 months after birth, starting with lower central incisors, and most primary dentition will be fully erupted by 24 months. It is thought that teeth begin to erupt once 1/3–2/3 of their entire development is complete (42). The eruption of permanent teeth commences at six years with the first molar and exfoliation proceeds in teeth with succedaneous teeth. The third molar, which is the most variable tooth in terms of anatomy and eruption, can erupt anywhere between 14 to 17 years, even as late as 20 years of age. Teeth continue to develop in their more apical portions for 2–3 years after eruption and are considered entirely complete only after third molar root closure is complete, which can be as late as 23 years of age (42).

#### *1.4.12* Root completion

Root completion for deciduous dentition starts from 18 months for central incisors up to 36 months in second molars. The permanent dentition commences root closure in central incisors from 9 years and third molars can continue root development, and the roots can close as late as at 25 years of age. Figure 3 illustrates average closure in individual teeth (43).

## 1.5 Tooth emergence as a method of age estimation

The eruption sequence is considered to be relatively accurate in age determination in growing children. Its use dates back 180 years to when Dr Edwin Saunders presented his findings to the British Parliament, which aided in age determination using the clinical picture of erupted teeth. It was called "The teeth, a test of age" (44, 45). The United Kingdom at this time was at the peak of the industrial revolution, and children between 9 to 13 years of age were allowed to work in factories, with limited working hours, provided they showed adequate documents indicating a birthdate. This was, however, more difficult to obtain as there was no civil registration of births until 1838, and birthdates obtained from parishes were of little reliability as christening may have occurred at various times after the birth. Thus the use of average heights for age and erupted teeth at 9 and 13 years were used as a method of assessment to establish age (45). To date, many studies have been done to assess the eruption sequence in various populations, and ethnic variability requires emergence standards to be obtained for specific populations (46). The exact time of tooth eruption is difficult to determine due to individual variability, and this is why we use the average of tooth eruption as a range (47). Some studies have found tooth emergence in African and North American black population faster than Caucasian populations (43, 48). Charts and tables were used for age estimation by assessing initial calcification, root formation, eruption and exfoliation during the developmental phase. Tables by Kronfild and Logan, modified by McCall and Schour, have been accepted as the standard for development and have appeared in a lot of the literature (49).

| PRIMARY DENTITION | | | | | | |
|---|---|---|---|---|---|---|
| | Calcification Begins At | Formation Complete At | Eruption | | Exfoliation | |
| | | | *Maxillary* | *Mandibular* | *Maxillary* | *Mandibular* |
| Central Incisors | 4th Fetal Month | 18-24 Month | 6-10 Month | 5-8 Month | 7-8 Year | 6-7 Year |
| Lateral Incisors | 4th Fetal Month | 18-24 Month | 8-12 Month | 7-10 Month | 8-9 Year | 7-8 Year |
| Canines | 4th Fetal Month | 30-39 Month | 16-20 Month | 16-20 Month | 11-12 Year | 9-11 Year |
| First Molars | 4th Fetal Month | 24-30 Month | 11-18 Month | 11-18 Month | 9-11 Year | 10-12 Year |
| Second Molars | 4th Fetal Month | 36 Month | 20-30 Month | 20-30 Month | 9-12 Year | 11-13 Year |

| PERMANENT DENTITION | | | | | |
|---|---|---|---|---|---|
| | Calcification Begins At | Crown (Enamel) Complete At | Roots Completed At | Eruption* | |
| | | | | *Maxillary* | *Mandibular* |
| Central Incisors | 3-4 Month | 4-5 Year | 9-10 Year | 7-8 Year (3) | 6-7 Year (2) |
| Lateral Incisors | Maxilla: 10-12 Month | 4-5 Year | 11 Year | 8-9 Year (5) | 7-8 Year (4) |
| | Mandible: 3-4 Month | 4-5 Year | 10 Year | | |
| Canines | 4-5 Month | 6-7 Year | 12-15 Year | 11-12 Year (11) | 9-11 Year (6) |
| First Premolars | 18-24 Month | 5-6 Year | 12-13 Year | 10-11 Year (7) | 10-12 Year (8) |
| Second Premolars | 24-30 Month | 6-7 Year | 12-14 Year | 10-12 Year (9) | 11-13 Year (10) |
| First Molars | Birth | 30-36 Month | 9-10 Year | 5.5-7 Year (1) | 5.5-7 Year (1a) |
| Second Molars | 30-36 Month | 7-8 Year | 14-16 Year | 12-14 Year (12) | 12-14 Year (12a) |
| Third Molars | Maxilla: 7-9 Year | | | 17-30 Year (13) | 17-30 Year (13a) |
| | Mandible: 8-10 Year | | | | |

* Figures in parentheses indicate order of eruption. Many otherwise normal infants do not conform strictly to the stated schedule.

**Figure 3.** Eruption sequence of the teeth (50). Adopted and interpreted from: Development of the human jaws and surrounding structures from birth to the age of fifteen years. J Am Dent Assoc 1933; (3):379-427.

Eruption sequence studies in South Africa have found trends similar to Kenyan and African-American children (51). South African blacks are made up of several ethnic groups, and a large of them comprise of the Tswana, and the Tswana in Botswana also originates from the same group (52). No eruption studies have been done on the Batswana population yet, but due to a common ethnic origin, it may be possible to use the South African studies for comparison. Eruption studies of South African Blacks have found a significantly faster emergence of the canine, premolars, and third molar formation, and this could imply that black children develop faster tooth eruption. However, dental radiographic age estimation studies are needed to confirm these findings with the Batswana sample (53). However, the eruption sequence is not only influenced by dental development and mineralization, but also by local factors including alveolar space, previous local trauma of deciduous teeth resulting in premature extraction of deciduous teeth, environmental and nutritional variations (54). Methods using dental radiographs are more appropriate because it is possible to evaluate the development of the whole dentition, before and after an eruption, in contrast to only seeing the clinical picture of the number of teeth that are located in the mouth at a given time. Eruption is a unique process in time for each individual tooth, whereas tooth formation occurs from in utero development to early adulthood and can be studied continuously through the help of radiographic images from when x-rays are able to be taken (55).

### 1.6 Dental radiographic age estimation

Tooth development, as with all aspects of age, is a complex process starting from 6 weeks of in utero development up to 20 years of age or more. Both developmental and regressive changes to the tooth can be related to chronological age (56). Radiographic interpretation of tooth development represents a more accurate depiction of the development of dentition as it is not only showing what teeth have erupted in the mouth but how far along in the development stages each tooth is, whereas, with eruption studies, we are only able to view what is clinically visible. Most of the research on dental development is performed by evaluating dental age (DA) by using specific radiographic methods on a total number of selected teeth. The methods analyze the degree of tooth mineralization attained or the size of open apices on radiographic records, mostly panoramic radiographs (OPTs). One of the first methods for dental age assessment in children using radiographs was the method by Demirjian et al. (57), which used the Tanner, Whitehouse and Healy method (58) for skeletal maturity to derive a score for each stage of each tooth. The figures below show how skeletal maturity scores in the left were used to establish tooth stages on the right (Figure 4).

| Stage | Image | Description |
|---|---|---|
| B |  | The centre is just visible as a single deposit of calcium, or more rarely as multiple deposits. The border is ill-defined. |
| C |  | The centre is distinct in appearance and disc-shaped, with a smooth continuous border. |
| D |  | The maximum diameter is half or more the width of the metaphysis. |
| E |  | The epiphysis is as wide as the metaphysis. The central potion of the proximal border has grown toward the end of the middle phalanx, so that the proximal border no longer consists of a single convex surface; no differentiation into palmar and dorsal surfaces, however, can yet be seen. |
| F |  | Palmar and dorsal proximal surfaces are distinct, and each has shaped to the trochlear articulation of the middle phalanx. The palmar surface appears as a projection proximal to the thickened white line representing the dorsal surface. |
| G |  | The epiphysis caps the metaphysis. |
| H |  | Fusion of epiphysis and metaphysis has now begun. |
| I |  | Fusion of epiphysis and metaphysis is completed. |

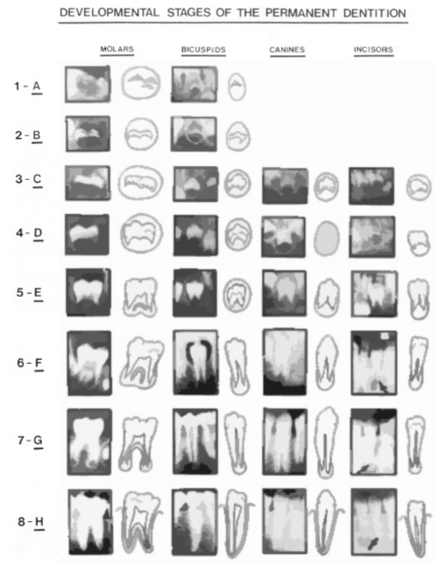



**Figure 4.** Charts adapted from Tanner, Whitehouse and Healy stages of skeletal maturation to Demirjian stages of mineralization of permanent teeth (57, 58). From: Tanner TM et al. A New System for Estimating the Maturity of the Hand and Wrist, with Standards Derived from 2600 Healthy British Children. Part II. The Scoring System method, 1962, Paris.

The population sample in Botswana consists predominantly of local (Batswana) children, children from other African countries and expatriate children from Europe, America and Asia. The local Batswana sample was sufficient; however, expatriates of Asian and European descent were insufficient in number to do a comparative study. Since it has been found in some African populations that tooth emergence occurs earlier in African populations (48, 59), as opposed to Europeans, mineralization of teeth may also occur earlier, thus standard age estimation methods based predominantly on European and Asian samples may not be accurate enough to be used on African samples. This heavily emphasizes the need to test on the Batswana sample population to determine the accuracy of the methods and to develop more specific tables for African populations. Some children and youth in Botswana were not born in proper health facilities, so their record and date of birth were never noted. This, in particular, happens in the indigenous (Bangwato) tribes of the central Kgalagadi district and there are many court cases where doctors and dentists have to be involved in determining the defendants' age and whether they are classified as an adult or underage (60). This would be the first study of dental development of all permanent teeth in children and adolescents of African origin in Botswana.

# 2. AIMS

## 2.1 The aims of the study

1. To evaluate the timing of tooth development among Batswana children and young adults.
2. To evaluate the accuracy of age estimation using the Demirjian, Willems and Cameriere methods on the Botswana sample.
3. To evaluate the accuracy of the Demirjian and Köhler staging methods and the Cameriere third molar maturity index of left mandibular third molars for estimating dental age.
4. To evaluate the Demirjian and Köhler stages and the third molar maturity index of left mandibular third molars for discriminating adults (≥18 years) from minors (<18 years) in the Batswana sample of children and young adults.

# 3. HYPOTHESES

### 3.1 The hypotheses of the study

1. The timing of mineralization stages of permanent teeth in Batswana children and adolescents is within age ranges of children and adolescents of Caucasian origin from Europe, according to the literature.

2. There is no difference between the dental age estimated by methods of Demirjian, Willems and Cameriere in Batswana children.

3. There is no difference among correlations of the Demirjian, Willems and Cameriere methods and chronological age.

4. There is no difference among correlations of third molar development evaluated by the Demirjian, Köhler and Cameriere third molar maturity index and chronological age.

5. There is no difference in the accuracy of staging systems by Demirjian, Köhler and Cameriere's open apices in discriminating participants between 18 years of age and older (adult) or under 18 years of age (child).

# 4. PARTICIPANTS AND METHODS

## 4.1 The sample

In this cross-sectional study, 2100 orthopantomographs (OPTs) of children and adolescents aged 6–23 years were investigated. The subjects visited two private orthodontic practices from 2001 to 2015, located in the city of Gaborone, the Republic of Botswana. During the time frame when these OPTs were taken (2001–2015), the only digital orthopantomographs in the country were located at these two premises. The national census was performed in 2011, with a population of 2,024,787 million (61). The sample collected indicates that a solid representation of the population was collected and analyzed in this study. The study was conducted following the ethical standards laid down by the Declaration of Helsinki (62). The approval for the study was granted by the HRDC (Human Research and Development Committee) of the Ministry of Health in Botswana. All OPTs from the sample were evaluated, and only black African subjects up to 23 years of age were investigated in the study, because of the few available OPTs of Caucasians and Asians and residents of unknown origin. In addition, there was no evidence of the development of permanent teeth in individuals older than 23 years. OPTs without supplementary subject's full dental records, lack of birth date and date of the OPTs were not included in the study. Also, the OPTs of children with proven hereditary or systematic illnesses, malnutrition, severe destruction, extraction, or hypodontia of permanent teeth or missing third molars were excluded from the study. In total, the final sample of 1760 OPTs, 807 males and 953 females were evaluated in this study.

## 4.2 Chronological age and dental methods used for age estimation

The chronological age (CA) of participants was calculated as the difference between the date the OPT was taken and their birthdate (rounded off to two decimal places), with age groups based on one-year increments.

## 4.3 Study design for the analysis of permanent teeth development and dental age estimation methods in Botswana's children

The analysis of all permanent teeth from the left side of the maxilla and mandible was performed using the Demirjian stages (63). The reason all teeth in the maxilla and mandible were studied was that all OPTs were digital and very clear, and there was little evidence of malposition or overlap, which normally occurs more so in the maxilla. Thus, all teeth could be evaluated. The dental age (DA) was calculated based on seven permanent mandibular teeth from the left side of the mandible using the Demirjian method from 1973 (57), the Willems method from 2001 (64), and the Cameriere method using the European formula from 2007 (65). Finally, the dental age (DA) of a subsample of third molars, aged 13 to 23 years, was analyzed in two steps. In the first step, the sample was randomly divided into the training dataset and test dataset. The training dataset was used to analyze the development of the mandibular third molars by the Demirjian stages (Demirjian3M) (57), the Köhler stages (Köhler3M) (66) and the Cameriere third molar maturity index (Cameriere$I_{3M}$) (67). The linear regression formula for calculating dental age was set using each method for males and females. The results of dental age using the linear regression formula was compared to the test dataset. Different methods for the analysis of third molars, Demirjian and Köhler stages and $I_{3M}$, were evaluated for indicating adult age (≥18 years) on the Batswana subsample of third molars.

### 4.4 Demirjian mineralization stages of teeth from the left side of the maxilla and mandible

The mineralization stages alphabetically abbreviated (A to H) according to the Demirjian et al. method from 1973 (Demirjian) (57) of all permanent teeth on the left side of the maxilla and mandible, were evaluated by JC. Furthermore, the crypt stage of the third molars was also assessed.

### 4.5 Dental age estimation using mandibular teeth by the Demirjian, Willems and Cameriere methods

The next step was to evaluate the efficacy of the original French-Canadian scores for dental stages and conversion tables for dental age in children as described by Demirjian (57), Willems adopted standards for age estimation based on Belgian children (Willems)(64) and Cameriere European formula for age estimation (Cameriere)(65, 68) in Batswana children. All children who have not finished mineralization of at least one of the seven permanent teeth from one side of the mandible, excluding third molars, were evaluated, and dental age (DA) was calculated.

## 4.6 Demirjian method

The stages of the seven left mandibular teeth were converted to exact values for each stage by sex-specific tables of self-weighted scores for dental stages by Demirjian et al. (57). These self-weighted sex-specific scores for each tooth were based on a mathematical balancing of tooth development across stages on French-Canadian samples of 2928 males and females age 2 to 20 years (57). The scores of all seven teeth were added together to get a total dental maturity score and were presented as percentages. Then the fiftieth percentile dental maturity score was converted to a dental age using sex-specific tables from Demirjian et al. (57).

### *4.6.1* Procedure for determining dental age according to Demirjian

Crown and root mineralization were evaluated according to the range of stages indicated by the letters of the alphabet from A to H, while the existence of a dental crypt without evidence of mineralization was assigned as zero (0.0). In the process of determining dental age, Demirjian proposed the order of assessment of teeth: 2nd molar, 1st molar, 2nd premolar, 1st premolar, canine, 2nd incisor, 1st incisor. The stage of development attained in an individual tooth is determined by strict instructions and an index that describes each individual stage and the tooth is compared with the diagram and radiological image of the tooth (Figure 5). The illustrations shown should be used as an aid and not as an exclusive sample for comparison. For each stage of tooth development reached, there are one, two or three conditions marked as a), b), c) assigned. If one condition is given, it must be met to confirm that the stage of tooth development has been reached. If two conditions are given, it is sufficient that at least one of them is met to confirm that the stage of tooth development has been reached. When all three conditions are given, at least two must be met to confirm that the stage of tooth development has been reached. As an additional condition for controlling the attainment of each stage of tooth development, the conditions from the lower stages of tooth development must be met. Absolute tooth sizes are not measured when determining the stage of tooth development. Crown height is defined as the greatest distance between the highest point on the cusp and the cement-enamel junction. Next, if the buccal and lingual cusps are not at the same height, the mid-height between them is taken as a reference point.

### *4.6.2* Instructions for assessing the developmental stage of a tooth according to Demirjian

**Stage 0** There is no evidence of calcification; the existence of a dental crypt is involved in determining the stage of development.

**Stage A** In both single-rooted and multi-rooted teeth, the start of calcification is seen at the superior level of the crypt in the form of an inverted cone or cones. There is no fusion of these calcified points.

**Stage B** The fusion of the calcified points forms one or several cusps, which unite to give a regularly outlined occlusal surface.

**Stage C** a) Enamel formation is complete at the occlusal surface. Its extension and convergence towards the cervical region are seen.

b) The beginning of the dentinal deposit is seen.

c) The outline of the pulp chamber has a curved shape at the occlusal margin.

**Stage D** a) Crown formation is complete, down to the cementoenamel junction.

b) The superior border of the pulp chamber in single-rooted teeth has a definite curved form, being concave towards the cervical region. The projection of the pulp horns, if present, gives an outline shaped like an umbrella top. In molars, the pulp chamber has a trapezoidal form.

c) The beginning of root formation is seen in the form of a spicule.

**Stage E** Uniradicular teeth:

a) The walls of the pulp chamber now form straight lines, whose continuity is broken by the presence of the pulp horn, which is larger than in the previous stage.

b) The root length is less than the crown height.

Molars:

a) The initial formation of the radicular bifurcation is seen in the form of either a calcified point or a semi-lunar shape.

b) The root length is still less than the crown height.

**Stage F** Uniradicular teeth:

a) The walls of the pulp chamber now form a more or less isosceles triangle. The apex ends in a funnel shape.

b) The root length is equal to or higher than the crown height.

Molars:

a) The calcified region of the bifurcation has developed further down from its semi-lunar stage to give the roots a more definite and distinct outline with funnel-shaped endings.

b) The root length is equal to or higher than the crown height.

**Stage G** a) The walls of the root canal are now parallel, and its apical end is still partially open (distal root in molars).

**Stage H** a) The apical end of the root canal is completely closed (distal root in molars).

b) The periodontal membrane has a uniform width around the root and the apex.

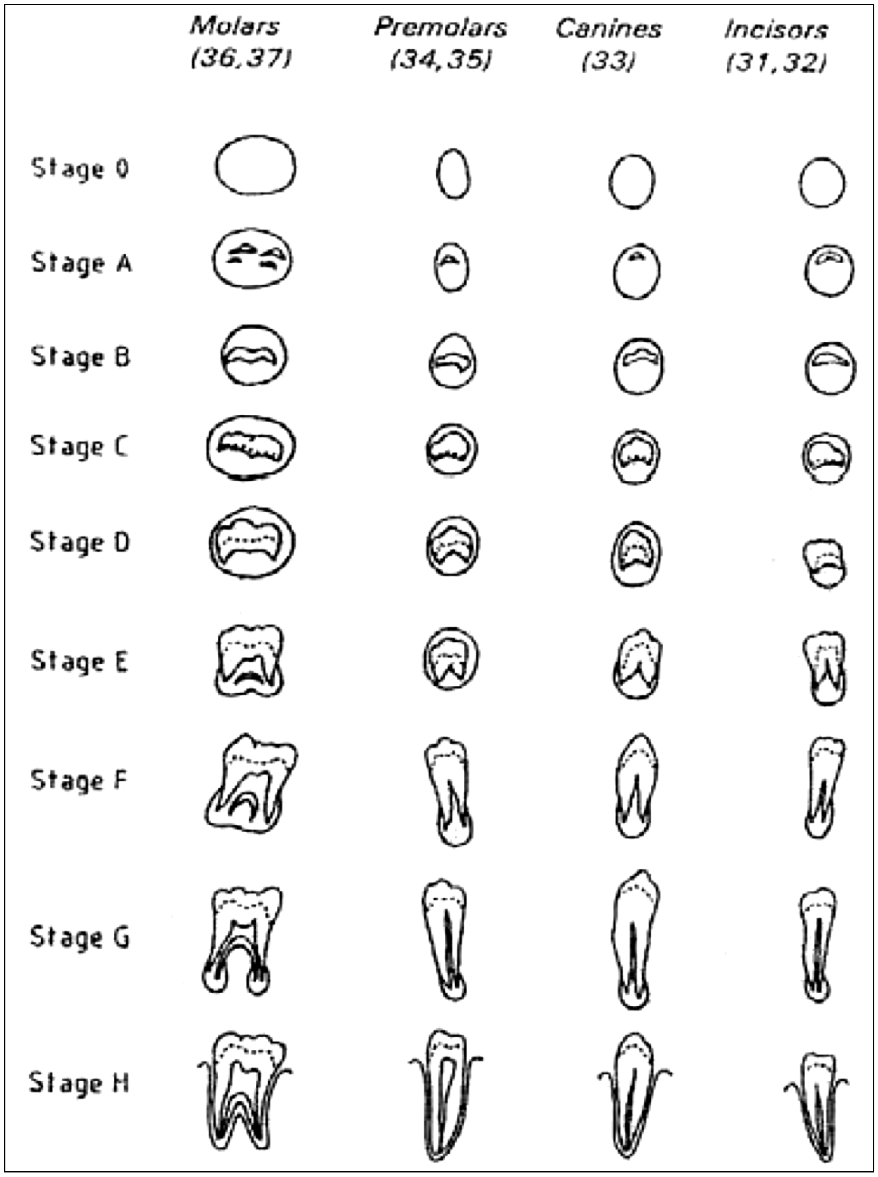



**Figure 5.** Developmental stages (0 to H) of permanent mandibular dentition

### *4.6.3* Scoring system for the Demirjian method

1. Each tooth will be assigned a stage, assessed by the procedure described.
2. The estimated stage is converted into a score using Table 1 for males or Table 2 for females. For example, if tooth 36 (1st lower left molar) of a boy is in stage E, it is given a score of 9.6.
3. The scores for all seven teeth are added together to give the maturity score.

4. The maturity score may be plotted on the centile charts (males or females as appropriate) where the age of the child is known. For example, a score of 35 for a boy aged 5.0 years lies just above the 90th centile.
5. The maturity score may be converted directly into a dental age either by reading off of the horizontal scale the age at which the 50th percentile attains that maturity score value or by using Table 3, which has been constructed by these means. Thus, a score of 45 for a boy is equivalent to the dental age of 6.9 years.

### *4.6.4* Demirjian method from 1973, an age estimation procedure

The degree of development of each permanent tooth is determined according to the described procedure, then assigned a numerical value according to separate Table 1 for males and Table 2 for females. Next, each stage of tooth development for males and females is translated into numerical results, according to Table 3.

**Table 1**. Self-weighted Demirjian scores for dental stages of the seven mandibular teeth from the left side in males

| Tooth | Individual tooth development stages | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **0** | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **H** |
| **37** | 0.0 | 2.1 | 3.5 | 5.9 | 10.0 | 12.5 | 13.2 | 13.6 | 15.4 |
| **36** | | | | 0.0 | 8.0 | 9.6 | 12.3 | 17.0 | 19.3 |
| **35** | 0.0 | 1.7 | 3.1 | 5.4 | 9.7 | 12.0 | 12.8 | 13.2 | 14.4 |
| **34** | | | 0.0 | 3.4 | 7.0 | 11.0 | 12.3 | 12.7 | 13.5 |
| **33** | | | | 0.0 | 3.5 | 7.9 | 10.0 | 11.0 | 11.9 |
| **32** | | | | 0.0 | 3.2 | 5.2 | 7.8 | 11.7 | 13.7 |
| **31** | | | | | 0.0 | 1.9 | 4.1 | 8.2 | 11.8 |

Note: Tags by Federation Dentaire International (FDI) abbreviation: 37, second molar; 36, first molar; 35, first premolar; 34, second premolar; 33, canine; 32, second incisive; 31, first incisive.

**Table 2.** Self-weighted Demirjian scores for dental stages of the seven mandibular teeth from the left side in females

| Tooth | Individual tooth development stages | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **0** | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **H** |
| **37** | 0.0 | 2.7 | 3.9 | 6.9 | 11.1 | 13.5 | 14.2 | 14.5 | 15.6 |
| **36** | | | | 0.0 | 4.5 | 6.2 | 9.0 | 14.0 | 16.2 |
| **35** | 0.0 | 1.8 | 3.4 | 6.5 | 10.6 | 12.7 | 13.5 | 13.8 | 14.6 |
| **34** | | | 0.0 | 3.7 | 7.5 | 11.8 | 13.1 | 13.4 | 14.1 |
| **33** | | | | 0.0 | 3.8 | 7.3 | 10.3 | 11.6 | 12.4 |
| **32** | | | | 0.0 | 3.2 | 5.6 | 8.0 | 12.2 | 14.2 |
| **31** | | | | | 0.0 | 2.4 | 5.1 | 9.3 | 12.9 |

Note: Tags by Federation Dentaire International (FDI) abbreviation: 37, second molar; 36, first molar; 35, first premolar; 34, second premolar; 33, canine; 32, second incisive; 31, first incisive.

The numerical values of all seven teeth are added together to give a result that determines the maturity score. The degree of maturity can be translated directly into the dental age by reading off of the finished tables or by reading from the percentile chart.

**Table 3.** Conversion of maturity score to dental age, according to Demirjian 1973 (57)

| Males | | | | | | | | Females | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | Score | Age | Score | Age | Score | Age | Score | Age | Score | Age | Score | Age | Score | Age | Score |
| 3.0 | 12.4 | 7.0 | 46.7 | 11.0 | 92.0 | 15.0 | 97.6 | 3.0 | 13.7 | 7.0 | 51.0 | 11.0 | 94.5 | 15.0 | 99.2 |
| 3.1 | 12.9 | 7.1 | 48.3 | 11.1 | 92.2 | 15.1 | 97.7 | 3.1 | 14.4 | 7.1 | 52.9 | 11.1 | 94.7 | 15.1 | 99.3 |
| 3.2 | 13.5 | 7.2 | 50.0 | 11.2 | 92.5 | 15.2 | 97.8 | 3.2 | 15.1 | 7.2 | 55.5 | 11.2 | 94.9 | 15.2 | 99.4 |
| 3.3 | 14.0 | 7.3 | 52.0 | 11.3 | 92.7 | 15.3 | 97.8 | 3.3 | 15.8 | 7.3 | 57.8 | 11.3 | 95.1 | 15.3 | 99.4 |
| 3.4 | 14.5 | 7.4 | 54.3 | 11.4 | 92.9 | 15.4 | 97.9 | 3.4 | 16.6 | 7.4 | 61.0 | 11.4 | 95.3 | 15.4 | 99.5 |
| 3.5 | 15.0 | 7.5 | 56.8 | 11.5 | 93.1 | 15.5 | 98.0 | 3.5 | 17.3 | 7.5 | 65.0 | 11.5 | 95.4 | 15.5 | 99.6 |
| 3.6 | 15.6 | 7.6 | 59.6 | 11.6 | 93.3 | 15.6 | 98.1 | 3.6 | 18.0 | 7.6 | 68.0 | 11.6 | 95.6 | 15.6 | 99.6 |
| 3.7 | 16.2 | 7.7 | 62.5 | 11.7 | 93.5 | 15.7 | 98.2 | 3.7 | 18.8 | 7.7 | 71.8 | 11.7 | 95.8 | 15.7 | 99.7 |
| 3.8 | 17.0 | 7.8 | 66.0 | 11.8 | 93.7 | 15.8 | 98.2 | 3.8 | 19.5 | 7.8 | 75.0 | 11.8 | 96.0 | 15.8 | 99.8 |
| 3.9 | 17.6 | 7.9 | 69.0 | 11.9 | 93.9 | 15.9 | 98.3 | 3.9 | 20.3 | 7.9 | 77.0 | 11.9 | 96.2 | 15.9 | 99.9 |
| 4.0 | 18.2 | 8.0 | 71.6 | 12.0 | 94.0 | 16.0 | 98.4 | 4.0 | 21.0 | 8.0 | 78.8 | 12.0 | 96.3 | 16.0 | 100.0 |
| 4.1 | 18.9 | 8.1 | 73.5 | 12.1 | 94.2 | | | 4.1 | 21.8 | 8.1 | 80.2 | 12.1 | 96.4 | | |
| 4.2 | 19.7 | 8.2 | 75.1 | 12.2 | 94.4 | | | 4.2 | 22.5 | 8.2 | 81.2 | 12.2 | 96.5 | | |
| 4.3 | 20.4 | 8.3 | 76.4 | 12.3 | 94.5 | | | 4.3 | 23.2 | 8.3 | 82.2 | 12.3 | 96.6 | | |
| 4.4 | 21.0 | 8.4 | 77.7 | 12.4 | 94.6 | | | 4.4 | 24.0 | 8.4 | 83.1 | 12.4 | 96.7 | | |
| 4.5 | 21.7 | 8.5 | 79.0 | 12.5 | 94.8 | | | 4.5 | 24.8 | 8.5 | 84.0 | 12.5 | 96.8 | | |
| 4.6 | 22.4 | 8.6 | 80.2 | 12.6 | 95.0 | | | 4.6 | 25.6 | 8.6 | 84.8 | 12.6 | 96.9 | | |
| 4.7 | 21.1 | 8.7 | 81.2 | 12.7 | 95.1 | | | 4.7 | 26.4 | 8.7 | 85.3 | 12.7 | 97.0 | | |
| 4.8 | 23.8 | 8.8 | 82.0 | 12.8 | 95.2 | | | 4.8 | 27.2 | 8.8 | 86.1 | 12.8 | 97.1 | | |
| 4.9 | 24.6 | 8.9 | 82.8 | 12.9 | 95.4 | | | 4.9 | 28.0 | 8.9 | 86.7 | 12.9 | 97.2 | | |
| 5.0 | 25.4 | 9.0 | 83.6 | 13.0 | 95.6 | | | 5.0 | 28.9 | 9.0 | 87.2 | 13.0 | 97.3 | | |
| 5.1 | 26.2 | 9.1 | 84.3 | 13.1 | 95.7 | | | 5.1 | 29.7 | 9.1 | 87.8 | 13.1 | 97.4 | | |
| 5.2 | 27.0 | 9.2 | 85.0 | 13.2 | 95.8 | | | 5.2 | 30.5 | 9.2 | 88.3 | 13.2 | 97.5 | | |
| 5.3 | 27.8 | 9.3 | 85.6 | 13.3 | 95.9 | | | 5.3 | 31.3 | 9.3 | 88.8 | 13.3 | 97.6 | | |
| 5.4 | 28.6 | 9.4 | 86.2 | 13.4 | 96.0 | | | 5.4 | 32.1 | 9.4 | 89.3 | 13.4 | 97.7 | | |
| 5.5 | 29.5 | 9.5 | 86.7 | 13.5 | 96.1 | | | 5.5 | 33.0 | 9.5 | 89.8 | 13.5 | 97.8 | | |
| 5.6 | 30.3 | 9.6 | 87.2 | 13.6 | 96.2 | | | 5.6 | 34.0 | 9.6 | 90.2 | 13.6 | 98.0 | | |
| 5.7 | 31.1 | 9.7 | 87.7 | 13.7 | 96.3 | | | 5.7 | 35.0 | 9.7 | 90.7 | 13.7 | 98.1 | | |
| 5.8 | 31.8 | 9.8 | 88.2 | 13.8 | 96.4 | | | 5.8 | 36.0 | 9.8 | 91.1 | 13.8 | 98.2 | | |
| 5.9 | 32.6 | 9.9 | 88.6 | 13.9 | 96.5 | | | 5.9 | 37.0 | 9.9 | 91.4 | 13.9 | 98.3 | | |
| 6.0 | 33.6 | 10.0 | 89.0 | 14.0 | 96.6 | | | 6.0 | 38.0 | 10.0 | 91.8 | 14.0 | 98.3 | | |
| 6.1 | 34.7 | 10.1 | 89.3 | 14.1 | 97.7 | | | 6.1 | 39.1 | 10.1 | 92.1 | 14.1 | 98.4 | | |
| 6.2 | 35.8 | 10.2 | 89.7 | 14.2 | 96.8 | | | 6.2 | 40.2 | 10.2 | 92.3 | 14.2 | 98.5 | | |
| 6.3 | 36.9 | 10.3 | 90.0 | 14.3 | 96.9 | | | 6.3 | 41.3 | 10.3 | 92.6 | 14.3 | 98.6 | | |
| 6.4 | 38.0 | 10.4 | 90.3 | 14.4 | 97.0 | | | 6.4 | 42.5 | 10.4 | 92.9 | 14.4 | 98.7 | | |
| 6.5 | 39.2 | 10.5 | 90.6 | 14.5 | 97.1 | | | 6.5 | 43.9 | 10.5 | 93.2 | 14.5 | 98.8 | | |
| 6.6 | 40.6 | 10.6 | 91.0 | 14.6 | 97.2 | | | 6.6 | 45.2 | 10.6 | 93.5 | 14.6 | 98.9 | | |
| 6.7 | 42.0 | 10.7 | 91.3 | 14.7 | 97.3 | | | 6.7 | 46.7 | 10.7 | 93.7 | 14.7 | 99.0 | | |
| 6.8 | 43.6 | 10.8 | 91.6 | 14.8 | 97.4 | | | 6.8 | 48.0 | 10.8 | 94.0 | 14.8 | 99.1 | | |
| 6.9 | 45.1 | 10.9 | 91.8 | 14.9 | 97.5 | | | 6.9 | 49.5 | 10.9 | 94.2 | 14.9 | 99.1 | | |

### *4.6.5* Willems method (2001)

Willems' dental age estimation method uses the same system of mineralization stages of crowns and roots according to Demirjian's 1973 system, the scale of developmental stages indicated by the letters of the alphabet from A to H (64). Willems et al. (64) created new calculation tables for dental age based on the regression analysis on a sample of Belgian children. A weighted ANOVA was performed in order to adapt the original Demirjian scoring system for the Belgian population (64).

In the Willems procedure, scores expressed in years are associated with individual developmental stages of the seven teeth on the left side of the mandible, again performed separately for males and females, Tables 4 and 5. Dental age is the result of the sum of the scores of all seven teeth (57, 64).

**Table 4.** Demirjian developmental stages with associated points expressed in years for the seven teeth of the left mandible for the Willems procedure in males (64)

| Stages | Tooth | | | | | | |
|---|---|---|---|---|---|---|---|
| | **31** | **32** | **33** | **34** | **35** | **36** | **37** |
| A | | | | 0.15 | 0.08 | | 0.18 |
| B | | | | 0.56 | 0.05 | | 0.48 |
| C | 1.68 | 0.55 | | 0.75 | 0.12 | | 0.71 |
| D | 1.49 | 0.63 | 0.04 | 1.11 | 0.27 | 0.69 | 0.8 |
| E | 1.5 | 0.74 | 0.31 | 1.48 | 0.33 | 1.14 | 1.31 |
| F | 1.86 | 1.08 | 0.47 | 2.03 | 0.45 | 1.6 | 2.0 |
| G | 2.07 | 1.32 | 1.09 | 2.43 | 0.4 | 1.95 | 2.48 |
| H | 2.19 | 1.64 | 1.9 | 2.83 | 1.15 | 2.15 | 4.17 |

Note: Tags by Federation Dentaire International (FDI) abbreviation: 31, first incisive; 32, second incisive; 33, canine; 34, first premolar; 35, second premolar; 36, first molar; 37, second molar.

**Table 5.** Demirjian developmental stages with associated points expressed in years for the seven teeth of the left mandible for the Willems procedure in females (64)

| Stages | Tooth | | | | | | |
|---|---|---|---|---|---|---|---|
| | **31** | **32** | **33** | **34** | **35** | **36** | **37** |
| A | | | | -0.95 | -0.19 | | 0.14 |
| B | | | | -0.15 | 0.01 | | 0.11 |
| C | 1.83 | | 0.6 | 0.16 | 0.27 | | 0.21 |
| D | 2.19 | 0.29 | 0.54 | 0.41 | 0.17 | 0.62 | 0.32 |
| E | 2.34 | 0.32 | 0.62 | 0.6 | 0.35 | 0.9 | 0.66 |
| F | 2.82 | 0.49 | 1.08 | 1.27 | 0.35 | 1.56 | 1.28 |
| G | 3.19 | 0.79 | 1.72 | 1.58 | 0.55 | 1.82 | 2.09 |
| H | 3.14 | 0.7 | 2 | 2.19 | 1.51 | 2.21 | 4.04 |

Note: Tags by Federation Dentaire International (FDI) abbreviation: 31, first incisive; 32, second incisive; 33, canine; 34, first premolar; 35, second premolar; 36, first molar; 37, second molar.

#### *4.6.6* Cameriere’s European formula method (2007)

Cameriere’s European formula method was based on a regression analysis of age as a dependent variable and normalized measurements of open apices of the first seven mandibular teeth on the

OPT, where sex ($g$) and the number of teeth with finished maturation of root apices ($N_0$) are important dependent variables in calculating dental age (65, 69). All teeth without completed root maturation were analyzed, and the distance between the inner side of the open apices ($A_i$. $i=1...5$) was measured. The sum of the distances between the inner sides of the two open apices was calculated for teeth with two roots ($A_i$, $i=6, 7$). Distances of open apices were normalized by dividing by the tooth length ($L_i$, $i=1...7$) to minimize the effects of differences among X-rays in magnification and angulation (Figure 6).

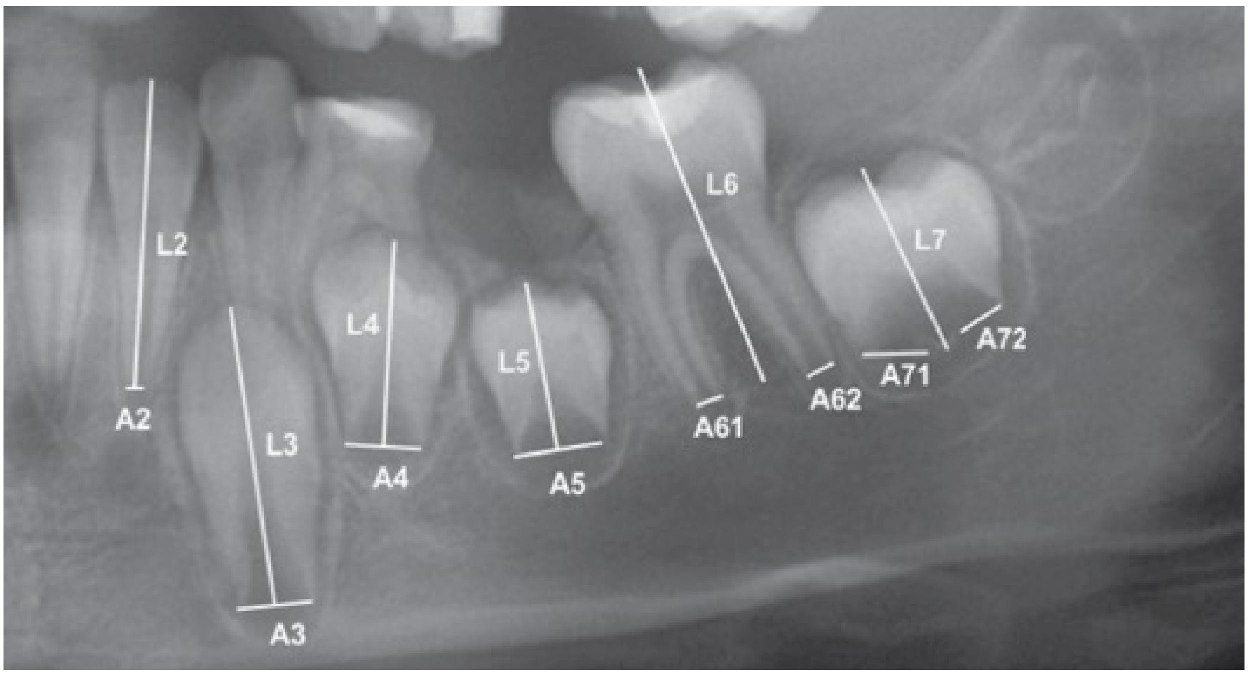



**Figure 6.** An example of Cameriere's measurements of mandibular teeth. xi = Ai/Li, i = 1.....7 of seven left mandibular teeth. Ai, i=1.....5 (teeth with one root) is the distance between the inner sides of the open apex; Ai, i=6. 7 teeth with two roots) is the sum of the distances between the inner sides of the two open apices; and Li, i=1.....7 is the length of the tooth.

Dental age was calculated according to the European formula:

$$Age = 8.387 + 0.282g - 1.692x_5 + 0.835 \text{ x } N_0 - 0.116s - 0.139s \text{ x } N_0 \qquad (1)$$

where g is a variable for biological sex, with $g$=1 for males and $g$=0 for females, $s$ is the sum of the normalized widths of apices of the seven left permanent developing mandibular teeth ($x_i = A_i / L_i$, $i = 1 \ldots 7$), and $x_5$ is the normalized measurement of the second premolar (69).

### *4.6.7* Three methods of development of third molars for age estimation

The aim of this part of the study was to compare three registration methods of third molar mineralization, two by staging methods and one by the measure of open apices, for the purpose of age estimation. Taking into account previously published research about the age range of crypt formation of third molars in various population samples occurring between 5 years to 12 years of age, this age range was not used for analysis to avoid participants without sound radiographic evidence of crown mineralization (70-73) being studied. In total, 1294 OPTs of participants aged 13 to 23 (582 males and 712 females) were evaluated. The sample was randomly divided into a training dataset, 900 OPTs, (Table 6.) and a test dataset, 394 OPTs, (Table 7.), taking into account similar distribution across age groups, Tables 3.12.1 and 3.12.2. The training dataset was used to generate the best linear regression model for age estimation, while the test dataset was used to study the performance of the model. The following registration methods were used for age estimation: Demirjian et al. (57), Köhler et al. (66) and Cameriere et al. (67). All methods were briefly and separately described.

**Table 6.** Sex-specific age distribution of training dataset in years

| Sex | N | Mean ± SD | Min | $Q_1$ | Median | $Q_3$ | Max |
|---|---|---|---|---|---|---|---|
| Males | 408 | 18.41 ± 3.13 | 13.01 | 15.71 | 18.33 | 21.28 | 23.80 |
| Females | 492 | 18.52 ± 3.11 | 13.01 | 15.97 | 18.48 | 21.19 | 23.96 |

Note: N, number of individuals; Mean, mean age; SD, standard deviation; Min, minimum age; $Q_1$, first quartile of age, Median, Median age; $Q_3$, third quartile of age; Max, maximum age.

**Table 7.** Sex-specific age distribution of test dataset in years

| Sex | N | Mean ± SD | Min | $Q_1$ | Median | $Q_3$ | Max |
|---|---|---|---|---|---|---|---|
| Males | 174 | 18.35 ± 3.11 | 13.06 | 15.74 | 18.20 | 21.26 | 23.80 |
| Females | 220 | 18.50 ± 3.11 | 13.11 | 15.92 | 18.52 | 21.29 | 23.96 |

Note: N, number of individuals; Mean, mean age; SD, standard deviation; Min, minimum age; $Q_1$, first quartile of age, Median, Median age; $Q_3$, third quartile of age; Max, maximum age.

### *4.6.8* Analysis of the development of the mandibular third molars by the Demirjian stages

An analysis of the development of third molars was based on Demirjian's eight stage method (A–H), described for the development of molars (Figure 7).

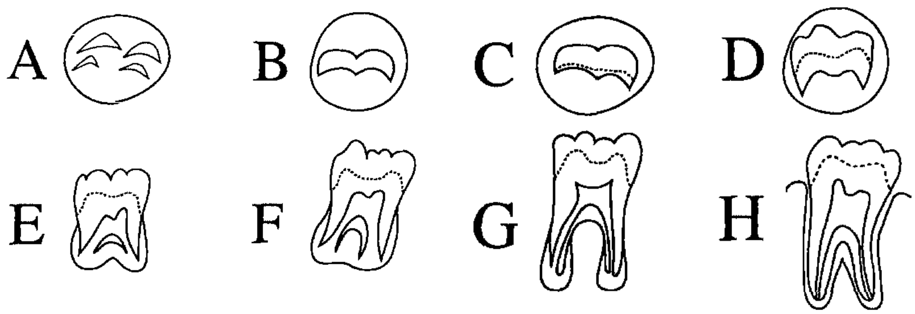



**Figure 7.** Graphic illustrations of the eight mineralization stages of crown and root formation used to score third molar development, according to Demirjian et al. (57). A definition of mineralization stages, modified from Mincer et al. (74); **A** – Cusp tips are mineralized but have not yet coalesced. **B** – Mineralized cusps are united, so the mature coronal morphology is well-defined. **C** – The crown is about half-formed; the pulp chamber is evident and dentinal deposition is occurring. **D** – Crown formation is complete to the dentinoenamel junction. **E** – Formation of the inter-radicular bifurcation has begun; root length is less than the crown length. **F** – Root length is at least as long as crown length, roots have funnel-shaped endings. **G** – Root walls are parallel, but apices remain open. **H** – Apical ends of the roots are completely closed, and the periodontal membrane has a uniform width around the root.

### *4.6.9* Analysis of the development of the mandibular third molars by the Köhler stages

Köhler et al. (66) introduced a ten-stage scoring system, based on the Gleiser and Hunt (75) method with three stages of crown formation and seven stages of root formation (Figure 8). According to Thevissen et al. (76), Köhler staging has been shown to be most suitable for age predictions in the late developmental stages of third molars.

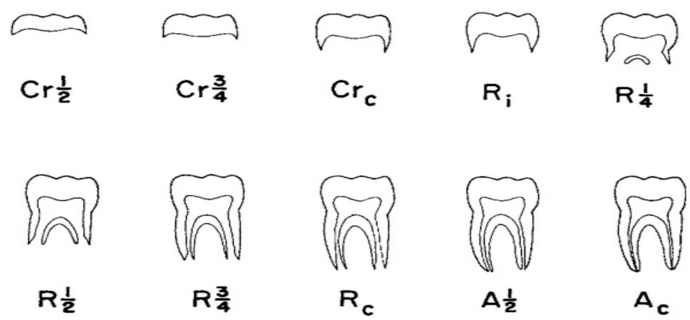



**Figure 8.** Graphic illustrations of ten stages of crown and root formation used to score third molar development, according to Köhler et al. (66). A definition of stages: **Cr½** – The crown is half-formed; the pulp chamber is evident and dentinal deposition is occurring; **Cr¾** – The crown is three-quarters formed; **$Cr_c$** – Crown formation is completed with a defined pulp roof; **$R_i$** – Initial root formation with diverging edges; **R¼** – root length less than crown length with a visible bifurcation area; **R½** – root length equals crown length; **R¾** – three-quarters of the root length developed with diverging ends; **$R_c$** – root length completed with parallel ends; **A½** – apex closed with a wide periodontal ligament space; **Ac** – apex closed with normal periodontal ligament space.

### *4.6.10* Analysis of the development of the mandibular third molars by the third molar maturity index ($I_{3M}$)

The left lower third molar was assessed using the $I_{3M}$ by JC without knowledge of the date of birth of subjects to avoid bias during measuring of specific projections of third molars on OPTs as proposed by Cameriere et al. (67). Briefly, $I_{3M}$ is a ratio of the sum of projections of open apices in multi-rooted teeth or apex width in single-rooted teeth and a tooth length of the mandibular third molar during growth (67). If the third molars were found with entirely closed roots, $I_{3M}$ =0.00 was assigned (Figure 9).

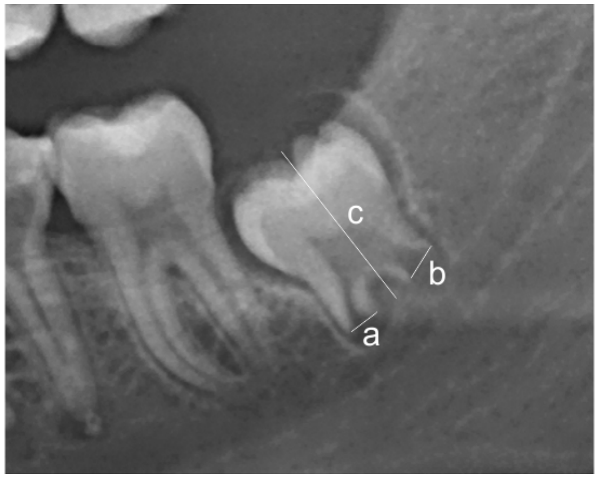



**Figure 9.** An example of third molar maturity index ($I_{3M}$) as a proportion of the sum of open apices (a + b) and height (c) of the projection of the third molar on a panoramic radiograph (77).

### 4.7 Third molars for assessing the age of majority (≥18 years)

The left lower third molar samples were studied by using the Demirjian et al. (57), Köhler et al. (66) and Cameriere et al. (67) methods for assessing the age of majority. For this purpose, specific stages of the Demirjian et al. (Demirjian) (57) and Köhler et al. (Köhler) (66) methods, as well as a set of specific cut-off values of Cameriere $I_{3M}$, were tested to discriminate majors (≥18 years) and minors (<18 years) (67). JC performed the staging of third molars by the Demirjian et al. and Köhler et al. stages and measurements of root apices and tooth lengths and calculated $I_{3M}$.

### 4.8 Statistical analysis for the dental age estimation methods

1. All OPTs were examined by the blind approach, without the possibility to evaluate age and sex.
2. The average age within each stage in all permanent teeth from the left side was calculated separately for males and females. In addition, an independent sample t-test was used to compare the means of chronological ages across developmental stages between the opposing teeth, as well as between sexes.
3. A paired-samples T-test was used to compare the accuracy of different methods, with the null hypothesis that there is no difference between dental age (DA) and chronological age (CA), and a Wilcoxon matched-pair signed-rank test was used if any group listed less than 20 participants within an age group (78). An independent-samples T-test was used to

compare the difference of age or dental age between sexes, with the null hypothesis that there is no difference, and a Mann-Whitney U test was used if any group listed less than 20 participants within an age group. The difference between samples with less than 5 participants in group was not compared.

4. A Repeated-measures ANOVA within the General Linear Model was used to compare DA-CA among three methods (79). The difference between DA and CA was presented as the difference in DA-CA or the accuracy of the method. For this purpose, the values of the chronological age of each child ($CA_i$, i = 1,. . ., n) was compared with their calculated dental age ($DA_i$, i = 1,. . ., n), and the difference between DA and CA (DA-CA) was presented as an error. Additionally, an error was presented as the absolute difference between DA-CA or the mean absolute error:

$$\text{MAE} = \frac{1}{n}\sum_{i=1}^{n}|DA_i - CA_i| \qquad (2)$$

where “n” is the number of participants in the tested sample. The results of MAE were presented for the males and females and age cohorts.

5. On the three third molar mineralization registration methods, the Spearman correlation coefficients were calculated to explore associations among the methods and between each method and age. In the absence of a lower left molar, the lower right molar was analyzed. On the training set, a linear regression model was fitted for each registration technique separately with age as response and the third molar development registration as an ordinal predictor; except for the $I_{3M}$ technique that was entered as a continuous predictor with logarithmic transformation according to Thevissen et al. (80). From each regression sex-specific model, the determination coefficient ($R^2$), indicating the proportion of variance in age explained by the tooth development registration technique, and the root mean squared error (RMSE) were calculated. The RMSE is a standard deviation, reflecting the variability of the predicted age around the true age. The RMSE was calculated as:

$$\text{RMSE}=\sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2} \qquad (3)$$

where $y_i$ represents actual age and $\hat{y}i$ represents the estimated age of the i-th case.

6. On the test dataset, linear regression models were tested, and the difference between DA and CA or mean error (DA-CA) and mean absolute error (MAE) were also calculated.

### *4.8.1* Statistical analysis for assessing the age of majority (≥18 years)

Scatterplot and box-plot graphs and tables were used to show relationships between chronological age and different mineralization stages or $I_{3M}$ indexes for both sexes.

Then the overall effectiveness of specific Demirjian et al. and Köhler et al. stages or $I_{3M}$ were evaluated by plotting the receiver operating characteristic (ROC) curves (67). The value of the area under the ROC curve shows the accuracy of the test or how well the test discriminates between participants being major or minor. An area of 1 represents a perfect test, while an area of 0.5 represents a worthless test (81). To test the performance of a specific cut-off value of $I_{3M}$, the results were summarized in a single 2×2 contingency table. Generally, the 2×2 tables displayed the number of participants who have a specific mineralization stage or a specific $I_{3M} < SCVI_{3M}$ and are 18 years and older (known as true positives); then participants with specific stages or $I_{3M} < SCVI_{3M}$ who are younger than 18 years of age (known as false positives); followed by those with specific stages or $I_{3M} \geq SCVI_{3M}$ who are 18 years and older (known as false negatives) and those with specific stages or $I_{3M} \geq SCVI_{3M}$ who are younger than 18 years of age (known as true negatives). The values of the test were presented with a 95% confidence interval (95% CI).

Measurements of accurately classified individuals (*AC*), the sensitivity of the test, the proportion of subjects equal to 18 years or more together with its specificity, the proportion of individuals younger than 18 years were evaluated. A single statistic that captures the performance of a diagnostic test is the Youden's index (*J-index*), a function of sensitivity and specificity (82). The positive predictive value (PPV) are tests that look at the probability that a subject which tests positive is truly positive, and negative predictive value (*NPV*) tests the probability that subjects who test negative are truly negative (83). The likelihood ratio of the positive test (LR+) and the likelihood ratio of the negative test (LR-) for the cut-off value of $I_{3M}$ were calculated. LR+ is equivalent to the probability that an individual 18 years and older was positive for the age of majority (true positive) divided by the probability that an individual younger than 18 was positive for the age of majority (false positive). LR- is equivalent to the probability that an individual 18 and older is selected negative for the age of majority (false negative) divided by the probability that an individual younger than 18 is selected negative for the age of majority (true negative). LR+ greater than 1 increases the probability of being a major (an adult), and a smaller LR+ decreases the probability (84). In this study, the likelihood ratios summarize how many times more or less likely majors are to have $I_{3M}$ <0.08 than minors and minors are to have $I_{3M}$ ≥0.08 than majors (84, 85). Values of likelihood ratios above 10 and below 0.1 are considered to provide strong evidence to accept or rule out an assessment in most situations (85).

Bayes' post-test probability (*p*) of being 18 years or older (i.e., the proportion of individuals who are 18 years or older and have reached a specific mineralization stage or $I_{3M}$ < cut-off may help to discriminate between those who are or are not 18 years or more (67). According to Bayes' theorem, *p* may be written as:

$$p = \frac{p_1 p_0}{p_1 p_0 + (1 - p_2)(1 - p_0)} \quad (4).$$

In the post-test probability *p*, $p_0$ *is* the probability that the participant in question is 18 years or older, given that he or she is aged between 13 and 23 years, which represents the target population. In this study, probability $p_0$ was calculated as the proportion of participants between 18 and 23 years of age who live in Botswana and those between 13 and 23 years, and this data was obtained from the 2011 census for Botswana from the Central Statistics Office (CSO) in Botswana (61) and is considered to be 0.53 and 0.54 in males and females respectively.

To test the performance of specific Demirjian and Köhler stages and cut-off values of $I_{3M}$ close to $I_{3M}$ <0.08, the results were summarized in a single condensed table, which consists of 2-by-2 contingency tables for the tested stages and $I_{3M}$ values. The results of accuracy, sensitivity, specificity, J-index, PPV, NPV, LR+LR- as well as p for different cut-off values of $I_{3M,}$ were also presented.

### *4.8.2* Intra-observer and inter-observer agreements

An evaluation of 100 randomly selected OPTs was conducted by JC for the second time to evaluate intra-rater observer, 2 months following initial evaluation, as well as by IG as an inter-rater observer. Based on these 100 OPTs intra-observer and inter-observer agreement of mineralization stages of Demirjian stages of all teeth from the left side (57), the Köhler stages of mandibular third molars (66), the number of teeth with finished maturation ($N_0$) was calculated using Kappa scores, while the Cameriere normalized values of open apices, $x_i$ (i=1…8) were calculated using intra-class correlation coefficient (ICC) (86).

The statistical significance was set to 0.05. For data management and statistical analysis, MS Excel 2010 (Microsoft Office 2010, Microsoft. and Redmond. WA) and SPSS Statistics 17.0 for Windows (SPSS Inc. Chicago, IL) were used.

# 5. RESULTS

## 5.1 The distribution of the sample

The final sample for the analysis consisted of 1760 OPTs of children and adolescents, aged 6–23 years old, from Botswana. The entire sample was analyzed using Demirjian's developmental stages and then the sample was separated for age estimation, first, by using the first seven mandibular developing teeth and the age range between 6 to 16 years, and second, by using only third molars for age estimation for the age range between 13 to 23 years of age. The distribution and frequencies of the final sample of OPTs were presented in Table 8.

**Table 8.** Distribution of panoramic radiographs of black African children from the city of Gaborone in the Republic of Botswana. The numbers in parentheses represent samples with completed mineralization of seven mandibular teeth from the left side.

| Age group | Males | Females | Total |
|---|---|---|---|
| 6.0 – 6.9 | 22 | 21 | 43 |
| 7.0 – 7.9 | 23 | 20 | 43 |
| 8.0 – 8.9 | 26 | 22 | 48 |
| 9.0 – 9.9 | 21 | 29 | 50 |
| 10.0 – 10.9 | 27 | 32 | 59 |
| 11.0 – 11.9 | 50 | 50 | 100 |
| 12.0 – 12.9 | 56 | 67 | 123 |
| 13.0 – 13.9 | 49 | 59 | 108 |
| 14.0 – 14.9 | 59 | 56 | 115 |
| 15.0 – 15.9 | 48 | 69 | 117 |
| 16.0 – 16.9 | 69 | 71 | 140 |
| 17.0 – 17.9 | 53 (4) | 68 (1) | 121 (5) |
| 18.0 – 18.9 | 45 (9) | 69 (20) | 114 (29) |
| 19.0 – 19.9 | 50 (33) | 74 (42) | 124 (75) |
| 20.0 – 20.9 | 47 (37) | 57 (42) | 104 (79) |
| 21.0 – 21.9 | 60 (48) | 64 (52) | 124 (100) |
| 22.0 – 22.9 | 55 (52) | 64 (57) | 119 (109) |
| 23.0 – 23.9 | 47 (41) | 61 (56) | 108 (97) |
| Total | 807 (224) | 953 (270) | 1760 (494) |

The Pearson Chi-Square homogeneity test indicates that the distribution of participants, males and females, is similar across age categories in the whole sample. $\chi^2$=11.166, df=17, p=0.848; in the sample of seven developing mandibular teeth. $\chi^2$ =5.182, df=10, p=0.879; and in the sample for age range 13 to 23 years, $\chi^2$ =6.955, df=10, p=0.730.

## 5.2 Intra-observer and inter-observer agreements

### *5.2.1* Intra-observer and inter-observer agreement for the Demirjian stages

The average Kappa coefficients for determining intra-observer agreement was 0.84 ($p < 0.05$) and inter-observer agreement was 0.79 ($p < 0.05$) for the Demirjian stages, indicating a high level of concordance, according to Altman et al. (87). Average Kappa values were slightly better for mandibular teeth than for maxillary teeth, both for the intra-rater and inter-rater agreement, but without statistical significance (Table 9.).

**Table 9.** Kappa scores for mineralization stages according to the Demirjian method of permanent teeth from the left side of the jaws based on an evaluation of 100 randomly selected panoramic radiographs

| **Tooth** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | **28** | **31** | **32** | **33** | **34** | **35** | **36** | **37** | **38** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Kappa** | | | | | | | | | | | | | | | |
| **Intra-observer** | 0.82 | 0.84 | 0.89 | 0.78 | 0.84 | 0.76 | 0.83 | 0.84 | 0.88 | 0.86 | 0.92 | 0.88 | 0.88 | 0.77 | 0.82 | 0.83 |
| **Inter-observer** | 0.72 | 0.88 | 0.84 | 0.80 | 0.83 | 0.78 | 0.72 | 0.74 | 0.76 | 0.88 | 0.80 | 0.80 | 0.79 | 0.81 | 0.74 | 0.78 |

### *5.2.2* Intra-observer and inter-observer agreement for the Köhler stages

The average Kappa for determining intra-rater and inter-rater agreements for Köhler stages of mandibular third molars were 0.93 ($p < 0.05$) and 0.92 ($p < 0.05$), respectively, indicating a higher level of concordance, according to Altman et al. (87).

### *5.2.3* Intra-observer and inter-observer agreement for the Cameriere normalized open apices of mandibular teeth

The intra-class correlation coefficients for determining intra-observer and inter-observer agreements for the Cameriere normalized open apices of mandibular teeth ($x_i$), $i=1…8$, were 0.88 ($p < 0.05$) and 0.88 ($p < 0.05$) respectively (Table 10.), indicating a high level of concordance, according to Altman et al. (87).

**Table 10.** Intra-class correlation coefficients (ICC) of intra-observer and inter-observer agreement of the normalized open apices of the mandibular teeth ($x_i$)

| **Cameriere normalized open apices of mandibular teeth** | $x_1$ | $x_2$ | $x_3$ | $x_4$ | $x_5$ | $x_6$ | $x_7$ | $x_8$ |
|---|---|---|---|---|---|---|---|---|
| Intra-observer agreement (ICC) | 0.81 | 0.78 | 0.92 | 0.93 | 0.91 | 0.91 | 0.85 | 0.92 |
| Inter-observer agreement (ICC) | 0.76 | 0.82 | 0.93 | 0.93 | 0.94 | 0.93 | 0.84 | 0.89 |

## 5.3 Demirjian's mineralization stages data for the chronological ages for the maxillary and mandibular teeth

The age within different Demirjian's developmental stages were presented for all permanent teeth on the left side of the maxilla and mandible, Table 11 for males and Table 12 for females. For stage H, only minimal age was recorded (63).

**Table 11.** Chronological age (years) at the tooth stage for the left maxillary and mandibular teeth in males according to the Demirjian method

| | Maxillary teeth | | | | | | | | | Mandibular teeth | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tooth | N | Mean ±SD | SEM | Min | $Q_1$ | Med | $Q_3$ | Max | Tooth | N | Mean ±SD | SEM | Min | $Q_1$ | Med | $Q_3$ | Max | t(df) | Mann-Whitney U test(Z) | *p* |
| **Stage** | | | | | | | | | | | | | | | | | | | | | |
| **E** | **21** | 2 | 6.40±**0.36** | 0.25 | 6.14 | | 6.40 | | 6.65 | **31** | | | | | | | | | | | - |
| **F** | | 27 | 6.80±0.54 | 0.10 | 6.10 | 6.41 | 6.61 | 7.26 | 8.13 | | 5 | 6.78±0.37 | 0.17 | 6.46 | 6.53 | 6.61 | 7.11 | 7.41 | | 0.03 | 0.976 |
| **G** | | 30 | 8.39±0.78 | 0.14 | 6.59 | 8.10 | 8.54 | 8.89 | 10.10 | | 18 | 6.90±0.70 | 0.16 | 6.14 | 6.41 | 6.71 | 7.46 | 8.58 | | 4.63 | <0.001 |
| **H** | | 748 | 7.45 | | | | | | | | 784 | | | 6.10 | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | |
| **D** | **22** | 2 | 6.40±0.36 | 0.26 | 6.14 | | 6.40 | | 6.65 | **32** | | | | | | | | | | | |
| **E** | | 21 | 6.97±0.73 | 0.16 | 6.10 | 6.41 | 6.61 | 7.50 | 8.38 | | 10 | 6.63±0.40 | 0.13 | 6.14 | 6.41 | 6.44 | 6.92 | 7.41 | | -0.08 | 0.94 |
| **F** | | 34 | 8.07±1.20 | 0.21 | 6.46 | 6.77 | 8.21 | 8.82 | 11.18 | | 12 | 6.72±0.42 | 0.12 | 6.21 | 6.54 | 6.61 | 6.77 | 7.60 | | 3.38 | <0.001 |
| **G** | | 32 | 9.15±1.09 | 0.19 | 7.45 | 8.38 | 9.16 | 10.00 | 11.14 | | 24 | 7.83±0.94 | 0.19 | 6.10 | 7.17 | 7.86 | 8.57 | 9.63 | 4.85(52.88) | | <0.001 |
| **H** | | 718 | | | 7.87 | | | | | | 761 | | | 7.45 | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | |
| **D** | **23** | | | | | | | | | **33** | 10 | 6.71±0.62 | 0.20 | 6.41 | 6.41 | 6.51 | 6.66 | 8.44 | | | |
| **E** | | 37 | 7.07±0.89 | 0.15 | 6.10 | 6.43 | 6.77 | 7.51 | 9.46 | | 33 | 7.37±0.93 | 0.16 | 6.10 | 6.59 | 7.26 | 8.03 | 9.29 | -1.37(66.30) | | 0.17 |
| **F** | | 76 | 9.38±1.37 | 0.16 | 7.35 | 8.39 | 9.13 | 10.33 | 12.58 | | 66 | 9.42±1.33 | 0.16 | 7.35 | 8.43 | 9.16 | 10.25 | 12.51 | -0.18(138.25) | | 0.86 |
| **G** | | 68 | 11.86±1.12 | 0.14 | 8.73 | 11.13 | 11.87 | 12.68 | 14.16 | | 56 | 11.77±1.13 | 0.15 | 8.73 | 10.98 | 11.83 | 12.47 | 14.16 | 0.44(117.88) | | 0.66 |
| **H** | | 626 | | | 10.28 | | | | | | 642 | | | 9.55 | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | |
| **D** | **24** | 23 | 6.75±0.58 | 0.12 | 6.14 | 6.41 | 6.61 | 6.82 | 8.89 | **34** | 14 | 6.89±0.62 | 0.17 | 6.41 | 6.41 | 6.55 | 7.25 | 8.44 | | 0.03 | 0.976 |
| **E** | | 48 | 8.27±0.96 | 0.14 | 6.10 | 7.59 | 8.34 | 8.89 | 10.21 | | 54 | 7.88±1.06 | 0.14 | 6.10 | 7.10 | 7.86 | 8.61 | 10.80 | 1.95(99.96) | | 0.054 |
| **F** | | 45 | 10.10±1.33 | 0.20 | 7.45 | 9.06 | 10.09 | 11.16 | 12.58 | | 44 | 9.93±1.19 | 0.18 | 7.53 | 9.03 | 9.83 | 11.04 | 12.19 | 0.64(86.33) | | 0.53 |
| **G** | | 56 | 11.77±1.12 | 0.15 | 8.32 | 11.13 | 11.80 | 12.43 | 14.16 | | 68 | 11.95±1.05 | 0.13 | 9.50 | 11.28 | 11.88 | 12.65 | 14.16 | -0.92(114.28) | | 0.36 |
| **H** | | 635 | | | 10.28 | | | | | | 627 | | | 10.25 | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | |
| **C** | **25** | 2 | 6.64±0.25 | 0.18 | 6.46 | | 6.64 | | 6.82 | **35** | | | | | | | | | | | |
| **D** | | 41 | 7.29±0.85 | 0.13 | 6.14 | 6.57 | 7.21 | 7.86 | 9.29 | | 37 | 7.08±0.76 | 0.13 | 6.10 | 6.44 | 6.77 | 7.59 | 8.89 | 1.15(76.00) | | 0.25 |
| **E** | | 50 | 8.98±1.13 | 0.16 | 6.10 | 8.36 | 8.92 | 9.65 | 11.42 | | 39 | 8.65±1.02 | 0.16 | 6.59 | 7.86 | 8.58 | 9.23 | 11.14 | 1.44(85.10) | | 0.15 |
| **F** | | 35 | 10.92±1.18 | 0.20 | 7.53 | 10.09 | 10.98 | 11.87 | 12.68 | | 59 | 10.61±1.40 | 0.18 | 7.53 | 9.50 | 10.87 | 11.70 | 13.89 | 1.15(81.27) | | 0.25 |
| **G** | | 68 | 11.94±0.99 | 0.12 | 8.32 | 11.58 | 11.92 | 12.56 | 13.91 | | 92 | 12.43±1.15 | 0.12 | 9.55 | 11.65 | 12.35 | 13.15 | 14.61 | -2.89(154.29) | | 0.004 |
| **H** | | 611 | | | 10.83 | | | | | | 580 | | | 11.04 | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | |
| **F** | **26** | 13 | 6.57 ± 0.41 | 0.11 | 6.14 | 6.31 | 6.41 | 6.62 | 7.42 | **36** | 14 | 6.80±0.58 | 0.16 | 6.14 | 6.41 | 6.63 | 6.93 | 8.38 | | 1.19 | 0.234 |
| **G** | | 75 | 8.45±1.18 | 0.14 | 6.10 | 7.58 | 8.56 | 9.29 | 11.06 | | 55 | 8.04±1.11 | 0.15 | 6.10 | 7.21 | 8.13 | 8.86 | 10.21 | 2.03(120.30) | | 0.045 |
| **H** | | 719 | | | 7.45 | | | | | | 738 | | | | 7.45 | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | |
| **D** | **27** | 46 | 7.19±0.78 | 0.11 | 6.10 | 6.54 | 7.14 | 7.86 | 8.89 | **37** | 50 | 7.35±0.97 | 0.14 | 6.10 | 6.55 | 7.24 | 7.88 | 10.21 | -0.89(92.39) | | 0.37 |
| **E** | | 73 | 9.61±1.25 | 0.15 | 7.45 | 8.77 | 9.46 | 10.31 | 12.53 | | 57 | 9.29±1.00 | 0.13 | 7.45 | 8.57 | 9.16 | 10.04 | 11.42 | 1.62(127.91) | | 0.11 |
| **F** | | 37 | 11.90±0.78 | 0.13 | 10.56 | 11.31 | 11.87 | 12.53 | 13.51 | | 59 | 11.87±0.93 | 0.12 | 9.55 | 11.27 | 11.83 | 12.53 | 14.16 | 0.17(86.25) | | 0.87 |
| **G** | | 136 | 13.00±1.17 | 0.10 | 10.28 | 12.12 | 12.98 | 13.93 | 16.58 | | 133 | 13.13±1.23 | 0.11 | 10.28 | 12.20 | 13.07 | 14.12 | 16.20 | -0.89(265.61) | | 0.38 |
| **H** | | 515 | | | 12.24 | | | | | | 508 | | | 12.48 | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | |
| **0**[b] | **28** | 2 | 7.42±0.22 | 0.16 | 7.26 | | 7.42 | | 7.58 | **38** | 23 | 7.18±0.90 | 0.19 | 6.10 | 6.41 | 6.82 | 7.60 | 8.89 | - | - | - |
| **A** | | 8 | 7.88±0.90 | 0.32 | 6.46 | 7.02 | 8.00 | 8.75 | 8.94 | | 16 | 7.64±0.93 | 0.23 | 6.55 | 6.77 | 7.45 | 8.71 | 8.94 | | 0.11 | 0.90 |
| **B** | | 26 | 8.41±1.53 | 0.30 | 6.55 | 7.35 | 8.15 | 8.93 | 12.51 | | 15 | 8.80±1.59 | 0.41 | 7.14 | 7.35 | 8.20 | 10.10 | 12.24 | | 0.88 | 0.38 |
| **C** | | 56 | 9.82±1.26 | 0.17 | 7.53 | 9.01 | 9.72 | 10.83 | 12.58 | | 54 | 9.96±1.19 | 0.16 | 7.87 | 9.09 | 9.83 | 10.95 | 12.34 | -0.60(107.95) | | 0.55 |
| **D** | | 137 | 12.75±1.56 | 0.13 | 9.90 | 11.73 | 12.44 | 13.59 | 19.49 | | 150 | 12.69±1.44 | 0.12 | 8.82 | 11.73 | 12.51 | 13.67 | 17.60 | 0.34(276.95) | | 0.41 |
| **E** | | 120 | 14.74±1.62 | 0.14 | 11.04 | 13.61 | 14.54 | 15.90 | 18.81 | | 115 | 15.03±1.48 | 0.14 | 11.73 | 13.90 | 14.92 | 16.11 | 18.81 | -1.43(232.47) | | 0.15 |
| **F** | | 84 | 16.37±1.28 | 0.14 | 14.34 | 15.37 | 16.22 | 17.03 | 20.52 | | 74 | 16.60±1.56 | 0.18 | 14.35 | 15.38 | 16.45 | 17.12 | 20.75 | -1.00(141.49) | | 0.32 |
| **G** | | 89 | 18.40±1.59 | 0.17 | 14.67 | 17.14 | 18.41 | 19.30 | 22.51 | | 94 | 18.30±1.57 | 0.16 | 14.67 | 17.15 | 18.13 | 19.18 | 22.60 | 0.43(180.18) | | 0.67 |
| **H** | | 256 | | | 15.72 | | | | | | 251 | | | 15.72 | | | | | | | |

Note: N, number of individuals; [a], crypt stage for the third molar; Mean, mean age; SD, standard deviation of mean age; SEM, standard error of the mean age; Min, minimal age; $Q_1$, first quartile of age; Med, median age; $Q_3$; third quartile of age; Max, maximum age; t, an independent samples t-test; df, degree of freedom; Mann-Whitney, an independent samples Mann-Whitney U test; *p*, statistically significant if <0.05.

**Table 12.** Chronological age (years) at the tooth stage for the left maxillary and mandibular teeth in females according to the Demirjian method

| | Maxillary teeth | | | | | | | | | Mandibular teeth | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Tooth** | **N** | **Mean ±SD** | **SEM** | **Min** | **$Q_1$** | **Med** | **$Q_3$** | **Max** | **Tooth** | **N** | **Mean ±SD** | **SEM** | **Min** | **$Q_1$** | **Med** | **$Q_3$** | **Max** | **t(df)** | **Mann-Whitney** | ***p*** |
| **Stage** | | | | | | | | | | | | | | | | | | | | | |
| **D** | **21** | 2 | 6.34±0.12 | 0.08 | 6.25 | | 6.34 | | 6.42 | **31** | | | | | | | | | | | - |
| **E** | | 9 | 6.70±0.29 | 0.10 | 6.35 | 6.37 | 6.77 | 6.97 | 7.09 | | 10 | 6.58±0.27 | 0.09 | 6.25 | 6.35 | 6.49 | 6.84 | 6.97 | | 0.77 | 0.44 |
| **F** | | 17 | 7.46±1.07 | 0.26 | 6.52 | 6.70 | 7.28 | 7.62 | 10.62 | | 6 | 7.25±0.37 | 0.15 | 6.74 | 6.87 | 7.31 | 7.58 | 7.70 | | 0.25 | 0.80 |
| **G** | | 33 | 8.02±0.86 | 0.15 | 6.08 | 7.64 | 8.23 | 8.60 | 9.36 | | 12 | 7.20±0.47 | 0.14 | 6.52 | 6.89 | 7.22 | 7.46 | 8.27 | | 3.17 | 0.002 |
| **H** | | 892 | | | 7.22 | | | | | | 925 | | | 6.08 | | | | | | | |
| **C** | **22** | 2 | 6.34±0.19 | 0.08 | 6.25 | | 6.34 | | 6.62 | **32** | | | | | | | | | | | - |
| **D** | | 5 | 6.74±0.25 | 0.12 | 6.40 | 6.48 | 6.80 | 6.97 | 6.97 | | 2 | 6.48±0.12 | 0.08 | 6.40 | | 6.48 | | 6.57 | | | - |
| **E** | | 17 | 7.45±1.09 | 0.26 | 6.35 | 6.75 | 7.28 | 7.62 | 10.62 | | 12 | 6.81±0.46 | 0.13 | 6.25 | 6.36 | 6.78 | 6.97 | 7.70 | | 1.81 | 0.07 |
| **F** | | 28 | 7.78±0.78 | 0.15 | 6.25 | 7.19 | 7.98 | 8.40 | 8.77 | | 13 | 7.32±1.14 | 0.32 | 6.25 | 6.54 | 7.22 | 7.46 | 10.62 | 1.32(17.42) | 2.13 | 0.03 |
| **G** | | 26 | 8.91±1.37 | 0.27 | 6.08 | 7.95 | 9.20 | 9.68 | 11.10 | | 15 | 7.72±0.79 | 0.20 | 6.65 | 7.15 | 7.64 | 8.31 | 9.49 | 3.53(38.99) | 2.81 | 0.005 |
| **H** | | 875 | | | 8.22 | | | | | | 911 | | | 6.08 | | | | | | | |
| **D** | **23** | 5 | 6.68±0.33 | 0.15 | 6.25 | 6.34 | 6.80 | 6.97 | 6.97 | **33** | 8 | 6.62±0.27 | 0.10 | 6.25 | 6.41 | 6.56 | 6.93 | 6.97 | | 0.29 | 0.77 |
| **E** | | 28 | 7.06±0.66 | 0.12 | 6.25 | 6.56 | 6.95 | 7.46 | 9.24 | | 26 | 7.26±0.78 | 0.15 | 6.25 | 6.66 | 7.12 | 7.73 | 9.24 | -1.01(49.17) | | 0.32 |
| **F** | | 66 | 9.18±1.33 | 0.16 | 6.08 | 8.25 | 9.16 | 9.90 | 12.47 | | 65 | 9.14±1.34 | 0.17 | 6.08 | 8.22 | 9.18 | 10.29 | 12.15 | 0.17(128.93) | | 0.86 |
| **G** | | 83 | 11.54±1.14 | 0.13 | 8.22 | 10.70 | 11.57 | 12.23 | 13.90 | | 72 | 11.37±0.94 | 0.11 | 9.00 | 10.70 | 11.54 | 12.02 | 12.89 | 1.04(152.97) | | 0.30 |
| **H** | | 771 | | | 9.83 | | | | | | 782 | | | 9.63 | | | | | | | |
| **D** | **24** | 17 | 6.68±0.28 | 0.07 | 6.25 | 6.41 | 6.66 | 6.94 | 7.28 | **34** | 12 | 6.68±0.43 | 0.13 | 6.25 | 6.36 | 6.56 | 6.93 | 7.83 | | 0.44 | 0.66 |
| **E** | | 49 | 7.91±0.85 | 0.12 | 6.08 | 7.35 | 8.05 | 8.44 | 9.89 | | 54 | 7.83±0.94 | 0.13 | 6.08 | 7.20 | 7.75 | 8.38 | 10.62 | 0.45(100) | | 0.65 |
| **F** | | 57 | 10.42±1.02 | 0.13 | 8.21 | 9.60 | 10.38 | 11.06 | 12.47 | | 52 | 10.39±1.15 | 0.16 | 8.22 | 9.51 | 10.33 | 11.08 | 14.10 | 0.14(101.6) | | 0.89 |
| **G** | | 57 | 11.79±1.09 | 0.14 | 9.25 | 10.95 | 11.68 | 12.64 | 14.10 | | 66 | 11.65±0.94 | 0.12 | 9.49 | 10.87 | 11.66 | 12.47 | 13.43 | 0.80(115.92) | | 0.43 |
| **H** | | 773 | | | 9.83 | | | | | | 769 | | | 9.83 | | | | | | | |
| **C** | **25** | 2 | 6.33±0.12 | 0.08 | 6.25 | | 6.34 | | 6.42 | **35** | 2 | 6.34±0.12 | 0.08 | 6.25 | | 6.34 | | 6.42 | | | - |
| **D** | | 34 | 7.20±0.78 | 0.13 | 6.25 | 6.63 | 6.99 | 7.58 | 9.24 | | 23 | 7.17±0.59 | 0.12 | 6.35 | 6.74 | 7.15 | 7.54 | 8.27 | 0.17(54.23) | | 0.87 |
| **E** | | 46 | 8.66±1.04 | 0.15 | 6.08 | 8.09 | 8.60 | 9.26 | 10.70 | | 46 | 8.09±1.10 | 0.16 | 6.08 | 7.30 | 8.23 | 8.73 | 10.70 | 2.55(89.72) | | 0.01 |
| **F** | | 50 | 10.89±1.12 | 0.16 | 8.22 | 10.04 | 10.80 | 11.68 | 14.10 | | 74 | 10.88±1.12 | 0.13 | 8.76 | 9.89 | 10.79 | 11.67 | 14.10 | 0.05(112.24) | | 0.96 |
| **G** | | 71 | 11.93±1.03 | 0.12 | 9.63 | 11.24 | 11.98 | 12.66 | 14.01 | | 96 | 12.32±1.16 | 0.12 | 9.49 | 11.49 | 12.54 | 13.26 | 14.63 | -2.33(161.09) | | 0.02 |
| **H** | | 750 | | | 10.69 | | | | | | 712 | | | 11.23 | | | | | | | |
| **F** | **26** | 13 | 6.59±0.24 | 0.07 | 6.25 | 6.37 | 6.57 | 6.78 | 6.97 | **36** | 8 | 6.76±0.66 | 0.23 | 6.25 | 6.35 | 6.58 | 6.87 | 8.27 | -0.70(8.15) | 0.04 | 1.00 |
| **G** | | 71 | 8.53±1.22 | 0.15 | 6.25 | 7.47 | 8.34 | 9.49 | 11.10 | | 53 | 7.81±1.01 | 0.14 | 6.25 | 7.04 | 7.65 | 8.44 | 10.62 | 3.59(120.64) | | 0.0005 |
| **H** | | 869 | | | 6.08 | | | | | | 892 | | | 6.08 | | | | | | | |
| **C** | **27** | 4 | 6.58±0.30 | 0.15 | 6.25 | 6.29 | 6.58 | 6.87 | 6.91 | **37** | 2 | 6.34±0.13 | 0.08 | 6.25 | | 6.34 | | 6.42 | - | - | - |
| **D** | | 45 | 7.37±0.71 | 0.11 | 6.25 | 6.78 | 7.30 | 7.85 | 9.24 | | 47 | 7.44±0.80 | 0.12 | 6.25 | 6.77 | 7.30 | 8.23 | 9.21 | -0.44(89.50) | | 0.66 |
| **E** | | 59 | 9.60±1.15 | 0.15 | 6.08 | 8.92 | 9.53 | 10.40 | 12.15 | | 55 | 9.42±1.23 | 0.17 | 6.08 | 8.64 | 9.49 | 10.32 | 12.15 | 0.81(109.91) | | 0.42 |
| **F** | | 49 | 11.37±0.99 | 0.14 | 9.62 | 10.70 | 11.33 | 12.00 | 14.10 | | 55 | 11.51±0.99 | 0.13 | 9.63 | 10.74 | 11.50 | 12.02 | 13.98 | -0.76(101.95) | | 0.45 |
| **G** | | 156 | 13.01±1.13 | 0.09 | 10.69 | 12.17 | 12.90 | 13.88 | 16.16 | | 158 | 13.07±1.29 | 0.10 | 10.10 | 12.14 | 13.06 | 13.89 | 16.80 | -0.44(305.54) | | 0.66 |
| **H** | | 640 | | | 11.73 | | | | | | 636 | | | 11.35 | | | | | | | |
| **0**[a] | **28** | 10 | 7.49±0.91 | 0.29 | 6.52 | 6.66 | 7.31 | 8.30 | 9.24 | **38** | 18 | 7.54±1.08 | 0.25 | 6.55 | 6.88 | 7.15 | 7.84 | 11.14 | | 0.05 | 1.00 |
| **A** | | 11 | 7.96±1.46 | 0.44 | 6.25 | 7.09 | 7.64 | 8.34 | 11.14 | | 14 | 7.76±1.37 | 0.37 | 6.25 | 6.55 | 7.29 | 8.72 | 10.70 | | 0.36 | 0.72 |
| **B** | | 13 | 8.06±0.84 | 0.23 | 6.55 | 7.38 | 8.10 | 8.57 | 9.60 | | 25 | 8.57±1.27 | 0.25 | 7.29 | 7.71 | 8.25 | 8.99 | 13.22 | | 1.12 | 0.26 |
| **C** | | 42 | 10.27±1.62 | 0.25 | 7.82 | 9.21 | 9.86 | 10.97 | 14.52 | | 41 | 10.29±1.19 | 0.19 | 8.22 | 9.33 | 9.90 | 11.05 | 12.69 | -0.06(75.28) | | 0.95 |
| **D** | | 147 | 12.24±1.78 | 0.15 | 6.08 | 11.21 | 12.06 | 13.15 | 18.21 | | 159 | 12.41±1.73 | 0.14 | 6.08 | 11.39 | 12.22 | 13.27 | 17.68 | -0.85(300.55) | | 0.40 |
| **E** | | 170 | 14.65±1.92 | 0.15 | 10.70 | 13.32 | 14.49 | 15.92 | 21.30 | | 153 | 14.79±1.69 | 0.14 | 11.23 | 13.59 | 14.67 | 15.93 | 20.70 | -0.70(320.85) | | 0.49 |
| **F** | | 113 | 16.91±1.54 | 0.14 | 13.71 | 15.80 | 16.85 | 17.99 | 22.21 | | 102 | 16.89±1.55 | 0.15 | 14.20 | 15.70 | 16.73 | 17.56 | 22.16 | 0.10(210.48) | | 0.92 |
| **G** | | 125 | 18.71±1.85 | 0.17 | 14.74 | 17.23 | 18.54 | 19.86 | 23.72 | | 120 | 18.45±1.53 | 0.14 | 15.30 | 17.20 | 18.30 | 19.39 | 23.07 | 0.21(0.55) | | 0.89 |
| **H** | | 288 | | | 15.11 | | | | | | 300 | | | 15.11 | | | | | | | |

Note: N, number of individuals; [a], crypt stage for the third molar; Mean, mean age; SD, standard deviation of mean age; SEM, standard error of the mean age; Min, minimal age; $Q_1$, first quartile of age; Med, median age; $Q_3$; third quartile of age; Max, maximum age; t, an independent samples t-test; df, degree of freedom; Mann-Whitney, an independent samples Mann-Whitney U test; *p*, statistically significant if <0.05.

A comparison of the mean age within developmental stages was performed in the total sample between the opposing two teeth. It was found that the mandibular teeth were slightly more advanced in the development compared to the maxillary teeth in both sexes, without significant differences in most stages. In males, the significant difference in advanced development of mandibular teeth was found only for stage G between the central incisors, stages E and G between lateral incisors and stage G between first molars while significantly advanced development of maxillary teeth was found in stage G in second premolars (Table 11).

In females, a significant difference in advanced development in mandibular teeth was only found in stage G between antagonists of both incisors, stage E of the second incisor, stage E in the second premolars and stage G in the first molars. Significantly, the advanced development of maxillary teeth was only found for stage G in the second premolar (Table 12).

In comparing males and females, mean ages within the same developmental stage (Tables 11 and 12) showed that permanent dentition develops at a similar developmental rate with a significant difference in the development of only a few teeth. In the maxilla, dental development in males was significantly early for stage F of the central incisor and stage F in the third molar, while in females only in stage F of the second molars and stage D of the third molars. In the mandible, dental development in females was significantly earlier at stage G for the canines, stage E for the second premolar and stage F for the second molars. There was no statistically significant difference in the mean age within the developmental stage of lower third molars between sexes. The crypt stage was only found and evaluated in the third molars. Mean ages within the crypt stage were 7.42 ± 0.22 years and 7.49 ± 0.91 years in maxillary and 7.18 ± 0.90 years and 7.54 ± 1.08 years in mandibular third molars in males and females respectively (Tables 11 and 12). The development of the third molars completed in an extensive time frame, from 14.67 to 22.51 years and from 14.74 to 23.72 years in maxillary, and from 14.67 to 22.60 years and from 15.30 to 23.07 years in mandibular teeth, in males and females respectively (Tables 11 and 12). No significant difference was found in mean ages at most of the developmental stages between maxillary and mandibular third molars. A statistically significant difference in mean ages was found only in the maxilla at stages D and F of maxillary third molars, without a particular pattern. Mandibular third molars develop at a similar rate in both sexes.

## 5.4 Dental age calculated by the Demirjian method (1973)

The first age estimation of Batswana children between 6 to 16 years of age was done by using the original Demirjian scores from the method published in 1973. The distribution of Demirjian scores of the sample versus real age (chronologic age) was presented in Figure 10.

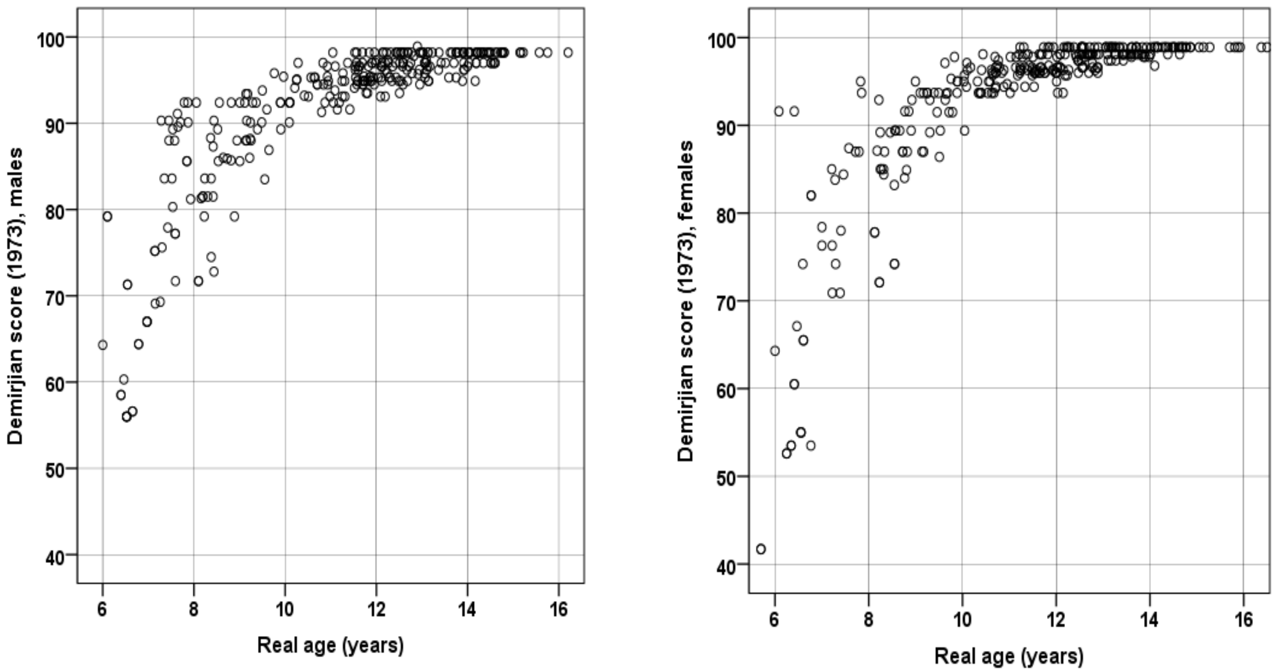



**Figure 10.** Scatterplot of the real age of the males and females versus the Demirjian maturity scores in males and females

Pearson's correlation coefficients between dental age calculated using Demirjian's maturity score and real age of the Batswana subsample between 6 to 16 years of age were 0.903 for males (n=299, p<0,001) and 0.909 for females (n=317, p<0.001) (Figure 11). Next, dental age was compared to real age and the difference and absolute differences were presented for the whole sample and across age groups. The histogram of the distribution of the difference between dental and real age showed near normal distribution, while the results of Kolmogorov-Smirnov's test of 0.067 (p=0.003) in males and 0.049 (p=0.059) in females indicate normal distribution only in females (Figure 12).

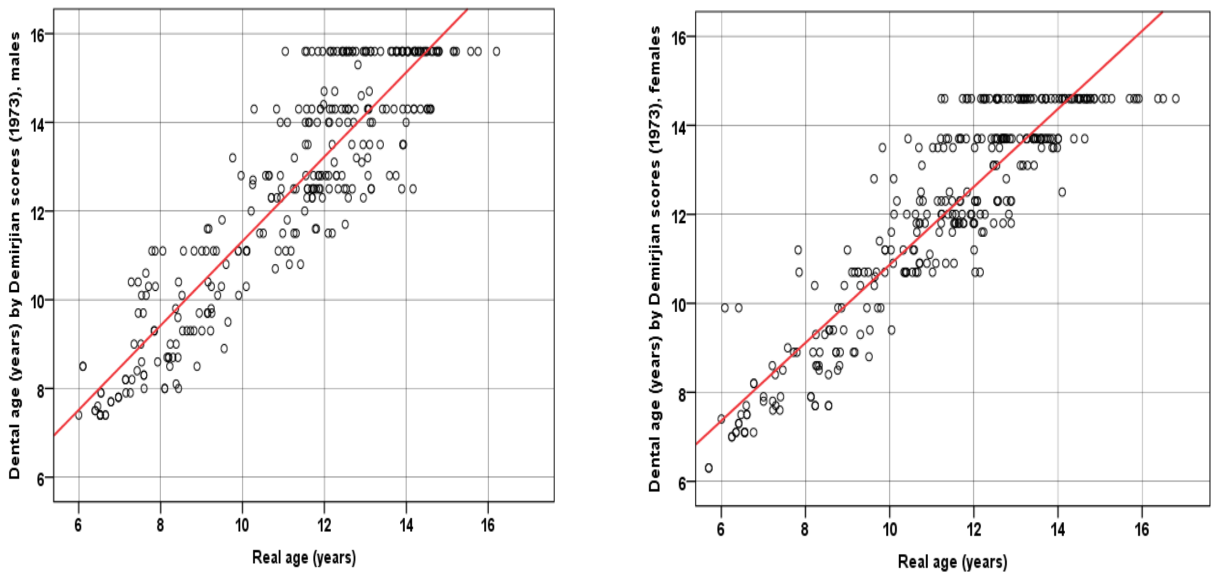



**Figure 11.** Scatterplot of actual age versus dental age calculated by the Demirjian maturity scores from 1973 in males and females

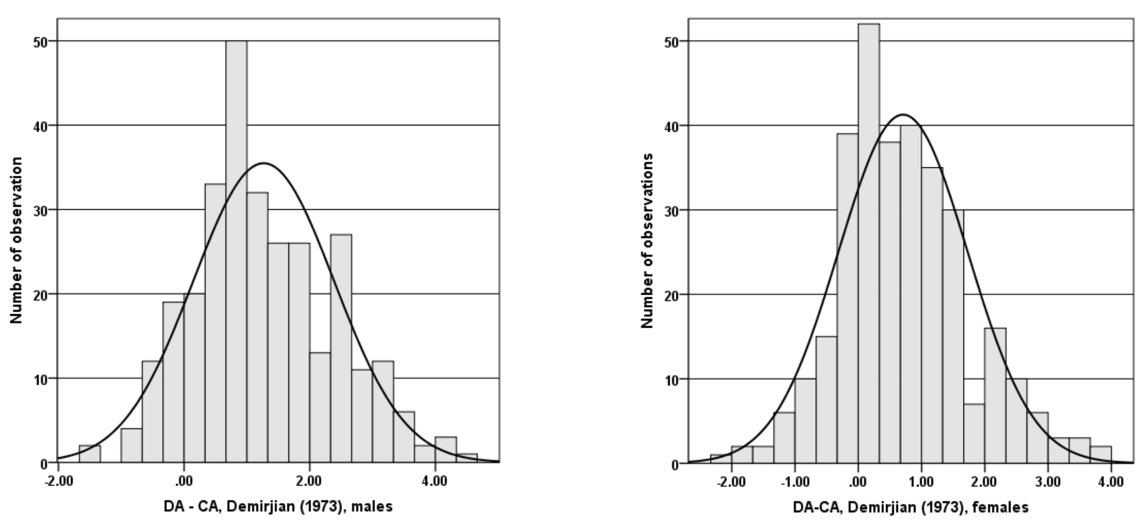



**Figure 12.** Distribution of the difference between dental age by Demirjian's method and real age (DA-CA) in Botswana males and females

The calculated dental age using Demirjian's method showed mean overestimation compared to chronological age in both sexes. When compared to real age, an overestimation was statistically significant ($p < 0.05$) in almost all age groups except in the last one of 16 years, with the mean overestimation of an average of 1.25 ± 1.11 years and 0.72 ± 1.02 years in males and females respectively (Table 13 and Figure 13).

**Table 13.** Comparison of chronological age (CA) and dental age (DA) (years) according to the Demirjian method on 299 males and 317 females of black African origin from the city of Gaborone, Republic of Botswana

| Age groups | *N* | Gender | CA±SD | DA±SD | (DA-CA) ±SD | SEM | Min | $Q_1$ | Med | $Q_3$ | Max | MAE ± SD | t(df) | Wilcoxon | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6–6.9 | 22 | M | 6.51±0.21 | 7.81±0.44 | 1.30±0.53 | 0.11 | -0.10 | 0.75 | 0.88 | 1.16 | 2.40 | 1.30±0.53 | 11.53(21) | | <0.001 |
| | 21 | F | 6.56±0.26 | 7.55±0.96 | 0.99±1.03 | 0.23 | -0.85 | 0.44 | 0.75 | 1.14 | 3.83 | 1.00±1.02 | 4.40(20) | | <0.001 |
| 7– 7.9 | 23 | M | 7.55±0.25 | 8.95±0.97 | 1.40±0.93 | 0.19 | 0.27 | 0.67 | 1.06 | 1.65 | 3.12 | 1.40±0.93 | 7.22(22) | | <0.001 |
| | 20 | F | 7.46±0.25 | 8.16±0.63 | 0.71±0.58 | 0.13 | -0.53 | 0.36 | 0.81 | 1.05 | 1.39 | 0.73±0.55 | 5.44(19) | | <0.001 |
| 8– 8.9 | 26 | M | 8.57±0.25 | 9.63±1.01 | 1.05±1.00 | 0.20 | -0.44 | 0.41 | 0.87 | 2.15 | 2.96 | 1.15±0.85 | 5.35(25) | | <0.001 |
| | 22 | F | 8.40±0.24 | 9.15±0.75 | 0.75±0.71 | 0.15 | -0.27 | 0.17 | 0.73 | 1.07 | 2.18 | 0.82±0.62 | 4.97(21) | | <0.001 |
| 9– 9.9 | 21 | M | 9.41±0.29 | 10.67±1.05 | 1.26±1.00 | 0.22 | -0.65 | 0.56 | 1.20 | 2.19 | 3.44 | 1.30±0.95 | 5.75(20) | | <0.001 |
| | 29 | F | 9.49±0.29 | 10.64±1.02 | 1.15±0.93 | 0.17 | -0.71 | 0.31 | 1.16 | 1.58 | 3.67 | 1.23±0.81 | 6.67(28) | | <0.001 |
| 10– 10.9 | 27 | M | 10.51±0.34 | 12.00±1.19 | 1.49±1.10 | 0.21 | -0.10 | 1.00 | 1.59 | 2.35 | 4.02 | 1.56±0.98 | 8.14(49) | | <0.001 |
| | 32 | F | 10.56±0.24 | 11.56±1.03 | 1.00±1.06 | 0.19 | -0.15 | 0.22 | 0.96 | 1.59 | 3.26 | 1.14±0.90 | 5.35(31) | | <0.001 |
| 11–11.9 | 50 | M | 11.60±0.27 | 13.13±1.36 | 1.53±1.31 | 0.19 | -0.62 | 0.60 | 1.01 | 2.43 | 4.56 | 1.58±1.24 | 8.23(49) | | <0.001 |
| | 48 | F | 11.50±0.27 | 12.61±1.12 | 1.10±1.11 | 0.16 | -0.63 | 0.19 | 0.71 | 2.18 | 3.37 | 1.18±1.03 | 6.87(47) | | <0.001 |
| 12–12.9 | 52 | M | 12.47±0.26 | 14.01±1.30 | 1.54±1.27 | 0.18 | -0.81 | 0.44 | 1.70 | 2.88 | 3.91 | 1.66±1.10 | 8.71(51) | | <0.001 |
| | 61 | F | 12.46±0.29 | 13.19±1.14 | 0.73±1.10 | 0.14 | -1.45 | -0.25 | 0.92 | 1.66 | 2.44 | 1.11±0.70 | 5.21(60) | | <0.001 |
| 13–13.9 | 38 | M | 13.47±0.34 | 14.50±1.14 | 1.03±1.12 | 0.18 | -1.39 | 0.14 | 1.22 | 1.87 | 2.59 | 1.32±0.74 | 5.70(37) | | <0.001 |
| | 46 | F | 13.46±0.27 | 14.04±0.54 | 0.58±0.66 | 0.10 | -0.48 | -0.01 | 0.44 | 1.28 | 1.54 | 0.68±0.55 | 6.00(45) | | <0.001 |
| 14–14.9 | 34 | M | 14.38±0.25 | 15.12±0.77 | 0.74±0.77 | 0.13 | -1.66 | 0.08 | 0.97 | 1.37 | 1.70 | 0.92±0.54 | 5.64(33) | | <0.001 |
| | 28 | F | 14.42±0.28 | 14.40±0.49 | -0.02±0.47 | 0.09 | -1.60 | -0.24 | 0.03 | 0.32 | 0.57 | 0.33±0.33 | -0.23(27) | | 0.814 |
| 15–15.9 | 5 | M | 15.37±0.27 | 15.60±0.00 | 0.23±0.27 | 0.12 | -0.15 | -0.06 | 0.38 | 0.44 | 0.45 | 0.29±0.19 | | 1.48 | 0.138 |
| | 7 | F | 15.54±0.37 | 14.60±0.00 | -0.94±0.37 | 0.14 | -1.32 | -1.27 | -1.10 | -0.54 | -0.45 | 0.94±0.37 | | 2.37 | 0.018 |
| 16–16.9 | 1 | M | 16.20 | 15.60 | -0.60 | - | - | - | - | - | - | - | - | | - |
| | 3 | F | 16.56±0.22 | 14.60±0.00 | -1.96±0.22 | 0.13 | -2.20 | | -1.90 | | -1.77 | 1.96±0.22 | -15.55(2) | 1.81 | <0.001 |
| Total | 299 | M | 11.18±2.44 | 12.44±2.59 | 1.26±1.10 | 0.06 | -1.66 | 0.51 | 1.10 | 2.04 | 4.56 | 1.36±0.96 | 19.69(298) | | <0.001 |
| | 317 | F | 11.29±2.43 | 12.01±2.34 | 0.72±1.02 | 0.06 | -2.20 | 0.03 | 0.62 | 1.31 | 3.82 | 0.96±0.80 | 12.58(316) | | <0.001 |

Note: N, number of individuals; Mean, mean age; SD, standard deviation of mean age; SEM, standard error of the mean age; Min, minimal age; $Q_1$, first quartile of age; Med, median age; $Q_3$; third quartile of age; Max, maximum age; t, a paired samples t-test; df, degree of freedom; Wilcoxon, a related samples Wilcoxon signed rank test; *p*, statistically significant if <0.05.

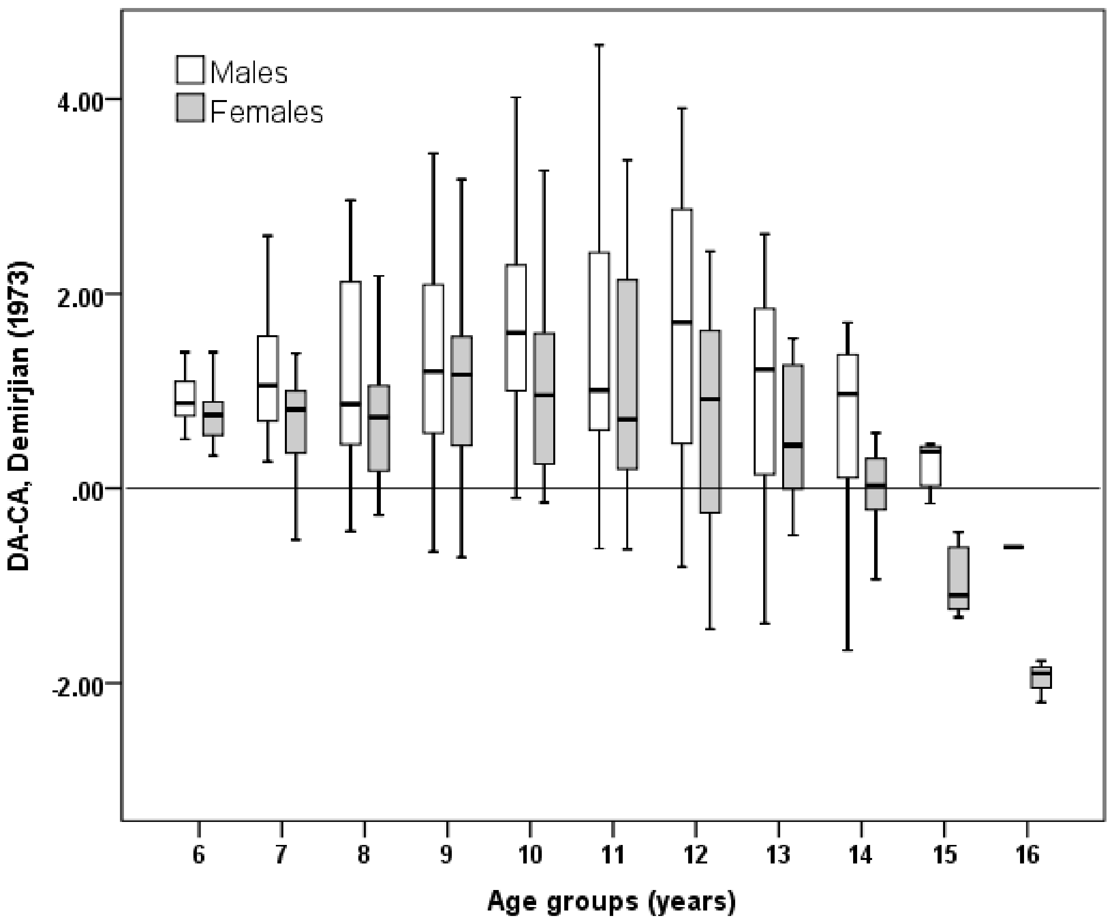



**Figure 13.** Boxplot of the differences (DA–CA), the dental age (DA) and chronological age (CA) according to the Demirjian method from 1973 for the participants between 6 and 16 years of age from Gaborone, Botswana. The boxplot shows median and interquartile range, while the whiskers indicate the range.

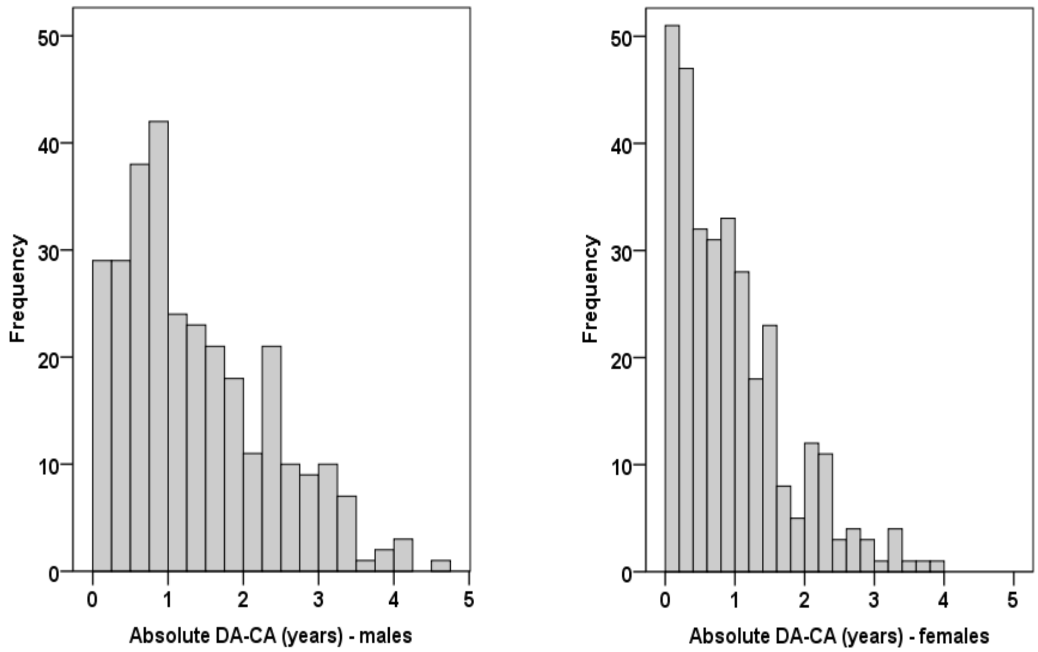



**Figure 14.** Distribution of absolute difference between dental age by the Demirjian method and chronological age for males and females

## 5.5 Dental age by the Willems method (2001)

The second method for age estimation tested on children from Botswana between 6 to 16 years was the Willems method, based on the original Demirjian stages and adopted from the Belgian sample of children. Pearson’s correlation coefficients between dental age calculated by the Willems (2001) method and real age of the Batswana subsample between 6 to 16 years of age were 0.912 for males (n=299, p<0,001) and 0.909 for females (n=317, p<0.001) (Figure 15).

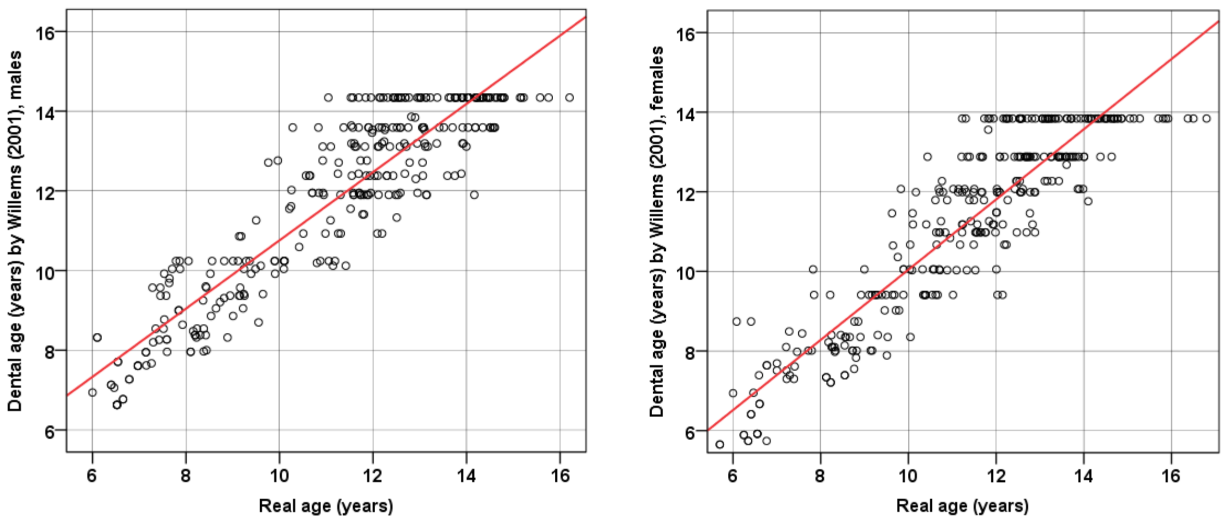



**Figure 15.** Scatterplot of actual age versus dental age calculated by the Willems (2001) method, for males and females

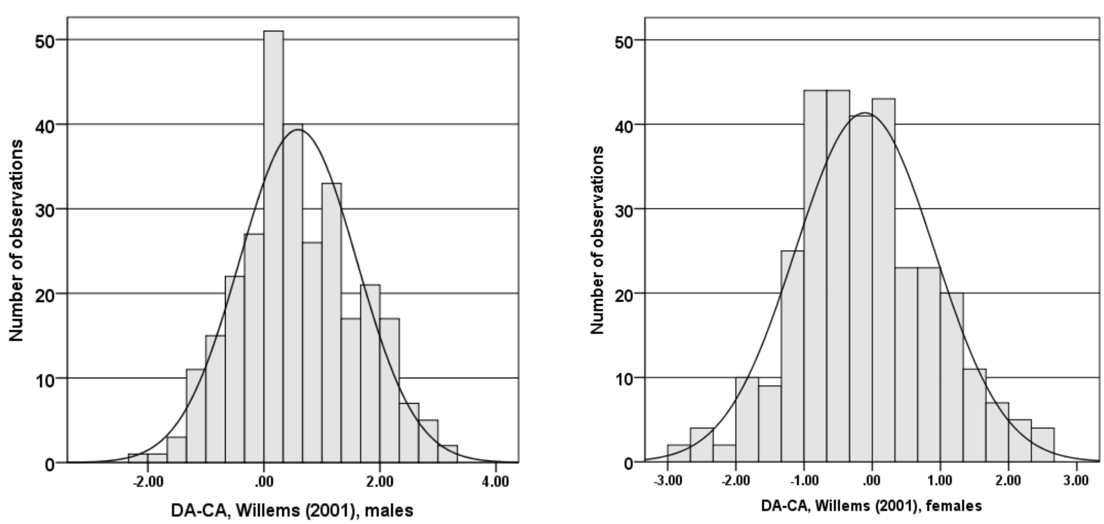



**Figure 16.** Distribution of the difference between dental age by the Willems method and real age (DA-CA) in Batswana males and females

The histogram of the distribution of the difference between dental and real age showed near normal distribution, while the results of Kolmogorov-Smirnov's test of 0.062 (p=0.008) in males and 0.050 (p=0.056) in females indicate normal distribution only in females (Figure 16). Next, dental age was compared to real age and the difference and absolute difference were presented for the whole sample of males and females and across age groups (Table 14 and Figures 17 and 18).

**Table 14.** Comparison of chronological age (CA) and dental age (DA) across different age groups according to the Willems method on 299 males and 317 females of black African origin from the city of Gaborone, the Republic of Botswana

| Age groups | *N* | Method | CA±SD | DA±SD | (DA-CA) ±SD | SEM | Min | $Q_1$ | Med | $Q_3$ | Max | MAE±SD[b] | t(df) | Wilcoxon | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6.0 – 6.9 | 22 | M | 6.51±0.21 | 7.43±0.62 | 0.92±0.69 | 0.15 | -0.14 | 0.11 | 0.48 | 0.78 | 2.22 | 0.92±0.69 | 6.23(21) | | <0.001 |
| | 21 | F | 6.56±0.26 | 6.67±1.00 | 0.11±1.11 | 0.24 | -1.16 | -0.71 | -0.36 | 0.43 | 2.66 | 0.93±0.58 | 0.46(20) | | 0.653 |
| 7.0 – 7.9 | 23 | M | 7.55±0.25 | 8.63±0.83 | 1.08±0.76 | 0.15 | 0.08 | 0.41 | 0.81 | 1.19 | 2.34 | 1.11±0.72 | 6.79(22) | | <0.001 |
| | 20 | F | 7.46±0.25 | 7.65±0.53 | 0.20±0.54 | 0.12 | -1.02 | -0.17 | 0.09 | 0.45 | 1.21 | 0.46±0.34 | 1.58(19) | | 0.129 |
| 8.0 – 8.9 | 26 | M | 8.57±0.25 | 9.26±0.80 | 0.68±0.76 | 0.15 | -0.57 | 0.08 | 0.65 | 1.49 | 2.39 | 0.83±0.59 | 4.60(25) | | <0.001 |
| | 22 | F | 8.40±0.24 | 8.29±0.59 | -0.11±0.56 | 0.12 | -1.21 | -0.71 | -0.17 | 0.30 | 1.19 | 0.44±0.34 | -0.95(21) | | 0.353 |
| 9.0 – 9.9 | 21 | M | 9.41±0.29 | 10.19±0.98 | 0.78±0.90 | 0.20 | -0.85 | 0.20 | 0.53 | 1.50 | 2.95 | 0.85±0.83 | 4(20) | | 0.001 |
| | 29 | F | 9.50±0.28 | 9.57±0.94 | 0.08±0.85 | 0.16 | -1.69 | -0.58 | 0.02 | 0.62 | 2.24 | 0.61±0.58 | 0.50(28) | | 0.622 |
| 10.0 – 10.9 | 27 | M | 10.51±0.34 | 11.40±1.22 | 0.89±1.10 | 0.21 | -0.63 | 0.17 | 1.25 | 1.81 | 3.31 | 1.11±0.87 | 4.21(26) | | <0.001 |
| | 32 | F | 10.56±0.24 | 10.67±1.12 | 0.11±1.12 | 0.20 | -1.26 | -0.81 | 0.81 | 1.15 | 2.44 | 0.93±0.61 | 0.49(31) | | 0.629 |
| 11.0 – 11.9 | 50 | M | 11.60±0.27 | 12.50±1.17 | 0.91±1.13 | 0.16 | -1.30 | 0.08 | 0.79 | 1.68 | 3.30 | 1.12±0.88 | 5.67(49) | | <0.001 |
| | 48 | F | 11.50±0.27 | 11.82±1.15- | 0.32±1.12 | 0.16 | -1.61 | -0.63 | 0.30 | 1.20 | 2.61 | 1.18±01.03 | 1.98(47) | | 0.053 |
| 12.0 – 12.9 | 52 | M | 12.47 ± 0.26 | 13.22±0.99 | 0.74±0.97 | 0.13 | -1.26 | -0.02 | 1.00 | 1.63 | 2.65 | 1.06±0.61 | 5.54(51) | | <0.001 |
| | 61 | F | 12.46±0.29 | 12.43±1.14 | -0.03±1.10 | 0.14 | -2.74 | -0.58 | 0.02 | 0.87 | 1.68 | 0.86±0.68 | -0.20(60) | | 0.840 |
| 13.0 – 13.9 | 38 | M | 13.47±0.34 | 13.62±0.79 | 0.15±0.77 | 0.13 | -1.46 | -0.33 | 0.42 | 0.61 | 1.33 | 0.65±0.42 | 1.18(37) | | 0.244 |
| | 46 | F | 13.46±0.27 | 13.21±0.63 | -0.25±0.76 | 0.11 | -1.91 | -0.84 | -0.38 | 0.52 | 0.78 | 0.67±0.41 | -2.27(45) | | 0.028 |
| 14.0 – 14.9 | 34 | M | 14.38±0.25 | 14.03±0.53 | -0.35±0.54 | 0.09 | -2.26 | -0.64 | -0.29 | 0.11 | 0.44 | 0.45±0.45 | -3.80(33) | | 0.001 |
| | 28 | F | 14.42±0.28 | 13.63±0.50 | -0.79±0.48 | 0.09 | -2.34 | -1.00 | -0.73 | -0.44 | -0.19 | 0.79±0.48 | -8.67(27) | | <0.001 |
| 15.0 – 15.9 | 5 | M | 15.37±0.27 | 14.34±0.00 | -1.15±0.31 | 0.14 | -1.41 | -1.32 | -0.88 | -0.82 | -0.81 | 1.03±0.27 | | 2.02 | 0.043 |
| | 7 | F | 15.54±0.37 | 13.84±0.00 | -1.70±0.37 | 0.14 | -2.08 | -2.03 | -1.86 | -1.30 | -1.21 | 1.70±0.37 | | 2.37 | 0.018 |
| 16.0 – 16.0 | 1 | M | 16.20 | 14.34 | -1.86 | - | - | - | - | - | - | 1.86 | - | | - |
| | 3 | F | 16.56±0.22 | 13.84±0.00 | -2.72±0.22 | 0.13 | -2.96 | | -2.66 | | -2.53 | 2.72±0.22 | - | 1.60 | <0.001 |
| 6.0 – 16.0 | 299 | M | 11.18±2.48 | 11.76±2.31 | 0.58±1.01 | 0.06 | -2.26 | -0.05 | 0.48 | 1.27 | 3.31 | 0.91±0.71 | 10.03(298) | | <0.001 |
| | 317 | F | 11.28±2.43 | 11.19±2.35 | -0.10±1.02 | 0.06 | -2.96 | -0.78 | -0.19 | 0.54 | 2.66 | 0.81±0.62 | -1.67(316) | | 0.095 |

Note: N, number of individuals; Mean, mean age; SD, standard deviation of mean age; SEM, standard error of the mean age; Min, minimal age; $Q_1$, first quartile of age; Med, median age; $Q_3$; third quartile of age; Max, maximum age; t, a paired samples t-test; df, degree of freedom; Wilcoxon, a related samples Wilcoxon signed rank test; *p*, statistically significant if <0.05.

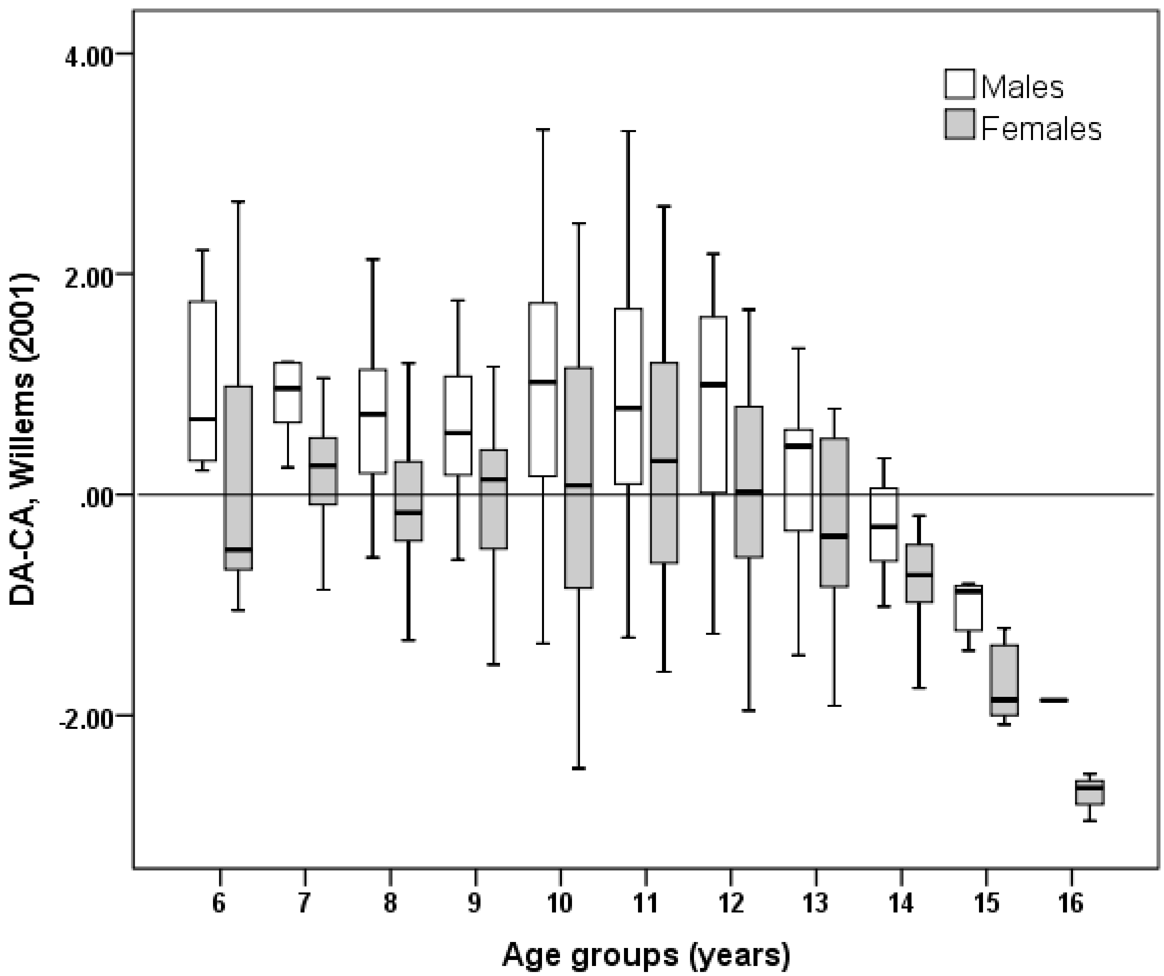



**Figure 17.** Boxplot of the differences (DA–CA), the dental age (DA) and chronological age (CA) according to the Willems method from 2001 for participants between 6 and 16 years of age from Gaborone, Botswana. The boxplot shows median and interquartile range, while the whiskers indicate the range.

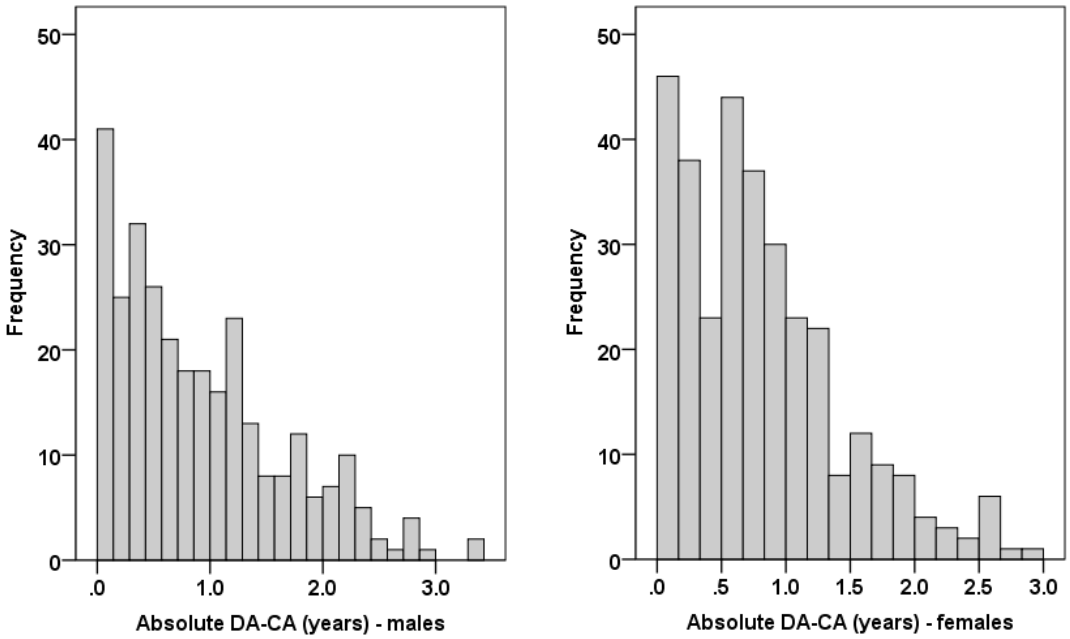



**Figure 18.** Distribution of absolute difference between dental age by the Willems method and chronological age for males and females

## 5.6 Dental age by the Cameriere European formula (2007)

The third method for age estimation tested on Batswana children between 6 to 16 years of age was the Cameriere method using the European formula, based on the linear regression analysis of the open apices of permanent teeth, originally based on the sample from Croatia, Germany, Kosovo, Italy, Slovenia, Spain and the UK. Pearson's correlation coefficients between dental age calculated by the Cameriere (2007) method and real age of the Batswana subsample between 6 to 16 years of age were 0.882 for males (n=299, p<0,001) and 0.886 for females (n=317, p<0.001) (Figure 19).

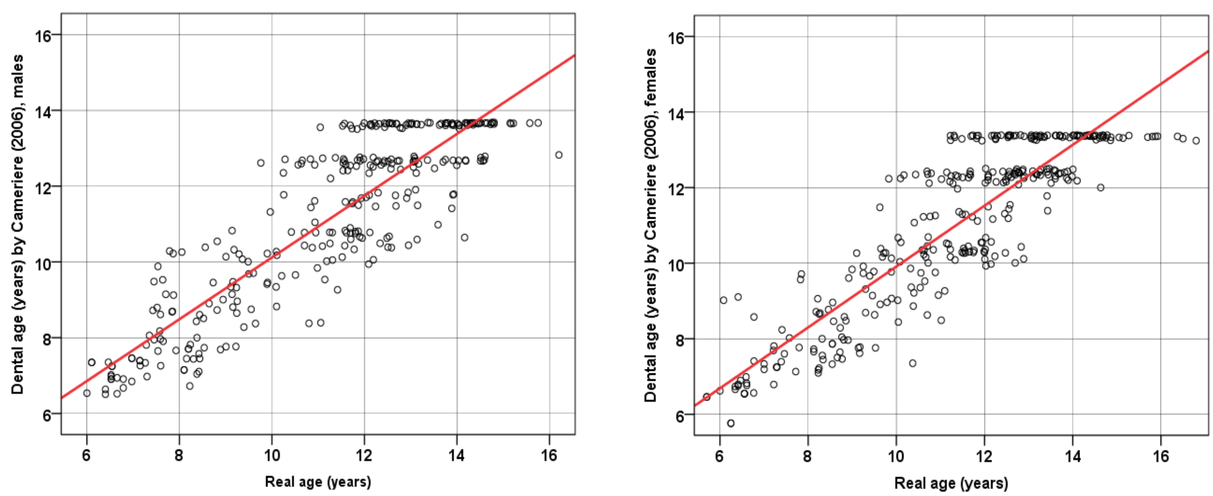



**Figure 19.** Scatterplot of actual age versus dental age calculated by the Cameriere (2007) method, for males and females

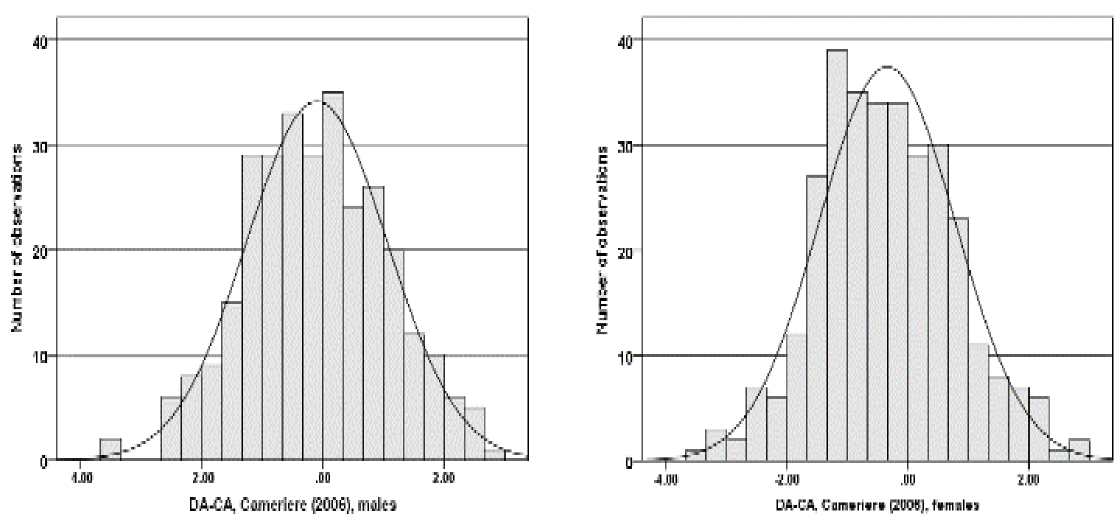



**Figure 20.** Distribution of the difference between dental age by the Cameriere (2007) method and real age (DA-CA) in Batswana males and females

The histogram of the distribution of the difference between dental age by Cameriere (2007) and real age showed near normal distribution, while the results of Kolmogorov-Smirnov's test of 0.062 ($p=0.008$) in males and 0.050 ($p=0.056$) in females indicate normal distribution only in females (Figure 20). Next, dental age was compared to real age and the difference and absolute difference was presented for the whole sample of males and females and across age groups (Table 15 and Figures 21 and 22).

**Table 15.** Comparison of chronological age (CA) and dental age (DA) across different age groups according to the Cameriere (2007) method of 299 males and 317 females of black African origin from the city of Gaborone, Republic of Botswana

| Age groups | *N* | Method | CA±SD | DA±SD | (DA-CA) ±SD | SEM | Min | $Q_1$ | Med | $Q_3$ | Max | MAE±SD | t(df) | Wilcoxon | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6.0 – 6.9 | 22 | M | 6.51±0.21 | 7.05±0.36 | 0.55±0.44 | 0.09 | -0.95 | -1.28 | 0.34 | 0.50 | 1.24 | 0.57±0.41 | 5.83(21) | | <0.001 |
| | 21 | F | 6.56±0.26 | 7.12±0.95 | 0.56±1.06 | 0.23 | -0.68 | -0.43 | -0.32 | 0.70 | 2.94 | 0.83±0.85 | 2.42(20) | | 0.025 |
| 7.0 – 7.9 | 23 | M | 7.55±0.25 | 8.10±0.93 | 0.55±0.86 | 0.18 | -1.50 | -0.26 | 0.37 | 1.02 | 2.03 | 0.75±0.68 | 3.09(22) | | 0.005 |
| | 20 | F | 7.45±0.25 | 7.50±0.64 | 0.05±0.62 | 0.14 | -1.13 | -0.35 | 0.08 | 0.40 | 0.83 | 0.46±0.41 | 0.34(19) | | 0.737 |
| 8.0 – 8.9 | 26 | M | 8.57±0.25 | 8.62±1.05 | 0.05±1.06 | 0.21 | -1.45 | -0.77 | 0.06 | 0.83 | 2.35 | 0.87±0.58 | 0.24(25) | | 0.815 |
| | 22 | F | 8.40±0.24 | 8.08±0.64 | -0.32±0.65 | 0.14 | -1.55 | -0.97 | -0.41 | 0.42 | 0.84 | 0.62±0.36 | -2.33(21) | | 0.030 |
| 9.0 – 9.9 | 21 | M | 9.41±0.29 | 9.57±1.13 | 0.16±1.09 | 0.24 | -1.27 | -0.61 | 0.18 | 0.81 | 2.85 | 0.84±0.69 | 0.66(20) | | 0.517 |
| | 29 | F | 9.49±0.29 | 9.64±0.97 | 0.15±0.91 | 0.17 | -1.77 | -0.77 | 0.27 | 0.70 | 2.41 | 0.72±0.55 | 0.85(28) | | 0.401 |
| 10.0 – 10.9 | 27 | M | 10.51±0.34 | 10.77±1.45 | 0.25±1.37 | 0.26 | -2.42 | -0.50 | 0.17 | 1.95 | 2.42 | 1.08±0.86 | 0.95(26) | | 0.325 |
| | 32 | F | 10.56±0.24 | 10.38±1.39 | -0.18±1.40 | 0.25 | -3.02 | -1.13 | -0.33 | 0.65 | 2.16 | 1.13±0.82 | -0.77(31) | | 0.446 |
| 11.0 – 11.9 | 50 | M | 11.60±0.27 | 11.67±1.34 | 0.07±1.28 | 0.18 | -2.62 | -1.05 | -0.17 | 1.08 | 2.51 | 1.13±0.58 | 0.40(49) | | 0.692 |
| | 48 | F | 11.50±0.27 | 11.59±1.32 | 0.08±1.31 | 0.19 | -2.53 | -1.21 | 0.26 | 1.25 | 2.15 | 1.19±0.53 | 0.43(47) | | 0.668 |
| 12.0 – 12.9 | 52 | M | 12.47±0.26 | 12.45±1.16 | -0.02±1.15 | 0.16 | -2.51 | -0.88 | 0.22 | 1.02 | 1.91 | 0.94±0.66 | -0.14(51) | | 0.891 |
| | 61 | F | 12.46±0.29 | 12.09±1.10 | -0.37±1.06 | 0.14 | -2.77 | -1.07 | -0.29 | 0.52 | 1.21 | 0.87±0.70 | -2.69(60) | | 0.009 |
| 13.0 – 13.9 | 38 | M | 13.47±0.34 | 12.88±0.87 | -0.59±0.88 | 0.14 | -2.61 | -1.05 | -0.38 | -0.01 | 0.64 | 0.75±0.75 | -4.10(37) | | <0.001 |
| | 46 | F | 13.46±0.27 | 12.78±0.56 | -0.68±0.66 | 0.10 | -2.04 | -1.30 | -0.80 | -0.01 | 0.33 | 0.76±0.57 | -6.99(45) | | <0.001 |
| 14.0 – 14.9 | 34 | M | 14.38±0.25 | 13.30±0.64 | -1.07±0.64 | 0.11 | -3.52 | -1.46 | -0.89 | -0.61 | -0.24 | 1.07±0.64 | -9.79(33) | | <0.001 |
| | 28 | F | 14.42±0.28 | 13.23±0.37 | -1.18±0.43 | 0.08 | -2.63 | -1.38 | -1.13 | -0.82 | -0.62 | 1.18±0.43 | -14.51(27) | | <0.001 |
| 15.0 – 15.9 | 5 | M | 15.37±0.27 | 13.66±0.01 | -1.71±0.27 | 0.12 | -2.09 | -1.99 | -1.56 | -1.50 | -1.48 | 1.71±0.27 | - | 2.02 | 0.043 |
| | 7 | F | 15.54±0.37 | 13.34±0.02 | -2.19±0.36 | 0.14 | -2.57 | -2.51 | -2.36 | -1.83 | -1.72 | 2.19±0.36 | - | 2.37 | 0.018 |
| 16.0 –16.0 | 1 | M | 16.20 | 12.82 | -3.38 | - | - | - | - | - | | 3.38 | - | | - |
| | 3 | F | 16.56±0.22 | 13.30±0.06 | -3.26±0.27 | 0.16 | -3.56 | - | -3.20 | - | -3.02 | 3.26±0.27 | -20.64(2) | 1.60 | 0.109 |
| 6.0 – 16.0 | 299 | M | 11.18±2.47 | 11.07±2.27 | -0.11±1.16 | 0.07 | -3.52 | -0.93 | -0.12 | 0.71 | 2.85 | 0.94±0.69 | -1.61(298) | | 0.110 |
| | 317 | F | 11.29±2.43 | 10.96±2.20 | -0.33±1.14 | 0.06 | -3.56 | -1.13 | -0.38 | 0.44 | 2.94 | 0.95±0.70 | -5.15(316) | | <0.001 |

Note: N, number of individuals; Mean, mean age; SD, standard deviation of mean age; SEM, standard error of the mean age; Min, minimal age; $Q_1$, first quartile of age; Med, median age; $Q_3$; third quartile of age; Max, maximum age; t, a paired samples t-test; df, degree of freedom; Wilcoxon, a related samples Wilcoxon signed rank test; *p*, statistically significant if <0.05.

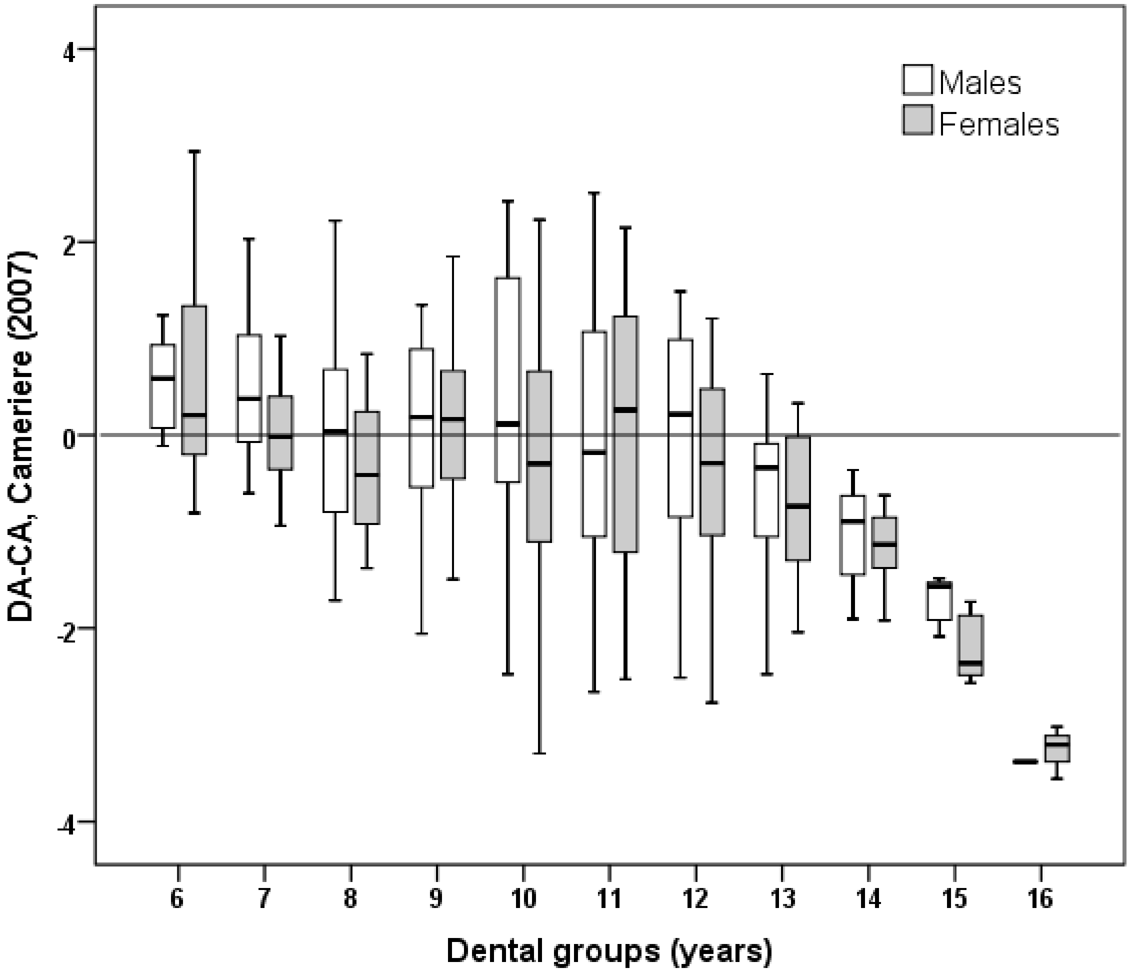



**Figure 21.** Boxplot of the differences (DA–CA), the dental age (DA) and chronological age (CA) according to the Cameriere method from 2007 for participants between 6 and 16 years of age from Gaborone, Botswana. The boxplot shows the median and interquartile range, while the whiskers indicate the range.

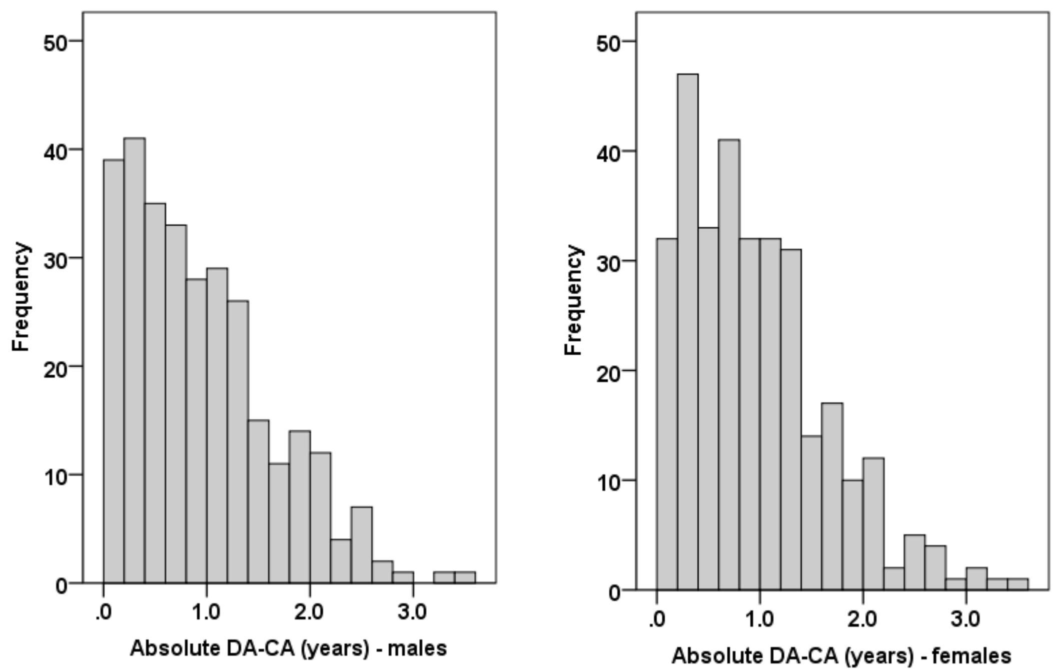



**Figure 22.** Distribution of absolute difference between dental age by the Cameriere method from 2007 and chronological age for males and females

## 5.7 Comparison of the Demirjian, Willems and Cameriere age estimation methods in Botswana children

The comparison of the accuracy of the methods was estimated as the difference between DA and CA or DA-CA. In the whole sample, we found significant differences between DA and CA (DA-CA) for the Demirjian method, the Willems method for males and the Cameriere method for females ($p < 0.05$). In males, the smallest DA-CA was found in the Cameriere method (-0.11 ± 01.16 years), followed by the Willems (0.58 ± 1.00 years) and the Demirjian (1.26 ± 1.10 years) methods respectively. In females, the smallest DA-CA was found in the Willems method (−0.10 ± 1.02 years), followed by the Cameriere (−0.33 ± 1.14 years) and Demirjian (0.72 ± 1.02 years) methods (Table 16).

**Table 16.** Comparison of chronological age (CA) and dental age (DA) according to Demirjian. The Willems and Cameriere methods of 299 males and 317 females of black African origin from the city of Gaborone, Republic of Botswana.

| Gender | *N* | Method | CA±SD | DA±SD | (DA-CA) ±SD | SEM | L | U | MAE ± SD | t(df) | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Males | 299 | Demirjian | 11.18±2.47 | 12.44±2.59 | 1.26±1.10 | 0.06 | 1.13 | 1.38 | 1.36±0.96 | 19.69(298) | <0.001 |
| | | Willems | | 11.76±2.31 | 0.58±1.00 | 0.06 | 0.47 | 0.70 | 0.91±0.71 | 10.03(298) | <0.001 |
| | | Cameriere | | 11.07±2.27 | -0.11±1.16 | 0.07 | -0.24 | 0.02 | 0.94±0.69 | -1.61(298) | 0.109 |
| Females | 317 | Demirjian | 11.29±2.43 | 12.01±2.34 | 0.72±1.02 | 0.06 | 0.61 | 0.83 | 0.96±0.80 | 12.58(316) | <0.001 |
| | | Willems | | 11.19±2.35 | -0.10±1.02 | 0.06 | -0.21 | 0.02 | 0.81±0.62 | -1.70(316) | 0.090 |
| | | Cameriere | | 10.96±2.20 | -0.33±1.14 | 0.06 | -0.46 | -0.20 | 0.95±0.70 | -5.16(316) | <0.001 |

Note: N, number of individuals; DA-CA, the difference between dental and chronological age; SEM, standard error of the mean age; L, lower interval; U, upper interval of 95% Confidence Interval of DA-CA; SD, standard deviation; MAE, mean absolute error between dental and chronological age; t, paired samples t-test between DA and CA; df, degrees of freedom; *P*, statistically significant if < 0.05.

Repeated measures ANOVA were performed to test the differences of DA-CA among the Demirjian, Willems and Cameriere methods in males and females. Mauchly's test indicated that the assumption of sphericity had been violated, $\chi^2$ (2)=73.04, p < 0.001 and $\chi^2$(2)=161.33, p <0.001 in males and females respectively. Therefore, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (ε=0.82 and ε=0.71 in males and females respectively). The results of repeated measures ANOVA for the within-subjects' variable shows that there was a significant difference of DA-CA among the three tested methods (p < 0.001) in males and females respectively (Table 17). Post-hoc pairwise comparisons, after Bonferroni adjustment, showed that there was a statistically significant difference of DA-CA between two different methods in both sexes (p<0.001).

The comparison of the absolute accuracy of the methods was estimated as the absolute difference between DA and CA or mean absolute error. The smallest mean absolute error or MAE was found for the Willems method, 0.91±0.71 years in males and 0.81±0.62 years in females. The greatest MAE was for the Demirjian method, 1.36±0.96 years in males and 0.96±0.80 years in females. Next, repeated measures ANOVA was also performed to test the differences of MAE among the Demirjian, Willems and Cameriere methods in males and females. Mauchly's test indicated that the assumption of sphericity had been violated, $\chi^2$ (2)=299.00, p <0.001 and $\chi^2$(2)=188.38, p <0.001 in males and females respectively. Therefore, degrees of freedom were corrected using

Greenhouse-Geisser estimates of sphericity (ε=0.61 and ε=0.69 in males and females respectively). The results of repeated measures ANOVA for the within-subjects variable shows that there was a significant difference of MAE among the Demirjian, Willems and Cameriere methods ($p < 0.001$) in males and females respectively (Table 17). Post-hoc pairwise comparisons, after Bonferroni adjustment, showed that there was a statistically significant difference of MAE only between the Demirjian and Willems methods (0.45 years, <0.001) and Demirjian and Cameriere (0.43 years, <0.001) in males and between the Demirjian and Willems methods (0.14 years, <0.001), the Willems and Cameriere methods (0.14 years, <0.001) in females.

**Table 17.** The summary of repeated measures ANOVA for the within-subjects' variables DA-CA and MAE of the Demirjian, Willems and Cameriere methods

| Gender | Source | Type III Sum of Squares | Degrees of freedom | Mean Square | F | *P* |
|---|---|---|---|---|---|---|
| Males | DA-CA | 278.81 | 1.64 | 169.80 | 1022.94 | <0.001 |
| | Error | 81.22 | 489.32 | 0.17 | | |
| Females | DA-CA | 192.141 | 1.43 | 134.58 | 798.49 | <0.001 |
| | Error | 76.04 | 451.17 | 0.17 | | |
| Males | MAE | 38.91 | 1.22 | 31.80 | 54.65 | <0.001 |
| | Error | 212.15 | 364.62 | 0.58 | | |
| Females | MAE | 4.15 | 1.38 | 3.01 | 7.25 | 0.003 |
| | Error | 180.87 | 435.83 | 0.41 | | |

Note: DA-CA, the difference between dental and chronological age; MAE, the absolute difference between dental and chronological age.

Tables 18 and 19 compare the data of dental age, DA-CA and MAE across age groups. The relationship between age groups and DA-CA of the Demirjian, Willems and Cameriere methods were presented in Figures 23 and 24.

**Table 18.** Comparison of chronological age (CA) and dental age (DA) across different age groups according to the Demirjian, Willems and Cameriere methods of 299 black African males from the city of Gaborone, Botswana

| Age groups | *N* | Method | CA±SD | DA±SD | (DA-CA)±SD | SEM | Min | $Q_1$ | Med | $Q_3$ | Max | MAE±SD | t(df) | Wilcoxon | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6.0 – 6.9 | 22 | Demirjian | 6.45±0.22 | 7.77±0.39 | 1.33±0.44 | 0.11 | -0.10 | 0.75 | 0.88 | 1.16 | 2.40 | 1.33±0.44 | 14.12(21) | | <0.001 |
| | | Willems | | 7.43±0.62 | 0.98±0.68 | 0.15 | -0.14 | 0.11 | 0.48 | 0.78 | 2.22 | 0.98±0.68 | 6.75(21) | | <0.001 |
| | | Cameriere | | 7.05±0.36 | 0.61±0.41 | 0.09 | -0.95 | -1.28 | 0.34 | 0.50 | 1.24 | 0.62±0.39 | 7.03(21) | | <0.001 |
| 7.0 – 7.9 | 23 | Demirjian | 7.55±0.25 | 8.82±0.91 | 1.26±0.85 | 0.19 | 0.27 | 0.67 | 1.06 | 1.65 | 3.12 | 1.26±0.85 | 7.09(22) | | <0.001 |
| | | Willems | | 8.63±0.83 | 1.07±0.74 | 0.15 | 0.08 | 0.41 | 0.81 | 1.19 | 2.34 | 1.10±0.69 | 6.95(22) | | <0.001 |
| | | Cameriere | | 8.10±0.93 | 0.55±0.86 | 0.18 | -1.50 | -0.26 | 0.37 | 1.02 | 2.03 | 0.75±0.68 | 3.04(22) | | 0.006 |
| 8.0 – 8.9 | 26 | Demirjian | 8.57±0.25 | 9.62±1.03 | 1.05±1.00 | 0.19 | -0.44 | 0.41 | 0.87 | 2.15 | 2.96 | 1.18±0.83 | 5.35(25) | | <0.001 |
| | | Willems | | 9.26±0.80 | 0.68±0.76 | 0.15 | -0.57 | 0.08 | 0.65 | 1.49 | 2.39 | 0.83±0.59 | 4.60(25) | | <0.001 |
| | | Cameriere | | 8.62±1.05 | 0.05±1.06 | 0.21 | -1.45 | -0.77 | 0.06 | 0.83 | 2.35 | 0.87±0.58 | 0.24(25) | | 0.815 |
| 9.0 – 9.9 | 21 | Demirjian | 9.41±0.29 | 10.66±1.05 | 1.25±1.00 | 0.22 | -0.65 | 0.56 | 1.20 | 2.19 | 3.44 | 1.29±0.94 | 5.75(20) | | <0.001 |
| | | Willems | | 10.19±0.98 | 0.78±0.90 | 0.20 | -0.85 | 0.20 | 0.53 | 1.50 | 2.95 | 0.85±0.83 | 4(20) | | 0.001 |
| | | Cameriere | | 9.57±1.13 | 0.16±1.09 | 0.24 | -1.27 | -0.61 | 0.18 | 0.81 | 2.85 | 0.84±0.69 | 0.66(20) | | 0.517 |
| 10.0 – 10.9 | 27 | Demirjian | 10.51±0.34 | 12.00±1.19 | 1.49±1.10 | 0.21 | -0.10 | 1.00 | 1.59 | 2.35 | 4.02 | 1.56±0.98 | 7.03(26) | | <0.001 |
| | | Willems | | 11.40±1.22 | 0.89±1.10 | 0.21 | -0.63 | 0.17 | 1.25 | 1.81 | 3.31 | 1.11±0.87 | 4.21(26) | | <0.001 |
| | | Cameriere | | 10.77±1.45 | 0.25±1.37 | 0.26 | -2.42 | -0.50 | 0.17 | 1.95 | 2.42 | 1.08±0.86 | 0.95(26) | | 0.325 |
| 11.0 – 11.9 | 50 | Demirjian | 11.59±0.27 | 13.13±1.36 | 1.54±1.34 | 0.19 | -0.62 | 0.60 | 1.01 | 2.43 | 4.56 | 1.60±1.27 | 8.14(49) | | <0.001 |
| | | Willems | | 12.50±1.17 | 0.91±1.13 | 0.16 | -1.30 | 0.08 | 0.79 | 1.68 | 3.30 | 1.13±0.91 | 5.67(49) | | <0.001 |
| | | Cameriere | | 11.24±3.52 | -0.35±3.46 | 0.49 | -2.62 | -1.05 | -0.17 | 1.08 | 2.51 | 1.58±3.09 | -0.72(49) | | 0.475 |
| 12.0 – 12.9 | 52 | Demirjian | 12.48±0.26 | 14.01±1.30 | 1.54±1.27 | 0.18 | -0.81 | 0.44 | 1.70 | 2.88 | 3.91 | 1.66±1.10 | 8.71(51) | | <0.001 |
| | | Willems | | 13.22±0.99 | 0.74±0.97 | 0.13 | -1.26 | -0.02 | 1.00 | 1.63 | 2.65 | 1.06±0.61 | 5.54(51) | | <0.001 |
| | | Cameriere | | 12.45±1.16 | -0.02±1.15 | 0.16 | -2.51 | -0.88 | 0.22 | 1.02 | 1.91 | 0.94±0.66 | -0.14(51) | | 0.891 |
| 13.0 – 13.9 | 38 | Demirjian | 13.47±0.34 | 14.50±1.14 | 1.03±1.12 | 0.18 | -1.39 | 0.14 | 1.22 | 1.87 | 2.59 | 1.32±0.74 | 5.70(37) | | <0.001 |
| | | Willems | | 13.62±0.79 | 0.15±0.77 | 0.13 | -1.46 | -0.33 | 0.42 | 0.61 | 1.33 | 0.66±0.42 | 1.18(37) | | 0.244 |
| | | Cameriere | | 12.88±0.87 | -0.59±0.88 | 0.14 | -2.61 | -1.05 | -0.38 | -0.01 | 0.64 | 0.74±0.75 | -4.10(37) | | <0.001 |
| 14.0 – 14.9 | 34 | Demirjian | 14.39±0.26 | 15.12±0.77 | 0.73±0.76 | 0.13 | -1.66 | 0.08 | 0.97 | 1.37 | 1.70 | 0.91±0.54 | 5.59(33) | | <0.001 |
| | | Willems | | 14.03±0.53 | -0.35±0.54 | 0.09 | -2.26 | -0.64 | -0.29 | 0.11 | 0.44 | 0.46±0.45 | -3.80(33) | | 0.001 |
| | | Cameriere | | 13.30±0.64 | -1.08±0.64 | 0.11 | -3.52 | -1.46 | -0.89 | -0.61 | -0.24 | 1.08±0.64 | -9.90(33) | | <0.001 |
| 15.0 – 15.9 | 5 | Demirjian | 15.49±0.31 | 15.60±0.00 | 0.11±0.31 | 0.14 | -0.15 | -0.06 | 0.38 | 0.44 | 0.45 | 0.25±0.19 | | 1.48 | 0.138 |
| | | Willems | | 14.34±0.00 | -1.15±0.31 | 0.14 | -1.41 | -1.32 | -0.88 | -0.82 | -0.81 | 1.15±0.31 | | 2.02 | 0.043 |
| | | Cameriere | | 13.66±0.01 | -1.83±0.31 | 0.14 | -2.09 | -1.99 | -1.56 | -1.50 | -1.48 | 1.83±0.31 | | 2.02 | 0.043 |
| 16.0 –16.0 | 1 | Demirjian | 16.20 | 15.60 | 0.60 | - | - | - | - | - | - | 0.60 | - | - | - |
| | | Willems | | 14.34 | -1.86 | - | - | - | - | - | - | 1.86 | - | - | - |
| | | Cameriere | | 12.82 | -3.38 | - | - | - | - | - | - | 3.38 | - | - | - |

Note: N, number of individuals; Mean, mean age; SD, standard deviation of mean age; SEM, standard error of the mean age; Min, minimal age; $Q_1$, first quartile of age; Med, median age; $Q_3$; third quartile of age; Max, maximum age; t, a paired samples t-test; df, degree of freedom; Wilcoxon, a related samples Wilcoxon signed rank test; *p*, statistically significant if <0.05.

**Table 19.** Comparison of chronological age (CA) and dental age (DA) across different age groups according to the Demirjian, Willems and Cameriere methods of 317 black African females from the city of Gaborone, Botswana

| Age groups | *N* | Method | CA±SD | DA±SD | (DA-CA) ±SD | SEM | Min | $Q_1$ | Med | $Q_3$ | Max | MAE±SD | t(df) | Wilcoxon | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6.0 – 6.9 | 21 | Demirjian | 6.56±0.26 | 7.55±0.96 | 0.99±1.07 | 0.23 | -0.85 | 0.44 | 0.75 | 1.14 | 3.83 | 1.00±1.06 | 4.25(20) | | <0.001 |
| | | Willems | | 6.67±1.00 | 0.11±1.11 | 0.24 | -1.16 | -0.71 | -0.36 | 0.43 | 2.66 | 0.93±0.58 | 0.46(20) | | 0.653 |
| | | Cameriere | | 7.12±0.95 | 0.56±1.06 | 0.23 | -0.68 | -0.43 | -0.32 | 0.70 | 2.94 | 0.83±0.85 | 2.42(20) | | 0.025 |
| 7.0 – 7.9 | 20 | Demirjian | 7.46±0.25 | 8.21±0.62 | 0.75±0.57 | 0.13 | -0.53 | 0.36 | 0.81 | 1.05 | 1.39 | 0.78±0.53 | 5.92(19) | | <0.001 |
| | | Willems | | 7.65±0.53 | 0.19±0.54 | 0.12 | -1.02 | -0.17 | 0.09 | 0.45 | 1.21 | 0.45±0.34 | 1.58(19) | | 0.129 |
| | | Cameriere | | 7.50±0.64 | 0.04±0.62 | 0.14 | -1.13 | -0.35 | 0.08 | 0.40 | 0.83 | 0.46±0.41 | 0.31(19) | | 0.758 |
| 8.0 – 8.9 | 22 | Demirjian | 8.40±0.24 | 9.10±0.69 | 0.70±0.65 | 0.14 | -0.27 | 0.17 | 0.73 | 1.07 | 2.18 | 0.77±0.56 | 5.01(21) | | <0.001 |
| | | Willems | | 8.29±0.59 | -0.11±0.56 | 0.12 | -1.21 | -0.71 | -0.17 | 0.30 | 1.19 | 0.44±0.34 | -0.95(21) | | 0.353 |
| | | Cameriere | | 8.08±0.64 | -0.32±0.65 | 0.14 | -1.55 | -0.97 | -0.41 | 0.42 | 0.84 | 0.62±0.36 | -2.33(21) | | 0.030 |
| 9.0 – 9.9 | 29 | Demirjian | 9.50±0.28 | 10.64±1.02 | 1.15±0.93 | 0.17 | -0.71 | 0.31 | 1.16 | 1.58 | 3.67 | 1.23±0.80 | 6.67(28) | | <0.001 |
| | | Willems | | 9.57±0.94 | 0.08±0.85 | 0.16 | -1.69 | -0.58 | 0.02 | 0.62 | 2.24 | 0.61±0.58 | 0.50(28) | | 0.622 |
| | | Cameriere | | 9.64±0.97 | 0.14±0.91 | 0.17 | -1.77 | -0.77 | 0.27 | 0.70 | 2.41 | 0.73±0.56 | 0.85(28) | | 0.401 |
| 10.0 – 10.9 | 32 | Demirjian | 10.56±0.24 | 11.56±1.03 | 1.00±1.06 | 0.19 | -0.15 | 0.22 | 0.96 | 1.59 | 3.26 | 1.14±0.90 | 5.35(31) | | <0.001 |
| | | Willems | | 10.67±1.12 | 0.10±1.13 | 0.20 | -1.26 | -0.81 | 0.81 | 1.15 | 2.44 | 0.93±0.61 | 0.49(31) | | 0.629 |
| | | Cameriere | | 10.38±1.39 | -0.19±1.41 | 0.25 | -3.02 | -1.13 | -0.33 | 0.65 | 2.16 | 1.13±0.82 | -0.77(31) | | 0.446 |
| 11.0 – 11.9 | 48 | Demirjian | 11.50±0.27 | 12.61±1.12 | 1.10±1.11 | 0.16 | -0.63 | 0.19 | 0.71 | 2.18 | 3.37 | 1.18±1.03 | 6.87(47) | | <0.001 |
| | | Willems | | 11.82±1.15 | 0.32±1.12 | 0.16 | -1.61 | -0.63 | 0.30 | 1.20 | 2.61 | 0.96±0.64 | 1.98(47) | | 0.053 |
| | | Cameriere | | 11.59±1.32 | 0.08±1.31 | 0.19 | -2.53 | -1.21 | 0.26 | 1.25 | 2.15 | 1.19±0.53 | 0.43(47) | | 0.668 |
| 12.0 – 12.9 | 61 | Demirjian | 12.46±0.29 | 13.19±1.14 | 0.73±1.10 | 0.14 | -1.45 | -0.25 | 0.92 | 1.66 | 2.44 | 1.11±0.71 | 5.21(60) | | <0.001 |
| | | Willems | | 12.43±1.14 | -0.02±1.10 | 0.14 | -2.74 | -0.58 | 0.02 | 0.87 | 1.68 | 0.86±0.68 | -0.17(60) | | 0.863 |
| | | Cameriere | | 12.09±1.10 | -0.37±1.06 | 0.14 | -2.77 | -1.07 | -0.29 | 0.52 | 1.21 | 0.87±0.70 | -2.69(60) | | 0.009 |
| 13.0 – 13.9 | 46 | Demirjian | 13.46±0.27 | 14.04±0.54 | 0.58±0.66 | 0.10 | -0.48 | -0.01 | 0.44 | 1.28 | 1.54 | 0.68±0.55 | 6.00(45) | | <0.001 |
| | | Willems | | 13.21±0.63 | -0.25±0.76 | 0.11 | -1.91 | -0.84 | -0.38 | 0.52 | 0.78 | 0.68±0.41 | -2.27(45) | | 0.028 |
| | | Cameriere | | 12.78±0.56 | -0.68±0.66 | 0.10 | -2.04 | -1.30 | -0.80 | -0.01 | 0.33 | 0.76±0.57 | -6.99(45) | | <0.001 |
| 14.0 – 14.9 | 28 | Demirjian | 14.42±0.28 | 14.40±0.49 | -0.02±0.47 | 0.09 | -1.60 | -0.24 | 0.03 | 0.32 | 0.57 | 0.32±0.33 | -0.23(27) | | 0.814 |
| | | Willems | | 13.63±0.50 | -0.79±0.48 | 0.09 | -2.34 | -1.00 | -0.73 | -0.44 | -0.19 | 0.46±0.45 | -8.67(27) | | <0.001 |
| | | Cameriere | | 13.23±0.37 | -1.18±0.43 | 0.08 | -2.63 | -1.38 | -1.13 | -0.82 | -0.62 | 1.18±0.43 | -14.51(27) | | <0.001 |
| 15.0 – 15.9 | 7 | Demirjian | 15.54±0.37 | 14.60±0.00 | -0.94±0.37 | 0.14 | -1.32 | -1.27 | -1.10 | -0.54 | -0.45 | 0.93±0.37 | -6.69(6) | | 0.001 |
| | | Willems | | 13.84±0.00 | -1.70±0.37 | 0.14 | -2.08 | -2.03 | -1.86 | -1.30 | -1.21 | 1.70±0.37 | -12.11(6) | | <0.001 |
| | | Cameriere | | 13.34±0.02 | -2.19±0.36 | 0.14 | -2.57 | -2.51 | -2.36 | -1.83 | -1.72 | 2.19±0.36 | -16.06(6) | | <0.001 |
| 16.0 – 16.0 | 3 | Demirjian | 16.56±0.22 | 14.60±0.00 | -1.96±0.22 | 0.13 | -2.20 | - | -1.90 | - | -1.77 | 1.96±0.22 | | 1.81 | <0.001 |
| | | Willems | | 13.84±0.00 | -2.72±0.22 | 0.13 | -2.96 | - | -2.66 | - | -2.53 | 2.72±0.22 | | 1.60 | <0.001 |
| | | Cameriere | | 13.30±0.06 | -3.26±0.27 | 0.16 | -3.56 | - | -3.20 | - | -3.02 | 3.26±0.27 | | 1.60 | <0.001 |

Note: N, number of individuals; Mean, mean age; SD, standard deviation of mean age; SEM, standard error of the mean age; Min, minimal age; $Q_1$, first quartile of age; Med, median age; $Q_3$; third quartile of age; Max, maximum age; t, a paired samples t-test; df, degree of freedom; Wilcoxon, a related samples Wilcoxon signed rank test; *p*, statistically significant if <0.05.

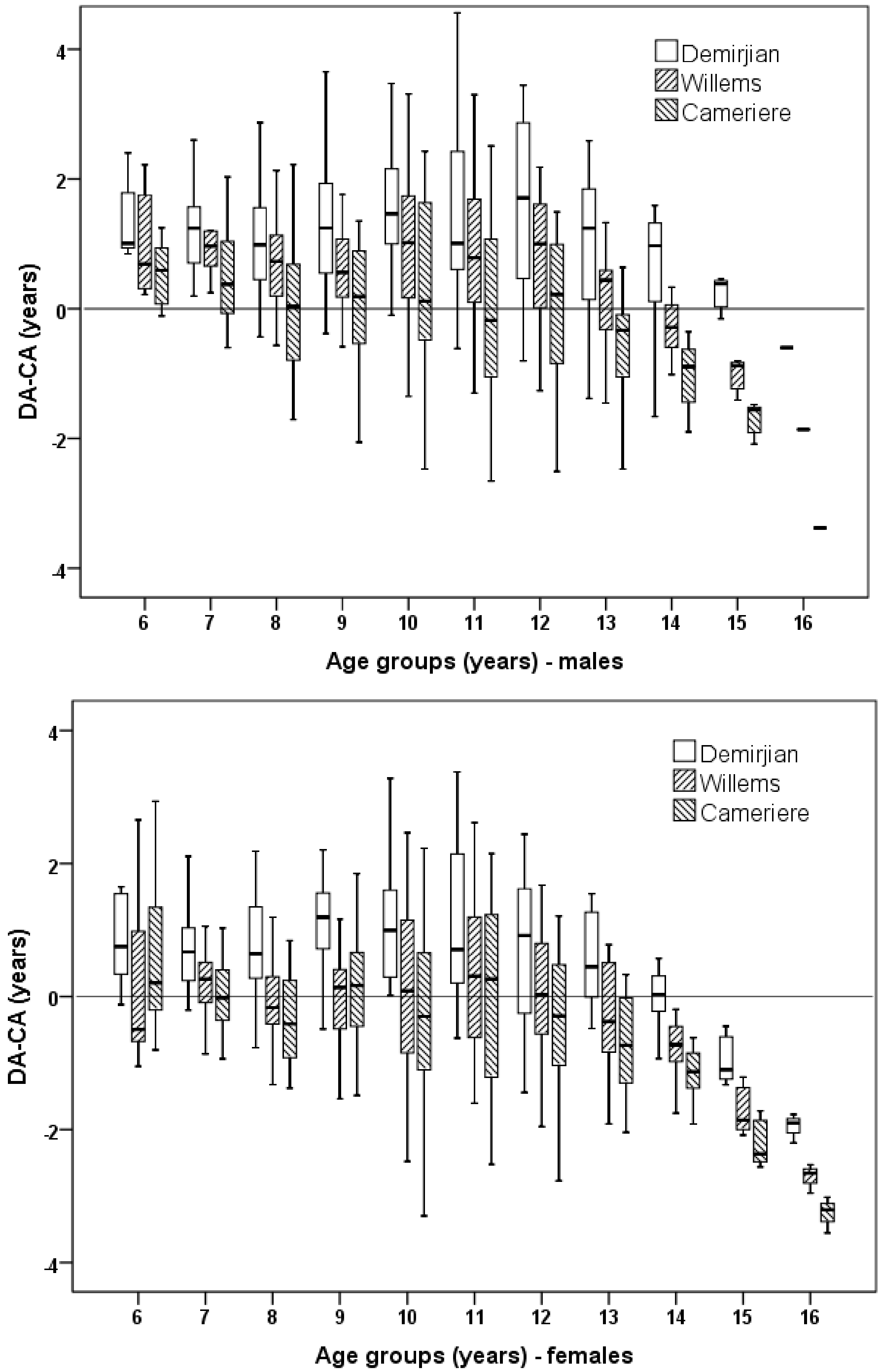



**Figure 23.** Boxplots of the relationship between age groups and the difference between dental age according to the Demirjian, Willems and Cameriere methods and chronological age (DA-CA) for males and females. The boxplot shows median and inter-quartile ranges, while whiskers are lines extending from the box to highest and lowest values, excluding outliers.

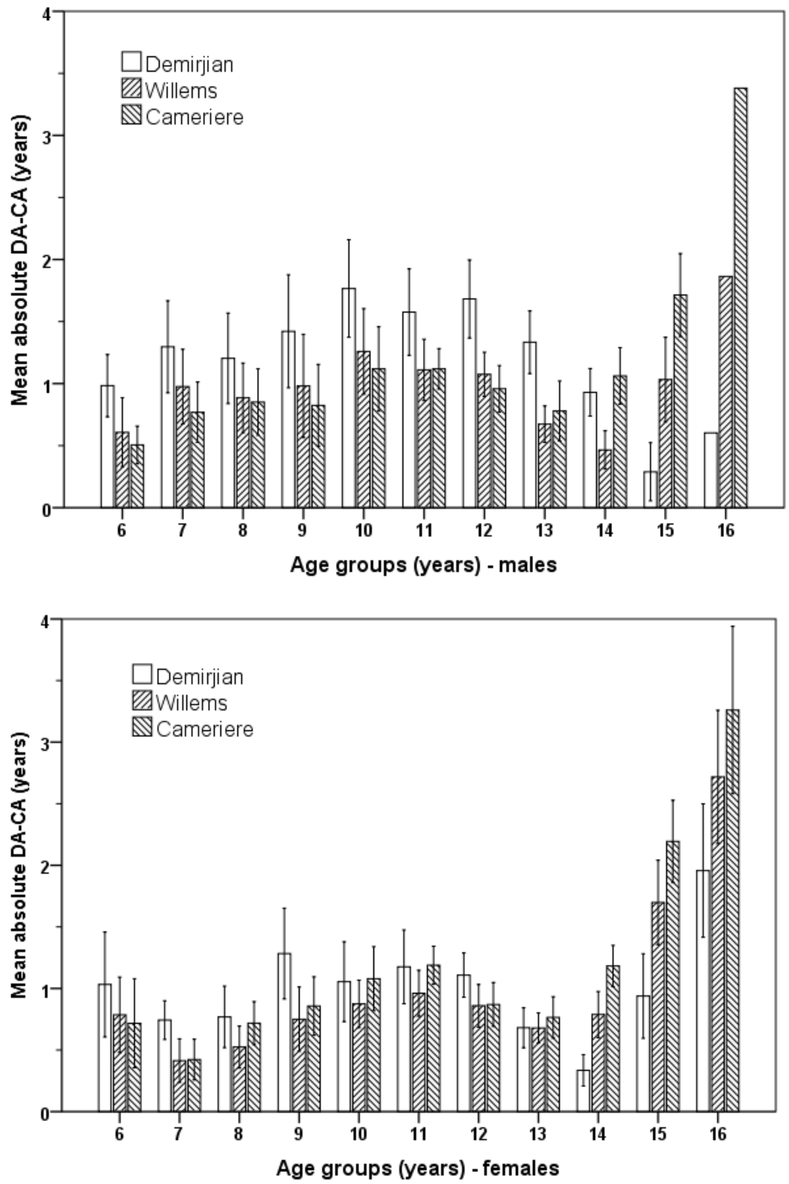



**Figure 24.** Distribution of mean absolute difference between dental age by the Demirjian, Willems and Cameriere methods (DA-CA) across different age groups in males and females.

## 5.8 Development of third molars in children and adolescents in Botswana’s children and adolescents

### *5.8.1* Development of third molars according to the Demirjian stages

The mean chronological age gradually increased for each higher mineralization stage according to Demirjian in third left mandibular molars (Figures 25 and 26). No statistically significant difference in the chronological age between males and females using the unpaired student t-test at specific mineralization stages by Demirjian was observed (Table 20) (57).

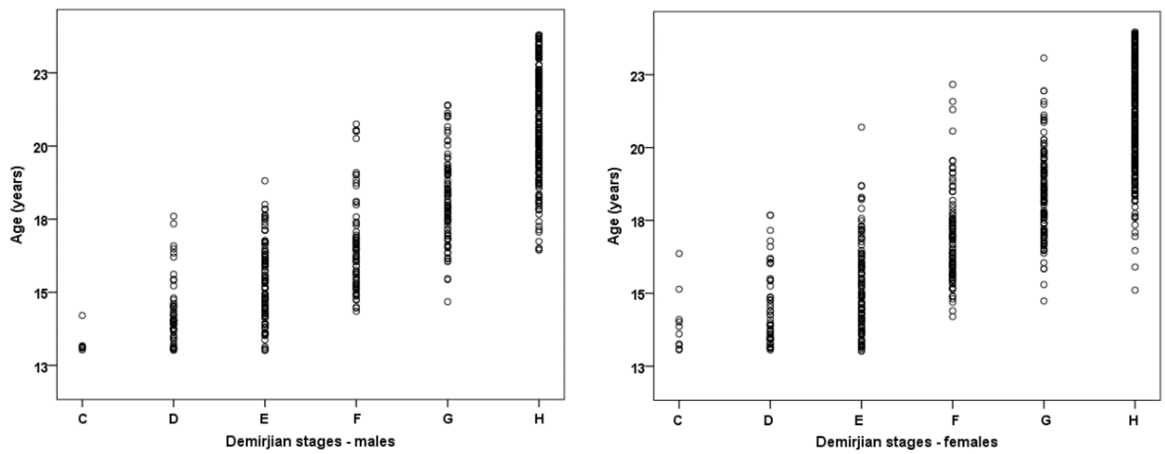



**Figure 25.** Scatterplot of the relationship between the Demirjian stages (C–H) of left mandibular third molars and chronological age in black African males and females from Botswana.

**Table 20.** Summary statistics and the difference between males and females of the mean chronological age (years) at specific Demirjian stages of the left mandibular third molars

| Stage | Males | | | | | | | Females | | | | | | | Difference | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Mean ± SD | Min | Q1 | Med | Q3 | Max | N | Mean ± SD | Min | Q1 | Med | Q3 | Max | Mean | 95% CI | t (df) | Mann-Whitney | *P* |
| C | 7 | 13.27 ± 0.42 | 13.01 | 13.09 | 13.13 | 13.17 | 14.20 | 10 | 13.97 ± 1.05 | 13.06 | 13.19 | 13.73 | 14.36 | 16.36 | -0.70 | -1.60 to 0.19 | | 1.76 | 0.088 |
| D | 53 | 14.32 ± 1.09 | 13.03 | 13.68 | 14.04 | 14.60 | 17.60 | 50 | 14.50 ± 1.29 | 13.08 | 13.44 | 14.04 | 15.44 | 17.68 | -0.18 | -0.64 to 0.29 | 0.75 (101) | | 0.453 |
| E | 100 | 15.29 ± 1.33 | 13.01 | 14.22 | 15.14 | 16.21 | 18.81 | 124 | 15.19 ± 1.47 | 13.01 | 14.04 | 14.93 | 16.06 | 20.70 | 0.10 | -0.27 to 0.47 | 0.529 (222) | | 0.598 |
| F | 75 | 16.60 ± 1.56 | 14.35 | 15.40 | 16.45 | 17.07 | 20.75 | 104 | 16.85 ±1.52 | 14.20 | 15.71 | 16.61 | 17.52 | 22.16 | -0.26 | -0.72 to 0.20 | 1.104 (177) | | 0.271 |
| G | 91 | 18.25 ± 1.52 | 14.67 | 17.14 | 18.07 | 19.17 | 21.39 | 120 | 18.42 ± 1.50 | 14.74 | 17.20 | 18.34 | 19.35 | 23.07 | -0.16 | -0.58 to 0.25 | 0.782 (209) | | 0.453 |
| H | 256 | 21.17 ± 1.76 | 16.44 | 19.88 | 21.47 | 22.51 | 23.80 | 304 | 21.29 ± 1.80 | 15.11 | 19.97 | 21.51 | 22.81 | 23.96 | -0.12 | -0.42 to 0.17 | 0.809 (558) | | 0.419 |

Note: N, number of individuals; Mean, mean age within the Demirjian stages; SD, standard deviation of mean age; Min, minimum age; $Q_1$, first quartile of age; Med, median age; $Q_3$, third quartile of age; Max, maximum age; t, independent samples T-test; df, degrees of freedom; Mann-Whitney, Independent samples Mann-Whitney U test; P, significance set at < 0.05.

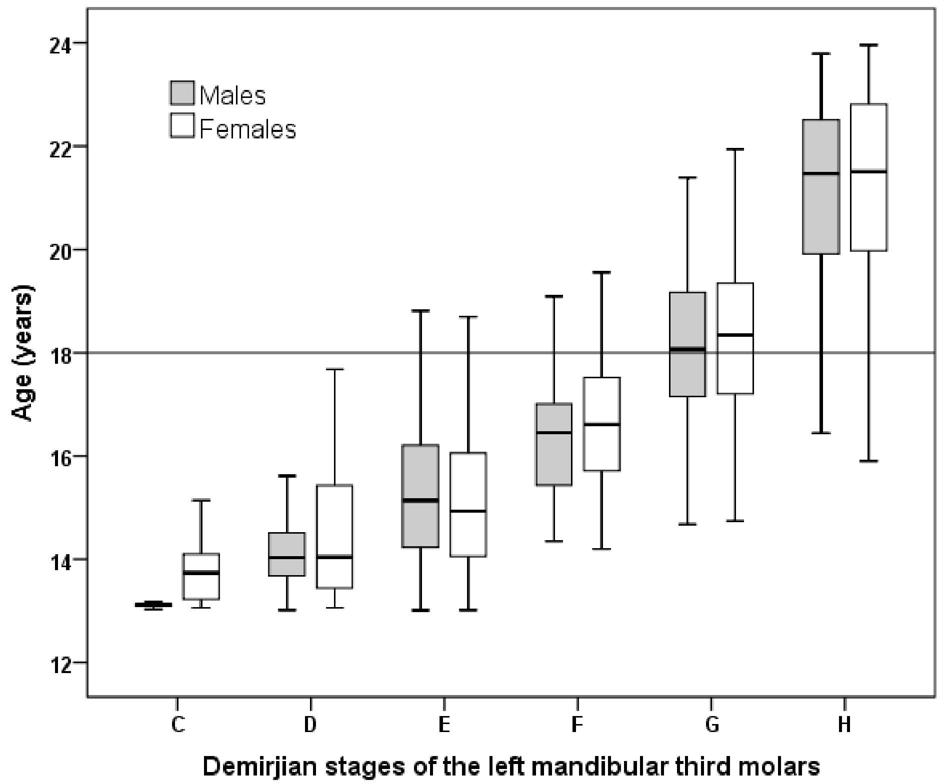



**Figure 26.** Boxplot of the relationship between the Demirjian stages of the left mandibular third molars and age (in years) in black African males and females from Botswana. The boxplot shows medians and interquartile ranges, while whiskers are lines extending from the box to maximum and minimum ages, excluding any outliers.

## 5.9 Development of third molars by the Köhler stages

The mean chronological age gradually increased for each higher mineralization stage according to Köhler in third left mandibular molars (Figures 27 and 28). There was no statistically significant difference in the chronological age between males and females by using the unpaired student t-test at which mineralization occurred at different developmental stages by Köhler et al. (66) (Table 21).

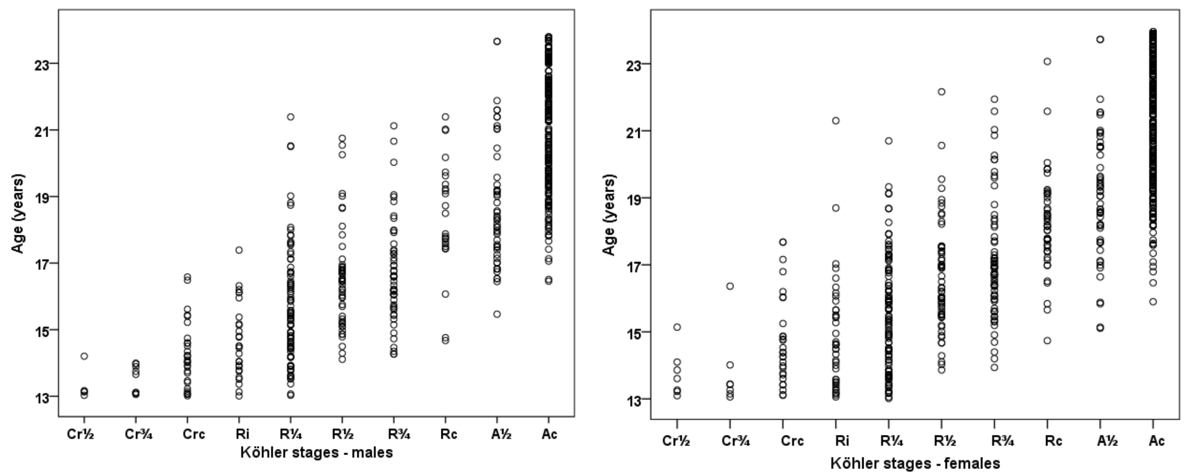



**Figure 27.** Scatterplot of the relationship between Köhler stages ($Cr_{1/2}$ to $A_c$) on the left mandibular third molars and chronological age in black African males and females from Botswana.

**Table 21.** Summary statistics of the chronological age of left mandibular third molars according to Köhler stages

| | Males | | | | | | | | Females | | | | | | | | Difference | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stages | N | Mean | Sd | Min | $Q_1$ | Med | $Q_3$ | Max | N | Mean | Sd | Min | $Q_1$ | Med | $Q_3$ | Max | Mean | 95%CI | t(df) | Mann-Whitney | P |
| $Cr_{1/2}$ | 5 | 13.33 | 0.49 | 13.03 | 13.08 | 13.14 | 13.69 | 14.29 | 7 | 13.76 | 0.71 | 13.09 | 13.22 | 13.61 | 14.10 | 15.14 | -0.42 | -1.24 to 0.40 | | 1.38 | 0.202 |
| $Cr_{3/4}$ | 9 | 13.51 | 0.41 | 13.05 | 13.10 | 13.66 | 13.93 | 14.00 | 7 | 13.82 | 1.16 | 13.06 | 13.15 | 13.43 | 14.01 | 16.36 | -0.30 | -1.19 to 0.59 | | 0.37 | 0.758 |
| $Cr_c$ | 30 | 14.24 | 0.96 | 13.01 | 13.45 | 14.07 | 14.64 | 16.58 | 28 | 14.76 | 1.36 | 13.11 | 13.73 | 14.38 | 15.83 | 17.68 | -0.52 | -1.14 to 0.09 | 1.71 (56) | | 0.093 |
| $R_i$ | 27 | 14.67 | 1.12 | 13.01 | 13.79 | 14.50 | 15.38 | 17.39 | 39 | 14.90 | 1.69 | 13.06 | 13.51 | 14.61 | 15.65 | 21.30 | -0.23 | -0.97 to 0.52 | 0.61 (64) | | 0.546 |
| $R_{1/4}$ | 87 | 15.67 | 1.74 | 13.03 | 14.42 | 15.41 | 16.69 | 21.39 | 104 | 15.51 | 1.67 | 13.01 | 14.10 | 15.37 | 16.74 | 20.70 | 0.16 | -0.32 to 0.65 | 0.66 (189) | | 0.508 |
| $R_{1/2}$ | 50 | 16.55 | 1.54 | 14.11 | 15.30 | 16.46 | 16.91 | 20.75 | 61 | 16.60 | 1.62 | 13.86 | 15.53 | 16.21 | 17.47 | 22.16 | -0.05 | -0.65 to 0.55 | 0.17 (109) | | 0.868 |
| $R_{3/4}$ | 51 | 16.72 | 1.55 | 14.26 | 15.69 | 16.58 | 17.40 | 21.12 | 69 | 17.13 | 1.77 | 13.94 | 15.97 | 16.90 | 17.98 | 21.94 | -0.41 | -1.02 to 0.21 | 1.31 (118) | | 0.191 |
| $R_c$ | 24 | 18.39 | 1.74 | 14.67 | 17.52 | 18.19 | 19.56 | 21.39 | 47 | 18.24 | 1.43 | 14.74 | 17.41 | 18.15 | 19.10 | 23.07 | 0.14 | -0.63 to 0.91 | 0.36 (69) | | 0.716 |
| $A_{1/2}$ | 50 | 18.72 | 1.91 | 15.47 | 17.22 | 18.30 | 19.73 | 23.66 | 54 | 19.07 | 1.88 | 15.11 | 17.73 | 19.06 | 20.51 | 23.73 | -0.35 | -1.09 to 0.39 | 0.94 (102) | | 0.347 |
| $A_c$ | 249 | 21.17 | 1.71 | 16.46 | 19.85 | 21.45 | 22.51 | 23.80 | 296 | 21.29 | 1.79 | 15.90 | 19.94 | 21.51 | 22.81 | 23.96 | -0.11 | -0.41 to 0.19 | 0.73(543) | | 0.464 |

Note: N, number of individuals; Mean, mean age within the Köhler stages; SD, standard deviation of mean age; Min, minimum age; Q1, first quartile of age; Med, median age; Q3, third quartile of age; Max, maximum age; t, independent samples T-test; df, degrees of freedom; Mann-Whitney, Independent samples Mann-Whitney U test; P, significance set at < 0.05.

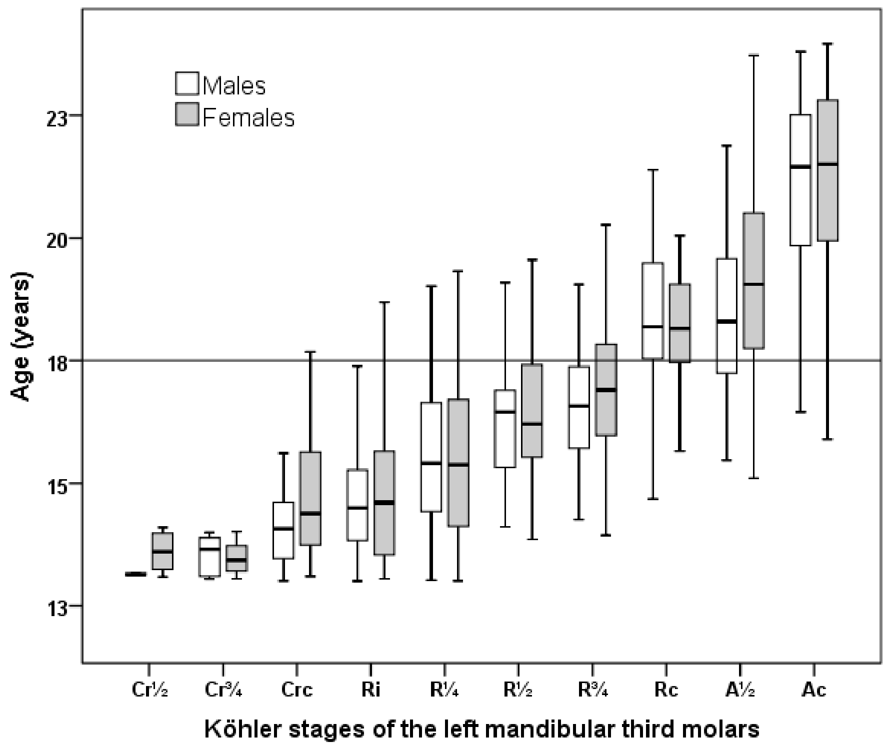



**Figure 28.** Boxplot of the relationship between Köhler stages of the left mandibular third molars and age (in years) in black African males and females from Botswana. The boxplot shows median and interquartile ranges, while whiskers are lines extending from the box to maximum and minimum ages, excluding any outliers.

### 5.10 Development of third molars by Cameriere's third molar maturity index ($I_{3M}$)

Real age gradually decreased as $I_{3M}$ increased across $I_{3M}$ classes in males and females respectively (Figures 29 and 30). Mean ages were not statistically significantly different between males and females across all $I_{3M}$ ranges ($p > 0.05$) (Table 22).

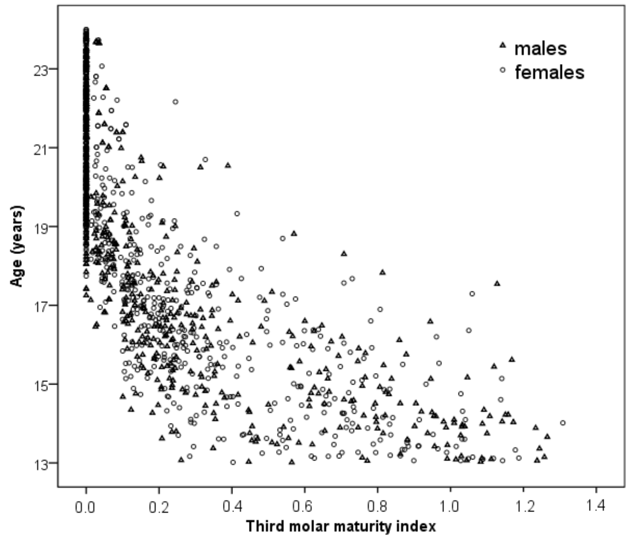



**Figure 29.** Scatterplot of the relationship between third molar maturity index of open apices of left mandibular third molars and chronological age in black African males and females from Botswana.

**Table 22.** Summary statistics of chronological age according to sex and third molar maturity index ($I_{3M}$) classes

| | Males | | | | | | | Females | | | | | | | Difference | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $I_{3M}$ | N | Mean±SD | Min | Q1 | Med | Q3 | Max | N | Mean±SD | Min | Q1 | Med | Q3 | Max | Mean | 95%CI | t(df) | P |
| [0.00. 0.05) | 238 | 21.24±1.66 | 16.46 | 20.02 | 21.48 | 22.50 | 23.79 | 293 | 21.39±1.63 | 17.74 | 19.99 | 21.50 | 22.83 | 23.98 | 0.15 | -0.13 to 0.43 | -1.05(529) | 0.294 |
| [0.05. 0.08) | 49 | 19.48±1.89 | 16.82 | 18.12 | 18.83 | 21.07 | 23.66 | 62 | 19.30±1.65 | 16.47 | 18.16 | 18.83 | 20.18 | 23.73 | 0.18 | -0.49 to 0.85 | 0.54(109) | 0.592 |
| [0.08. 0.3) | 142 | 16.99±1.70 | 13.07 | 15.97 | 16.75 | 17.80 | 21.39 | 183 | 16.98±1.67 | 13.17 | 15.91 | 16.92 | 17.73 | 22.21 | 0.01 | -0.36 to 0.38 | 0.17(323) | 0.986 |
| [0.3. 0.5) | 46 | 15.67±1.66 | 13.03 | 14.42 | 15.41 | 16.69 | 20.54 | 57 | 15.88±1.60 | 13.01 | 14.68 | 15.70 | 17.06 | 20.70 | 0.21 | -0.43 to 0.85 | -0.64(101) | 0.522 |
| [0.5. 0.7) | 33 | 15.08±1.29 | 13.01 | 13.90 | 14.91 | 16.02 | 18.81 | 46 | 14.91±1.29 | 13.12 | 13.81 | 14.67 | 15.98 | 18.70 | 0.17 | -0.42 to 0.76 | 0.56(77) | 0.574 |
| [0.7. 0.9) | 31 | 14.86±1.21 | 13.05 | 14.03 | 14.61 | 15.58 | 18.30 | 43 | 14.48±1.20 | 13.06 | 13.51 | 14.16 | 15.10 | 17.68 | 0.38 | -0.19 to 0.95 | 1.33(72) | 0.186 |
| [0.9. 1.3] | 43 | 14.06±0.92 | 13.03 | 13.41 | 13.97 | 14.22 | 17.53 | 28 | 14.08±1.14 | 13.06 | 13.18 | 13.60 | 14.72 | 17.29 | 0.02 | -0.47 to 0.51 | -0.08(69) | 0.939 |

Note: N, number of individuals; Mean, mean age within $I_{3M}$ class; SD, standard deviation of mean age; Min, minimum age; $Q_1$, 1st quartile of age; Med, median age; $Q_3$, 3rd quartile of age; Max, maximum age; t, independent samples test; df, degrees of freedom; p, significance set at < 0.05.

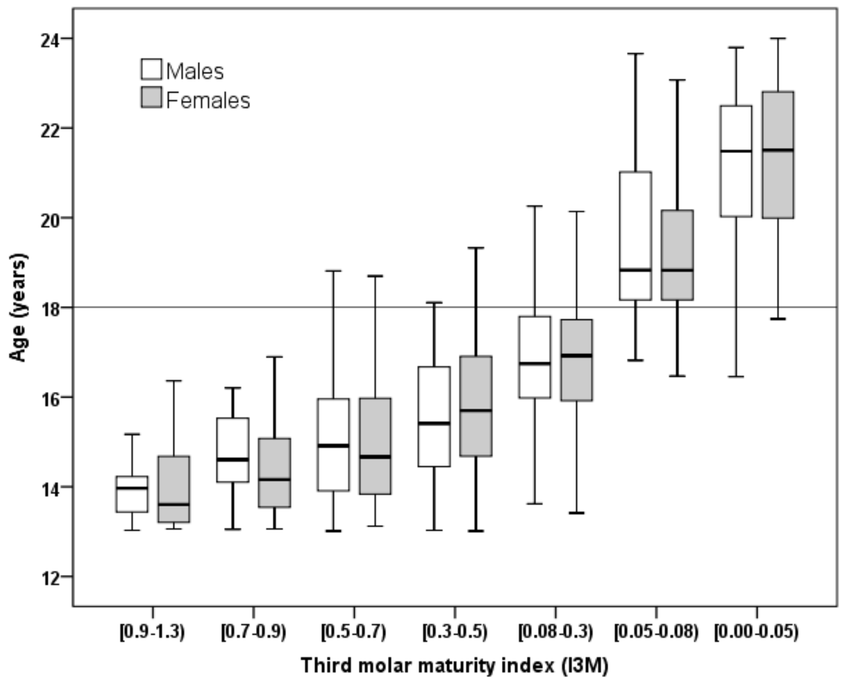



**Figure 30.** Boxplot of the relationship between chronological age and third molar maturity index of open apices of the mandibular left third molar in black African males and females from Botswana. The boxplot shows medians and interquartile ranges, while whiskers are lines extending from the box to maximum and minimum ages, excluding outliers.

## 5.11 Comparison of the Demirjian and Köhler stages and Cameriere's third molar maturity index ($I_{3M}$) for dental age estimation and indicating the legal adult age of 18 years

In males, the Spearman correlation coefficients between chronological age and third molar development registration techniques by Demirjian et al. (57), Köhler et al. (66) and Cameriere et at. (88) were (N=582) 0.866, 0.851 and -0.867, while in females they were (N=712) 0.858, 0.838 and -0.866 respectively. A linear regression analysis on the test sample of 900 OPTs proposed new specific linear regression formulas for males and females for each method (Table 24). Demirjian stages A to H are coded to 2-9 ordinal variables, Köhler stages $Cr_{½}$ to $A_c$ are coded to 1-10 ordinal variables for regression analysis.

**Table 24.** Regression formulas (assuming linearity) for Demirjian et al. (Demirjian3M), Köhler et al. (Köhler3M) and Cameriere et al. (Cameriere$I_{3M}$) tooth development registration methods for age estimation

| Method | Males | | Females | |
|---|---|---|---|---|
| | **Formula** | **RMSE** | **Formula** | **RMSE** |
| Demirjian3M | 4.669 + 1.787 * (DEM) | 1.668 | 4.604 + 1.809*(DEM) | 1.683 |
| Köhler3M | 10.595 + 1.019 * (KÖH) | 1.680 | 10.707 + 1.022*(KÖH) | 1.738 |
| Cameriere $I_{3M}$ | 20.066 – 7.041 * $I_{3M}$ | 2.085 | 20.198 – 7.868 * $I_{3M}$ | 2.064 |

Note: DEM, Demirjian stages; KOH, Kohler stages; RMSE, root mean square error.

In males, the Spearman correlation coefficients between chronological age and third molar development registration techniques by Demirjian et al. (57), Köhler et al. (66) and Cameriere et at. (88) were 0.866, 0.858 and -0.873, while in females they were 0.862, 0.845 and -0.878 respectively. In males and females, Demirjian et al. was the best age prediction model with the highest $R^2$ (Males 71.6%, Females 70.7%) and the lowest RMSE (Males, 1.66 years, Females 1.679 years) values (Table 23).

**Table 23.** Sex-specific listing of Spearman correlation coefficients between tooth development registration methods and age (***ρ***), determination coefficient ($R^2$), and root mean square error (RMSE) in years from the linear regression models with age as a response and each third molar development registration variable as a predictor

| Statistics | Tooth development registration methods | | |
|---|---|---|---|
| | **Demirjian3M** | **Köhler3M** | **Cameriere$I_{3M}$** |
| | **Males** | | |
| ***P*** | 0.866 | 0.858 | -0.873 |
| **$R^2$** | 0.716 | 0.712 | 0.557 |
| **RMSE** | 1.664 | 1.676 | 2.080 |
| | **Females** | | |
| ***P*** | 0.866 | 0.858 | -0.878 |
| **$R^2$** | 0.707 | 0.688 | 0.560 |
| **RMSE** | 1.679 | 1.735 | 2.059 |

Note: RMSE expressed in years.

The comparison of the accuracy of linear regression formulas was estimated as the difference between DA and CA or DA-CA. In the whole sample, we found significant differences between DA and CA for the Demirjian3M method for females ($p = 0.04$). In males, the smallest DA-CA was seen in the Köhler3M (-0.05 ± 1.81 years) and the Cameriere$I_{3M}$ method (0.05 ± 2.11 years), while the Demirjian3M method was the least accurate (-0.20 ± 1.58 years). In females, the smallest DA-CA was seen in the Köhler3M method (−0.07 ± 1.89 years), followed by the Cameriere $I_{3M}$ (0.17 ± 2.17 years) and Demirjian3M (-0.24 ± 1.74 years) (Table 25).

**Table 25.** Comparison of chronological age (CA) and dental age (DA) according to Demirjian3M, Köhler3M and Cameriere $I_{3M}$ linear regression formulas on 174 males and 220 females of black African origin from the city of Gaborone, the Republic of Botswana

| Gender | *N* | Method | CA±SD | DA±SD | (DA-CA) ±SD | SEM | L | U | MAE ± SD | t (df) | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Males | 174 | Demirjian3M | 18.35±3.11 | 18.14±2.55 | -0.20±1.58 | 0.12 | -0.44 | 0.03 | 1.32±0.89 | -1.71(173) | 0.090 |
| | | Köhler3M | | 18.30±2.48 | -0.05±1.81 | 0.14 | -0.32 | 0.22 | 1.45±1.08 | -0.38(173) | 0.706 |
| | | Cameriere $I_{3M}$ | | 18.39±2.38 | 0.05±2.11 | 0.16 | -0.27 | 0.36 | 1.74±1.18 | 0.303(173) | 0.762 |
| Females | 220 | Demirjian3M | 18.50±3.11 | 18.26±2.44 | -0.24±1.74 | 0.12 | -0.47 | -0.01 | 1.40±1.03 | -2.06(219) | 0.040 |
| | | Köhler3M | | 18.43±2.39 | -0.07±1.89 | 0.13 | 0.32 | 0.18 | 1.54±1.09 | -0.57(219) | 0.568 |
| | | Cameriere $I_{3M}$ | | 18.63±2.16 | 0.17±2.17 | 0.15 | -0.12 | 0.46 | 1.81±1.17 | 1.13(219) | 0.259 |

Note: N, number of participants; SD, standard deviation; DA-CA, the difference between dental and chronological age; SEM, standard error of mean age; L, lower interval; U, upper interval of 95% Confidence Interval of DA-CA; MAE, mean absolute error between dental and chronological age; t, a paired samples t-test between DA and CA; df, degrees of freedom; P, statistically significant if < 0.05.

Repeated measures ANOVA was performed to test the differences of DA-CA among the Demirjian3M, Köhler3M and Cameriere$I_{3M}$ methods in males and females. Mauchly's test indicated that the assumption of sphericity had been violated, $\chi^2$ (2)=77.24, $p < 0.001$ and $\chi^2(2)$ 87.16, $p < 0.001$ in males and females respectively. Therefore, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon=0.73$ and $\varepsilon=0.75$ in males and females respectively). The results of repeated measures ANOVA for within-subjects' variables show that there was a significant difference of DA-CA among the three tested methods ($p < 0.001$) in males and females respectively, Table 4.7.1. Post-hoc pairwise comparisons, after Bonferroni adjustment, showed that there was a statistically significant difference of DA-CA between the Demirjian3M and Köhler3M methods (p=0.02), the Demirjian and Cameriere$I_{3M}$ methods (p=0.048) in males and the Demirjian3M and Köhler3M methods (p<0.001), and between the Demirjian and Cameriere$I_{3M}$ methods (p=0.01) in females.

The smallest mean absolute error or MAE was found for the Demirjian3M method, 1.32±0.89 years in males, and 1.40±1.03 years in females. The greatest MAE was for the CameriereI$_{3M}$ method, 1.74±1.18 years in males, and 1.81±1.17 years in females. Next, the repeated-measures ANOVA was also performed to test the differences of MAE among the Demirjian3M, Köhler3M and CameriereI$_{3M}$ methods in males and females. Mauchly's test indicated that the assumption of sphericity had been violated, $\chi^2$ (2)=54.58, p <0.001, and $\chi^2$(2)=61.32, p <0.001 in males and females, respectively. Therefore, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (ε=0.79 and ε=0.80 in males and females, respectively). The results of repeated measures ANOVA for within-subjects' variables show that there was a significant difference of MAE among Demirjian3M, Köhler3M and CameriereI$_{3M}$ methods (p < 0.001) in males and females respectively (Table 26). Post-hoc pairwise comparisons, after Bonferroni adjustment, showed that there was a statistically significant difference of MAE between each of two different methods (p <0.001).

**Table 26.** The summary of repeated measures ANOVA for the within-subjects' variables DA-CA and MAE of Demirjian3M, Köhler3M and CameriereI$_{3M}$ methods

| Gender | Source | Type III Sum of Squares | Degrees of freedom | Mean Square | F | *P* |
|---|---|---|---|---|---|---|
| Males | DA-CA | 5.64 | 1.47 | 3.84 | 3.96 | 0.032 |
| | Error | 246.65 | 254.08 | 0.97 | | |
| Females | DA-CA | 8.84 | 1.50 | 5.88 | 6.44 | <0.004 |
| | Error | 300.56 | 329.43 | 0.91 | | |
| Males | MAE | 16.13 | 1.57 | 10.26 | 17.31 | <0.001 |
| | Error | 161.26 | 272.04 | 0.59 | | |
| Females | MAE | 18.23 | 1.61 | 11.35 | 20.76 | <0.001 |
| | Error | 192.32 | 351.75 | 0.55 | | |

Note: DA-CA, the difference between dental and chronological age; MAE, the absolute difference between dental and chronological age.

Tables 27 and 28 compare the data of dental age, DA-CA and MAE across age groups of the test sample. The relationship between age groups and DA-CA for the Demirjian3M, the Köhler3M and the CameriereI$_{3M}$ methods were presented in Figures 31 and 32.

**Table 27.** Comparison of chronological age (CA) and dental age (DA) (years) on the test sample, according to the Demirjian, Köhler stages and the Cameriere third molar maturity index ($I_{3M}$) linear regression formulas across different age groups of 174 black African males from the city of Gaborone, the Republic of Botswana

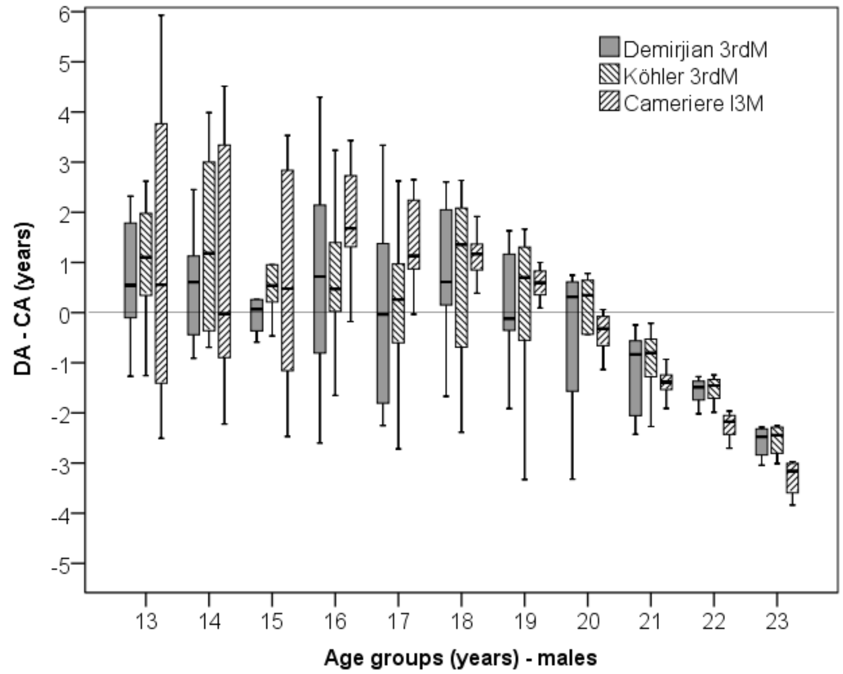
| Age groups | *N* | Method | CA±SD | DA±SD | (DA-CA) ±SD | SEM | Min | $Q_1$ | Med | $Q_3$ | Max | MAE±SD | Wilcoxon | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | 15 | Demirjian 3M | 13.53±0.31 | 14.32±1.13 | 0.79±1.10 | 0.28 | -1.27 | -0.15 | 0.55 | 1.80 | 2.32 | 1.07±0.81 | 2.21 | 0.027 |
| | | Köhler 3M | | 14.54±1.27 | 1.01±1.23 | 0.32 | -1.25 | 0.19 | 1.10 | 2.07 | 2.62 | 1.37±0.76 | 2.50 | 0.012 |
| | | Cameriere $I_{3M}$ | | 14.65±2.86 | 1.13±2.89 | 0.75 | -2.51 | -1.71 | 0.55 | 4.24 | 5.93 | 2.47±1.79 | 1.93 | 0.233 |
| 14 | 17 | Demirjian 3M | 14.42±0.32 | 14.97±1.37 | 0.55±1.13 | 0.27 | -0.91 | -0.47 | 0.61 | 1.16 | 2.45 | 1.03±0.68 | 1.92 | 0.055 |
| | | Köhler 3M | | 15.75±1.78 | 1.33±1.62 | 0.39 | -0.69 | -0.37 | 1.18 | 3.31 | 3.99 | 1.59±1.34 | 2.53 | 0.011 |
| | | Cameriere $I_{3M}$ | | 15.42±2.60 | 1.00±2.42 | 0.59 | -2.22 | -0.98 | -0.03 | 3.38 | 4.51 | 2.09±1.52 | 1.11 | 0.266 |
| 15 | 14 | Demirjian 3M | 15.47±0.28 | 15.77±1.25 | 0.30±1.29 | 0.35 | -1.80 | -0.41 | 0.07 | 0.62 | 3.50 | 0.83±1.01 | 0.28 | 0.778 |
| | | Köhler 3M | | 16.27±1.48 | 0.80±1.43 | 0.38 | -1.75 | 0.15 | 0.54 | 1.28 | 4.30 | 1.12±1.18 | 2.23 | 0.026 |
| | | Cameriere $I_{3M}$ | | 16.19±2.29 | 0.71±2.18 | 0.58 | -2.47 | -1.30 | 0.48 | 2.89 | 3.53 | 1.96±1.08 | 1.29 | 0.198 |
| 16 | 21 | Demirjian 3M | 16.46±0.28 | 17.17±1.79 | 0.72±1.72 | 0.37 | -2.60 | -0.82 | 0.72 | 2.14 | 4.30 | 1.53±1.02 | 1.58 | 0.114 |
| | | Köhler 3M | | 17.24±1.66 | 0.79±1.61 | 0.35 | -1.65 | -0.02 | 0.47 | 2.04 | 4.33 | 1.26±1.25 | 2.10 | 0.035 |
| | | Cameriere $I_{3M}$ | | 18.18±1.24 | 1.73±1.19 | 0.26 | -1.08 | 1.21 | 1.68 | 2.80 | 3.43 | 1.86±0.96 | 3.77 | <0.001 |
| 17 | 16 | Demirjian 3M | 17.47±0.29 | 17.51±1.75 | 0.04±1.73 | 0.43 | -2.25 | -1.85 | -0.04 | 1.46 | 3.34 | 1.46±0.84 | 0.21 | 0.836 |
| | | Köhler 3M | | 17.66±1.56 | 0.19±1.57 | 0.39 | -2.72 | -0.70 | 0.26 | 0.98 | 3.37 | 1.18±1.00 | 0.52 | 0.605 |
| | | Cameriere $I_{3M}$ | | 18.85±0.82 | 1.38±0.85 | 0.21 | -0.03 | 0.82 | 1.13 | 2.36 | 2.65 | 1.38±0.85 | 3.46 | 0.001 |
| 18 | 14 | Demirjian 3M | 18.45±0.28 | 19.22±1.38 | 0.77±1.35 | 0.36 | -1.67 | -0.11 | 0.61 | 2.09 | 2.60 | 1.27±0.85 | 1.79 | 0.074 |
| | | Köhler 3M | | 19.33±1.68 | 0.88±1.61 | 0.43 | -2.38 | 2.64 | 1.36 | 2.12 | 2.64 | 1.68±0.63 | 1.92 | 0.056 |
| | | Cameriere $I_{3M}$ | | 19.54±0.59 | 1.09±0.56 | 0.15 | -0.19 | 1.92 | 1.17 | 1.40 | 1.92 | 1.12±0.50 | 3.23 | 0.001 |
| 19 | 15 | Demirjian 3M | 19.33±0.24 | 19.44±1.26 | 0.11±1.12 | 0.29 | -1.91 | -0.40 | -0.12 | 1.26 | 1.63 | 0.89±0.64 | 0.17 | 0.865 |
| | | Köhler 3M | | 19.49±1.61 | 0.17±1.49 | 0.39 | -3.33 | -0.62 | 0.70 | 1.31 | 1.66 | 1.24±0.79 | 1.02 | 0.307 |
| | | Cameriere $I_{3M}$ | | 19.86±0.45 | 0.53±0.40 | 0.10 | -0.49 | 0.33 | 0.59 | 0.87 | 1.00 | 0.60±0.29 | 3.07 | 0.002 |
| 20 | 14 | Demirjian 3M | 20.33±0.22 | 19.86±1.36 | -0.47±1.43 | 0.38 | -3.32 | -1.60 | 0.31 | 0.61 | 0.75 | 1.08±1.02 | -0.47 | 0.638 |
| | | Köhler 3M | | 19.84±1.76 | -0.49±1.84 | 0.49 | -4.81 | -1.06 | 0.34 | 0.64 | 0.78 | 1.18±1.46 | -0.53 | 0.594 |
| | | Cameriere $I_{3M}$ | | 19.76±0.66 | -0.57±0.76 | 0.20 | -2.64 | -0.78 | -0.32 | -0.07 | 0.06 | 0.58±0.76 | -3.17 | 0.002 |
| 21 | 17 | Demirjian3M | 21.36±0.22 | 20.23±0.84 | -1.13±0.76 | 0.18 | -2.43 | -2.07 | -0.84 | -0.56 | -0.25 | 1.13±0.76 | -3.62 | <0.001 |
| | | Köhler3M | | 20.01±1.46 | -1.35±1.42 | 0.34 | -5.70 | -1.78 | -0.80 | -0.53 | -0.21 | 1.35±1.42 | -3.62 | <0.001 |
| | | Cameriere $I_{3M}$ | | 19.95±0.23 | -1.41±0.29 | 0.07 | -2.03 | -1.54 | -1.39 | -1.23 | -0.93 | 1.41±0.29 | -3.62 | <0.001 |
| 22 | 16 | Demirjian3M | 22.31±0.23 | 20.75±0.00 | -1.55±0.76 | 0.06 | -2.02 | -1.76 | -1.49 | -1.36 | -1.28 | 1.55±0.23 | -3.51 | <0.001 |
| | | Köhler3M | | 20.79±0.00 | -1.52±0.23 | 0.06 | -1.99 | -1.72 | -1.46 | -1.33 | -1.24 | 1.52±0.23 | -3.52 | <0.001 |
| | | Cameriere $I_{3M}$ | | 20.07±0.00 | -2.24±0.23 | 0.06 | -2.70 | -2.44 | -2.17 | -2.05 | -1.96 | 2.24±0.23 | -3.52 | <0.001 |
| 23 | 15 | Demirjian3M | 23.32±0.29 | 20.75±0.00 | -2.57±0.29 | 0.08 | -3.04 | -2.91 | -2.48 | -2.31 | -2.29 | 2.57±0.29 | -3.41 | <0.001 |
| | | Köhler3M | | 20.79±0.00 | -2.54±0.29 | 0.08 | -3.01 | -2.87 | -2.44 | -2.28 | -2.25 | 2.54±0.29 | -3.41 | <0.001 |
| | | Cameriere $I_{3M}$ | | 20.04±0.08 | -3.29±0.34 | 0.09 | -3.84 | -3.73 | -3.16 | -2.99 | -2.97 | 3.29±0.34 | -3.41 | 0.001 |

Note: N, number of participants; DA-CA, the difference between dental and chronological age; SD, standard deviation; SEM, standard error of mean age; Min, Minimal DA-CA; $Q_1$, first quartile of DA-CA, Med, Median DA-CA; $Q_3$, third quartile of DA-CA; Max, Maximal DA-CA; MAE, mean absolute error between dental and chronological age; Wilcoxon, a related samples Wilcoxon Signed Rank Test between DA and CA; *P*, statistically significant if < 0.05.

**Table 28.** Comparison of chronological age (CA) and dental age (DA) (years) on the test sample, according to the Demirjian, Köhler stages and the Cameriere third molar maturity index ($I_{3M}$) linear regression formulas across different age groups of 220 black African females from the city of Gaborone, the Republic of Botswana

| Age groups | *N* | Method | CA±SD | DA±SD | (DA-CA) ±SD | SEM | Min | $Q_1$ | Med | $Q_3$ | Max | MAE±SD | Wilcoxon | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | 18 | Demirjian3M | 13.48±0.23 | 14.65±0.92 | 1.18±0.94 | 0.22 | -0.23 | 0.20 | 1.75 | 1.93 | 2.29 | 1.21±0.90 | 3.42 | 0.001 |
| | | Köhler3M | | 15.08±0.91 | 1.60±0.83 | 0.20 | 0.17 | 0.66 | 2.02 | 2.22 | 2.65 | 1.60±0.83 | 3.72 | <0.001 |
| | | Cameriere $I_{3M}$ | | 14.53±2.16 | 1.05±2.10 | 0.49 | -2.17 | -0.33 | 0.61 | 2.76 | 4.79 | 1.73±1.55 | 1.67 | 0.094 |
| 14 | 18 | Demirjian3M | 14.49±0.23 | 15.36±1.45 | 0.87±1.42 | 0.33 | -1.11 | -0.61 | 0.81 | 1.30 | 4.34 | 1.31±1.00 | 2.50 | 0.012 |
| | | Köhler3M | | 15.65±1.62 | 1.16±1.56 | 0.37 | -0.98 | -0.48 | 1.32 | 2.16 | 4.15 | 1.53±1.81 | 2.42 | 0.016 |
| | | Cameriere $I_{3M}$ | | 16.32±2.08 | 1.83±2.02 | 0.48 | -2.65 | 0.32 | 2.14 | 3.67 | 4.92 | 2.19±1.58 | 3.03 | 0.002 |
| 15 | 22 | Demirjian3M | 15.58±0.29 | 16.69±1.41 | 1.11±1.34 | 0.29 | -0.53 | -0.03 | 1.29 | 1.82 | 4.98 | 1.241.041.21 | 3.07 | 0.002 |
| | | Köhler3M | | 17.35±1.57 | 1.77±1.51 | 0.32 | -0.06 | 0.48 | 1.36 | 2.44 | 5.03 | 1.77±1.51 | 4.07 | <0.001 |
| | | Cameriere $I_{3M}$ | | 17.96±1.65 | 2.38±1.58 | 0.34 | -2.14 | 1.88 | 2.68 | 3.41 | 4.21 | 2.63±1.10 | 3.75 | <0.001 |
| 16 | 22 | Demirjian3M | 16.56±0.34 | 16.77±2.03 | 0.22±1.89 | 0.40 | -4.52 | -0.91 | 0.65 | 2.09 | 2.56 | 1.56±1.04 | 0.83 | 0.408 |
| | | Köhler3M | | 16.84±1.73 | 0.28±1.69 | 0.36 | -3.61 | -1.01 | 0.71 | 1.50 | 3.26 | 1.40±0.93 | 0.80 | 0.426 |
| | | Cameriere $I_{3M}$ | | 17.63±2.03 | 1.08±2.03 | 0.43 | -4.43 | 0.95 | 1.67 | 2.18 | 3.14 | 2.08±0.86 | 2.09 | 0.021 |
| 17 | 20 | Demirjian3M | 17.49±0.27 | 17.90±1.58 | 0.41±1.54 | 0.34 | -4.03 | -0.23 | 0.26 | 1.36 | 3.14 | 1.15±1.08 | 1.57 | 0.117 |
| | | Köhler3M | | 17.86±1.72 | 0.37±1.70 | 0.38 | -3.90 | -0.65 | 0.17 | 1.43 | 3.19 | 1.31±1.07 | 1.08 | 0.279 |
| | | Cameriere $I_{3M}$ | | 18.40±1.52 | 0.92±1.55 | 0.35 | -3.22 | 0.31 | 1.58 | 1.89 | 2.46 | 1.55±0.89 | 2.39 | 0.017 |
| 18 | 22 | Demirjian3M | 18.53±0.27 | 18.66±1.93 | 0.13±1.92 | 0.41 | -3.22 | -1.43 | 0.42 | 2.09 | 2.73 | 1.66±0.91 | 0.21 | 0.833 |
| | | Köhler3M | | 19.07±1.83 | 0.54±1.87 | 0.40 | -2.86 | -1.50 | 1.29 | 2.14 | 2.77 | 1.78±0.69 | 1.25 | 0.211 |
| | | Cameriere $I_{3M}$ | | 19.53±0.79 | 1.00±0.80 | 0.17 | -1.03 | 0.68 | 1.29 | 1.48 | 2.04 | 1.18±0.48 | 3.62 | <0.001 |
| 19 | 22 | Demirjian3M | 19.40±0.26 | 19.49±1.24 | 0.09±1.17 | 0.25 | -2.27 | -0.41 | -0.05 | 1.25 | 1.66 | 0.89±0.73 | 0.05 | 0.961 |
| | | Köhler3M | | 19.90±1.34 | 0.51±1.23 | 0.26 | -3.30 | 0.34 | 0.78 | 1.30 | 1.70 | 1.07±0.76 | 2.58 | 0.010 |
| | | Cameriere $I_{3M}$ | | 19.95±0.47 | 0.55±0.50 | 0.11 | -0.50 | 0.26 | 0.65 | 0.94 | 1.14 | 0.67±0.29 | 3.52 | <0.001 |
| 20 | 17 | Demirjian 3M | 20.45±0.32 | 20.35±1.40 | -0.10±1.51 | 0.37 | -5.24 | 0.02 | 0.38 | 0.64 | 1.15 | 0.88±1.22 | 1.30 | 0.193 |
| | | Köhler3M | | 20.27±1.40 | -0.19±1.49 | 0.36 | -4.88 | -0.60 | 0.30 | 0.68 | 1.19 | 0.94±1.15 | 0.59 | 0.554 |
| | | Cameriere $I_{3M}$ | | 19.87±0.67 | -0.58±0.77 | 0.19 | -3.07 | -0.73 | -0.43 | -0.09 | 0.14 | 0.60±0.74 | -3.24 | 0.001 |
| 21 | 20 | Demirjian3M | 21.52±0.27 | 20.34±1.03 | -1.17±0.95 | 0.21 | -4.03 | -1.74 | -0.78 | -0.60 | -0.22 | 1.17±0.95 | -3.92 | <0.001 |
| | | Köhler3M | | 20.31±1.53 | -1.20±1.46 | 0.33 | -6.51 | -1.03 | -0.70 | -0.53 | -0.18 | 1.20±1.46 | -3.92 | <0.001 |
| | | Cameriere $I_{3M}$ | | 19.99±0.64 | -1.53±0.65 | 0.15 | -3.86 | -1.72 | -1.35 | -1.25 | -0.84 | 1.53±0.65 | -3.92 | <0.001 |
| 22 | 19 | Demirjian 3M | 22.49±0.30 | 20.88±0.00 | -1.61±0.30 | 0.07 | -2.10 | -1.90 | -1.50 | -1.39 | -1.21 | 1.61±0.30 | -3.82 | <0.001 |
| | | Köhler 3M | | 20.93±0.00 | -1.57±0.30 | 0.07 | -2.06 | -1.86 | -1.46 | -1.34 | -1.17 | 1.57±0.30 | -3.82 | <0.001 |
| | | Cameriere $I_{3M}$ | | 20.20±0.00 | -2.30±0.30 | 0.07 | -2.79 | -2.59 | -2.19 | -2.07 | -1.90 | 2.30±0.30 | -3.82 | <0.001 |
| 23 | 20 | Demirjian 3M | 23.50±0.31 | 20.79±0.40 | -2.70±0.42 | 0.09 | -3.99 | -2.92 | -2.75 | -2.39 | -2.11 | 2.70±0.42 | -3.92 | <0.001 |
| | | Köhler 3M | | 20.77±0.50 | -2.72±0.53 | 0.12 | -4.19 | -1.86 | -1.46 | -1.35 | -1.17 | 2.72±0.53 | -3.92 | <0.001 |
| | | Cameriere $I_{3M}$ | | 20.16±0.10 | -3.34±0.32 | 0.07 | -3.79 | -2.59 | -2.19 | -2.07 | -1.90 | 3.34±0.32 | -3.92 | <0.001 |

Note: N, number of participants; DA-CA, the difference between dental and chronological age; SD, standard deviation; SEM, standard error of mean age; Min, Minimal DA-CA; $Q_1$, first quartile of DA-CA, Med, Median DA-CA; $Q_3$, third quartile of DA-CA; Max, Maximal DA-CA; MAE, mean absolute error between dental and chronological age; Wilcoxon, a related samples Wilcoxon Signed Rank Test between DA and CA; *P*, statistically significant if < 0.05.

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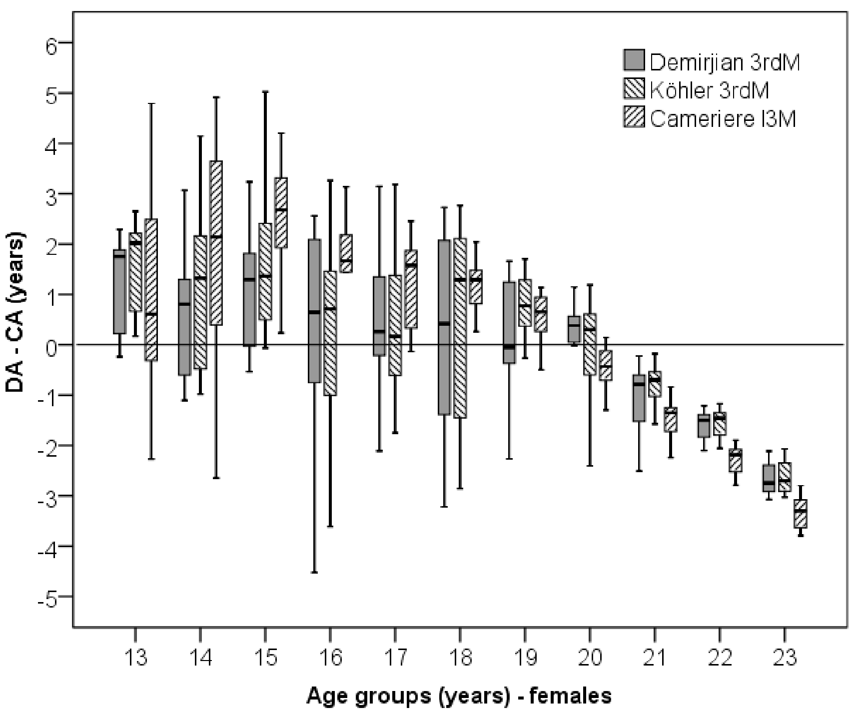
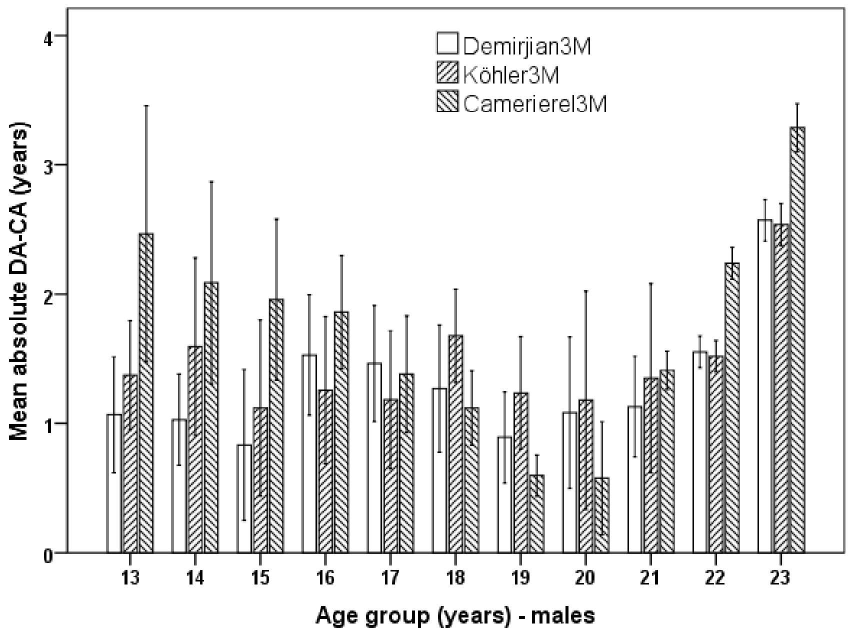



**Figure 31.** Boxplots of the relationship between the difference between dental age by the linear regression formula using Demirjian3M, Köhler3M, and CameriereI$_{3M}$ registration methods of the development of the third molars and real age (DA-CA) across different age groups. The boxplot shows median and inter-quartile ranges, while the whiskers are lines extending from the box to the highest and lowest values of extending outliners.



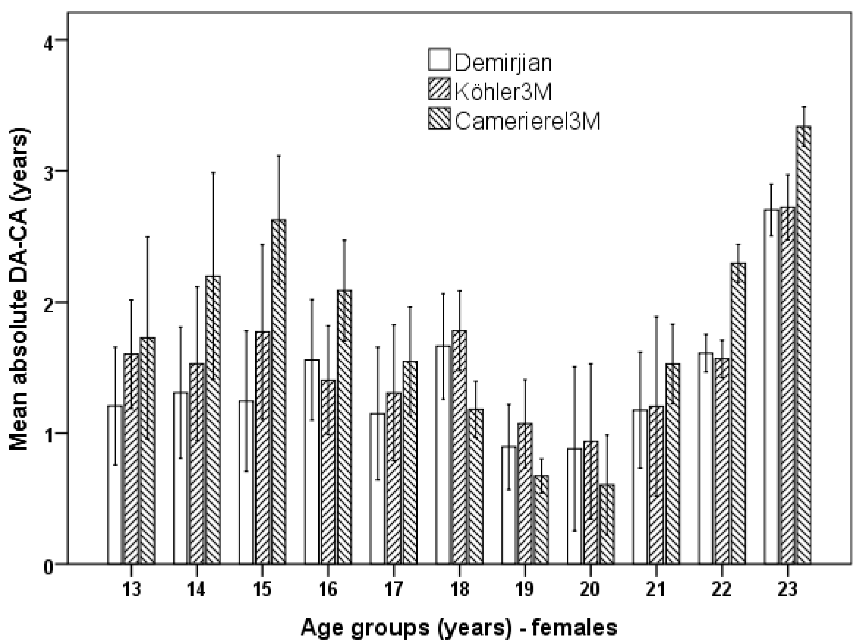




**Figure 32.** Distribution of the mean absolute difference between dental age by the Demirjian3M, Köhler3M, and Cameriere$I_{3M}$ or MAE across different age groups in males and females.

## 5.12 Third molars stages of mineralization by Demirjian and Köhler and third molar maturity index ($I_{3M}$) for estimating adult age (≥18 years)

The sample of third molars was evaluated separately in males and females for accuracy of specific Demirjian and Köhler stages and $I_{3M}$ values for estimating adult age in the Botswana sample. The areas under the ROC curve were 0.946, 0.943, and 0.961in males and 0.942, 0.939, and 0.964 in females for the Demirjian, Köhler, and Cameriere methods, respectively (Figures 33–35), (Table 29) (81).

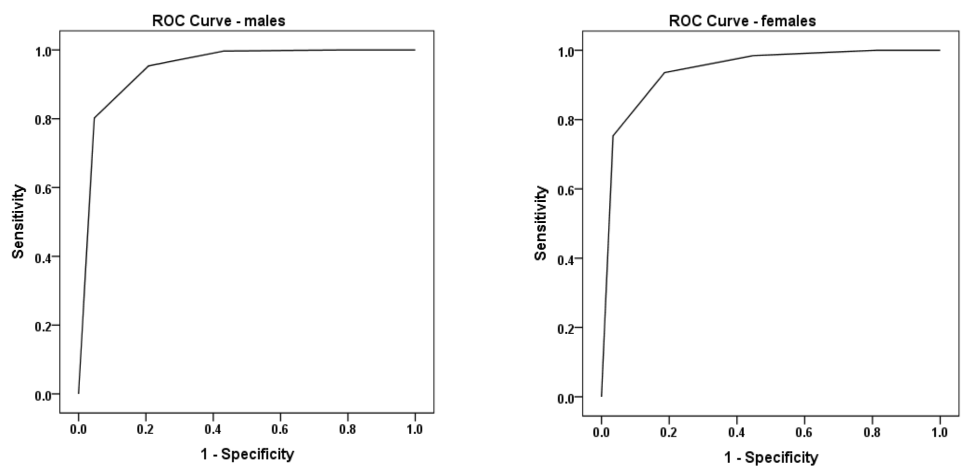



**Figure 33.** Receiver operating characteristic (ROC) curves for the Demirjian stages of tooth number 38 for predicting adult age of ≥18 years in black Africans males and females from Botswana

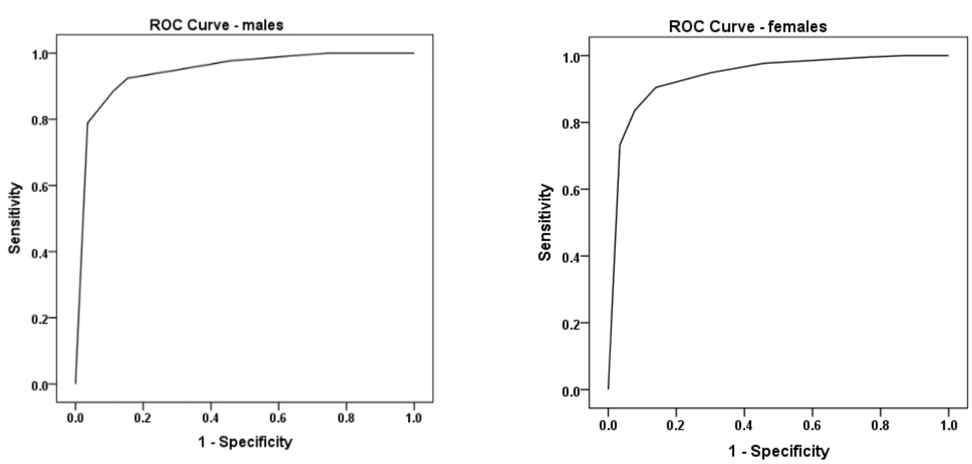



**Figure 34.** Receiver operating characteristic (ROC) curves for the Köhler stages of tooth number 38 for predicting adult age of ≥18 years in black Africans males and females from Botswana

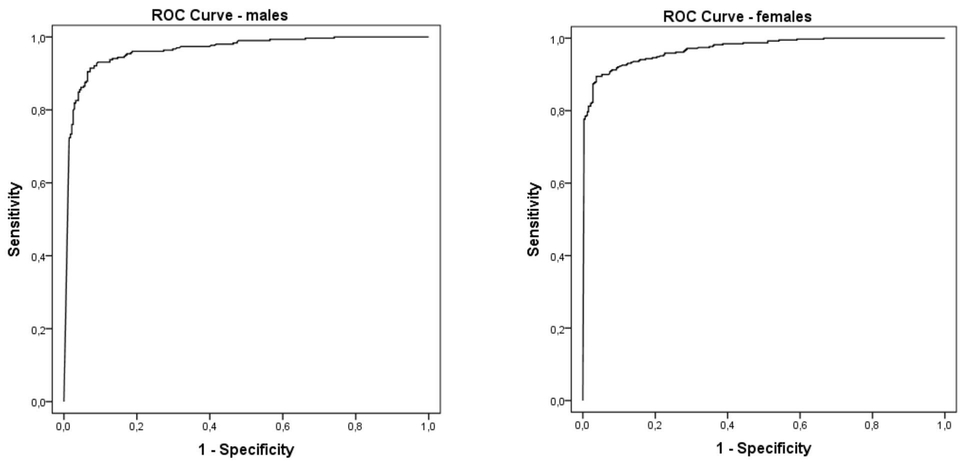



**Figure 35.** Receiver operating characteristic (ROC) curves for the third molar maturity index ($I_{3M}$) of tooth number 38 for predicting adult age of ≥18 years in black Africans males and females from Botswana

**Table 29.** The Area Under the Curve for the Demirjian, Köhler and Cameriere methods for tooth number 38 in predicting adult age of ≥18 years in black Africans males and females from Botswana

| Method | Sex | AuC | SE | 95%CI | *P* |
|---|---|---|---|---|---|
| Demirjian | Males | 0.946 | 0.009 | 0.928 to 0.965 | <0.001 |
| Köhler | | 0.943 | 0.010 | 0.924 to 0.963 | <0.001 |
| Cameriere $I_{3M}$ | | 0.961 | 0.008 | 0.946 to 0.977 | <0.001 |
| Demirjian | Females | 0.942 | 0.009 | 0.924 to 0.959 | <0.001 |
| Köhler | | 0.939 | 0.009 | 0.921 to 0.957 | <0.001 |
| Cameriere $I_{3M}$ | | 0.964 | 0.006 | 0.952 to 0.976 | <0.001 |

Note: AuC, Area under the Curve; SE, standard error of estimate; 95%CI, 95% confidence interval of AuC.

The results of specific Demirjian and Köhler stages and a specific cut-off value of $I_{3M}$ to discriminate adults from minors were presented in 2×2 contingency tables (Table 30, 32 and 34) and derived values of the test were presented in Tables 31, 33 and 35.

The best performance of the test to discriminate adults from minors for the Demirjian stages were for stage H in males, where 507 of 582 (87%) individuals were accurately classified. The sensitivity and specificity were 0.80 and 0.95 respectively with Bayes' post-test probability of 0.95. In females, the best performance was for stage G, 627 of 712 (88%) individuals were accurately selected. The sensitivity and specificity were 0.94 and 0.81 respectively, with Bayes' post-test probability of 0.86. In cases where better sensitivity is necessary, the Demirjian stage H in females can provide the specificity of 0.97 (Tables 30 and 31).

For Köhler's method, the best performance to discriminate adults from minors was for the A½ stage in both sexes. In total, 514 of 582 (88%) males and 623 of 712 (88%) females were accurately classified. The values of the sensitivity, specificity and Bayes' post-test probabilities were 0.88, 0.89, and 0.90 in males and 0.84, 0.92, and 0.93 in females, respectively. In cases where better sensitivity is necessary, the Köhler stage $A_c$ can provide the specificity of 0.96 in men and 0.97 in females (Tables 32 and 33).

For the third molar maturity index or $I_{3M}$, the best performance to discriminate adults from minors was for the cut-off value of $I_{3M}$ <0.08 in both sexes. In total, 529 of 582 (91%) males and 654 of 712 females (92%) were accurately selected (Tables 34 and 35). This value of $I_{3M}$ shows the best performance compared to other $I_{3M}$ cut-off values and the other Demirjian and Köhler stages. This indicates a close association between the age of majority, and the test is positive or $I_{3M}$ <0.08. In males, the sensitivity or proportion of correctly classified participants being 18 years and older was 0.88 (95% CI, 0.86 to 0.90), while the specificity or proportion of correctly classified participants younger than 18 years of age was 0.94 (95% CI, 0.91 to 0.96). The PPV of the test, indicating that the participants whose $I_{3M}$ <0.08 were classified as adults, was 0.94 (95% CI. 0.91 to 0.96), while the NPV of the test, signifying that the participants whose $I_{3M}$ ≥0.08 are minors, was 0.88 (95% CI, 0.85 to 0.90). The highest value of J-index was 0.83 (95% CI, 0.81 to 0.87) for the cut-off value of $I_{3M}$ <0.10 (Table 35). The positive likelihood ratio (LR+) was 13.67 (95% CI. 9.21 to 21.02), while the negative likelihood ratio (LR-) was 0.12 (95% CI, 0.10 to 0.16). Bayes' post-test probability *p* was 0.94 (95% CI, 0.90 to 0.98). In females, accuracy was 0.92 (95% CI. 0.90 to 0.93), sensitivity and specificity were 0.88 (95% CI. 0.86 to 0.89) and 0.96 (95% CI, 0.94 to 0.98) respectively. The values of PPV and NPV were 0.97 (95% CI, 0.94 to 0.98) and 0.87 (95% CI, 0.85 to 0.89) respectively, while the highest value of J-index was 0.85 (95% CI. 0.80 to 0.88) for the cut-off value of $I_{3M}$ =0.10 (Table 35), as in males. The values of (LR+) and (LR-) were 23.73 (95% CI, 14.20 to 42.28) and 0.12 (95% CI, 0.11 to 0.15) respectively, while Bayes' post-test probability *p* was 0.97 (95% CI, 0.93 to 1.00). The results of additional cut-off values of $I_{3M}$ (0.02 to 0.14) were also presented in 2×2 contingency tables. Table 35 also showed that the cut-off value of $I_{3M}$ <0.10 has similar accuracy and a better J-index and appears more accurate generally. However, it is less appropriate for practical use as the obtained specificity, and Bayes' post-test probability *p* is less than for the cut-off value of $I_{3M}$ <0.08 for both sexes. Explicitly, in most medico-legal matters of whether an individual in question is a major (adult) or minor, it is more important that a discrimination test shows a low proportion of false-positive subjects (minors who are selected as majors) or higher specificity. From this point of view, the cut-off value of $I_{3M}$ <0.08, which better represents specificity and Bayes' post-test probability, may be recommended for discriminating adults from minors in black Africans in Botswana.

**Table 30.** Contingency table describing the discrimination performance of the test for different cut-off values of Demirjian stages of third molars

| **Test** | **Age males ≥18** | **<18** | **Total males** | **Age females ≥18** | **<18** | **Total females** |
|---|---|---|---|---|---|---|
| ≥Stage E | 305 | 217 | 522 | 389 | 263 | 652 |
| < Stage E | 0 | 60 | 60 | 0 | 60 | 60 |
| Stage F | 302 | 120 | 422 | 383 | 145 | 528 |
| < Stage F | 3 | 157 | 160 | 6 | 178 | 184 |
| ≥Stage G | 289 | 58 | 347 | 364 | 60 | 424 |
| < Stage G | 16 | 219 | 235 | 25 | 263 | 288 |
| Stage H | 243 | 13 | 256 | 293 | 11 | 304 |
| < Stage H | 62 | 264 | 326 | 96 | 312 | 408 |
| Total | 304 | 278 | 582 | 389 | 323 | 712 |

**Table 31.** The quantities derived from 2-by-2 contingency tables (95% confidence interval) of performance of different Demirjian stages to discriminate adults from minors in black Africans from Botswana

| Quantities | Sex | Demirjian | | | |
|---|---|---|---|---|---|
| | | Stage E | Stage F | Stage G | Stage H |
| Accuracy | **Males** | 0.63 (0.59 to 0.67) | 0.79 (0.76 to 0.82) | 0.87 (0.85 to 0.90) | 0.87 (0.84 to 0.90) |
| Sensitivity | | 1.00 (0.99 to 1.00) | 0.99 (0.98 to 1.00) | 0.95 (0.92 to 0.97) | 0.80 (0.75 to 0.84) |
| Specificity | | 0.22 (0.17 to 0.27) | 0.57 (0.51 to 0.63) | 0.79 (0.74 to 0.84) | 0.95 (0.93 to 0.98) |
| J-index | | 0.22 (0.19 to 0.22) | 0.56 (0.52 to 0.57) | 0.74 (0.68 to 0.78) | 0.75 (0.69 to 0.79) |
| PPV | | 0.58 (0.54 to 0.63) | 0.72 (0.67 to 0.76) | 0.83 (0.79 to 0.87) | 0.95 (0.92 to 0.98) |
| NPV | | 1.00 (0.93 to 1.00) | 0.98 (0.96 to 1.00) | 0.93 (0.90 to 0.96) | 0.81 (0.77 to 0.85) |
| LR+ | | 1.28 (1.20 to 1.36) | 2.29 (2.00 to 2.62) | 4.53 (3.59 to 5.70) | 16.98 (9.96 to 28.95) |
| LR- | | 0.00 (0.00 to 0.07) | 0.02 (0.01 to 0.05) | 0.07 (0.04 to 0.11) | 0.21 (0.17 to 0.27) |
| Bayes PTP | | 0.59 (0.57 to 0.61) | 0.72 (0.68 to 0.76) | 0.84 (0.80 to 0.88) | 0.95 (0.91 to 0.99) |
| | | | | | |
| Accuracy | **Females** | 0.63 (0.60 to 0.67) | 0.79 (0.76 to 0.82) | 0.88 (0.86 to 0.90) | 0.85 (0.82 to 0.88) |
| Sensitivity | | 1.00 (0.99 to 1.00) | 0.98 (0.97 to 1.00) | 0.94 (0.91 to 0.96) | 0.75 (0.71 to 0.80) |
| Specificity | | 0.19 (0.14 to 0.23) | 0.55 (0.50 to 0.61) | 0.81 (0.77 to 0.86) | 0.97 (0.95 to 0.99) |
| J-index | | 0.19 (0.16 to 0.19) | 0.54 (0.50 to 0.56) | 0.75 (0.70 to 0.79) | 0.72 (0.67 to 0.75) |
| Positive PV | | 0.60 (0.56 to 0.63) | 0.73 (0.69 to 0.76) | 0.86 (0.83 to 0.89) | 0.96 (0.94 to 0.98) |
| Negative PV | | 1.00 (0.93 to 1.00) | 0.97 (0.94 to 0.99) | 0.91 (0.88 to 0.95) | 0.76 (0.72 to 0.81) |
| LR+ | | 1.23 (1.17 to 1.29) | 2.19 (1.94 to 2.48) | 5.04 (4.00 to 6.34) | 22.12 (12.34 to 39.64) |
| LR- | | 0.00 (0.00 to 0.06) | 0.03 (0.01 to 0.06) | 0.08 (0.05 to 0.12) | 0.26 (0.21 to 0.30) |
| Bayes PTP | | 0.59 (0.57 to 0.61) | 0.72 (0.69 to 0.75) | 0.86 (0.82 to 0.89) | 0.96 (0.93 to 1.00) |

Note: J-index, Youden index; Positive PV, positive predictive value; Negative PV, negative predictive value; LR+, positive likelihood ratio; LR+, negative likelihood ratio; Bayes PTP, Bayes' post-test probability.

**Table 32.** Contingency table describing the discrimination performance of the test for different cut-off values of the Köhler stages of third molars

| **Test** | **Age males ≥18** | **<18** | **Total males** | **Age females ≥18** | **<18** | **Total females** |
|---|---|---|---|---|---|---|
| Stage $R_{1/2}$ | 297 | 127 | 424 | 380 | 147 | 527 |
| <Stage $R_{1/2}$ | 8 | 150 | 158 | 9 | 176 | 185 |
| Stage $R_{3/4}$ | 288 | 86 | 374 | 369 | 97 | 466 |
| <Stage $R_{3/4}$ | 17 | 191 | 208 | 20 | 226 | 246 |
| Stage $A_{rc}$ | 280 | 43 | 323 | 352 | 45 | 397 |
| < Stage $A_{rc}$ | 25 | 234 | 259 | 37 | 278 | 315 |
| Stage $A_{1/2}$ | 268 | 31 | 299 | 325 | 25 | 350 |
| < Stage $A_{1/2}$ | 37 | 246 | 283 | 64 | 298 | 362 |
| Stage $A_c$ | 239 | 12 | 251 | 285 | 11 | 296 |
| < Stage $A_c$ | 66 | 265 | 331 | 104 | 312 | 416 |
| Total | 305 | 277 | 582 | 389 | 323 | 712 |

**Table 33.** The quantities derived from 2-by-2 contingency tables (95% confidence interval) of the performance of different Köhler stages to discriminate adults from minors black Africans from Botswana

| Quantities | Sex | Köhler Stage $R_{½}$ | Stage $R_{¾}$ | Stage $R_c$ | Stage $A_{½}$ | Stage $A_c$ |
|---|---|---|---|---|---|---|
| **Accuracy** | **Males** | 0.77 (0.73 to 0.80) | 0.82 (0.79 to 0.85) | 0.88 (0.86 to 0.91) | 0.88 (0.86 to 0.94) | 0.87 (0.84 to 0.89) |
| **Sensitivity** | | 0.97 (0.96 to 0.99) | 0.94 (0.92 to 0.97) | 0.92 (0.89 to 0.95) | 0.88 (0.84 to 0.92) | 0.78 (0.74 to 0.83) |
| **Specificity** | | 0.54 (0.48 to 0.60) | 0.69 (0.64 to 0.74) | 0.84 (0.80 to 0.89) | 0.89 (0.85 to 0.93) | 0.96 (0.93 to 0.98) |
| **J-index** | | 0.52 (0.47 to 0.54) | 0.64 (0.57 to 0.68) | 0.76 (0.70 to 0.81) | 0.77 (0.71 to 0.82) | 0.74 (0.69 to 0.78) |
| **PPV** | | 0.70 (0.66 to 0.74) | 0.77 (0.73 to 0.81) | 0.87 (0.84 to 0.89) | 0.90 (0.86 to 0.93) | 0.95 (0.93 to 0.98) |
| **NPV** | | 0.95 (0.92 to 0.98) | 0.92 (0.88 to 0.96) | 0.90 (0.87 to 0.93) | 0.87 (0.83 to 0.91) | 0.80 (0.76 to 0.84) |
| **LR+** | | 2.12 (1.87 to 2.42) | 3.04 (2.55 to 3.63) | 5.91 (4.77 to 7.24) | 7.85 (5.62 to 10.97) | 18.09 (10.37 to 31.56) |
| **LR-** | | 0.05 (0.02 to 0.10) | 0.08 (0.05 to 0.13) | 0.1 (0.07 to 0.14) | 0.14 (0.10 to 0.19) | 0.23 (0.18 to 0.28) |
| **Bayes PTP** | | 0.71 (0.67 to 0.74) | 0.77 (0.74 to 0.81) | 0.87 (0.83 to 0.91) | 0.90 (0.86 to 0.94) | 0.95 (0.91 to 0.99) |
| | | | | | | |
| **Accuracy** | **Females** | 0.78 (0.75 to 0.81) | 0.84 (0.81 to 0.86) | 0.88 (0.86 to 0.91) | 0.88 (0.85 to 0.90) | 0.84 (0.81 to 0.87) |
| **Sensitivity** | | 0.98 (0.96 to 0.99) | 0.95 (0.93 to 0.97) | 0.90 (0.88 to 0.93) | 0.84 (0.80 to 0.87) | 0.73 (0.69 to 0.78) |
| **Specificity** | | 0.54 (0.49 to 0.60) | 0.70 (0.65 to 0.75) | 0.86 (0.82 to 0.90) | 0.92 (0.89 to 0.95) | 0.97 (0.95 to 0.99) |
| **J-index** | | 0.52 (0.48 to 0.55) | 0.65 (0.60 to 0.69) | 0.77 (0.71 to 0.81) | 0.76 (0.70 to 0.80) | 0.70 (0.65 to 0.73) |
| **Positive PV** | | 0.72 (0.68 to 0.76) | 0.79 (0.75 to 0.83) | 0.89 (0.86 to 0.92) | 0.93 (0.90 to 0.96) | 0.96 (0.94 to 0.98) |
| **Negative PV** | | 0.95 (0.92 to 0.98) | 0.92 (0.88 to 0.95) | 0.88 (0.85 to 0.92) | 0.82 (0.78 to 0.86) | 0.75 (0.71 to 0.79) |
| **LR+** | | 2.15 (1.90 to 2.42) | 3.16 (2.67 to 3.74) | 6.50 (4.94 to 8.53) | 10.79 (7.39 to 15.77) | 21.51 (12.00 to 38.57) |
| **LR-** | | 0.04 (0.02 to 0.08) | 0.07 (0.05 to 0.11) | 0.11 (0.08 to 0.15) | 0.18 (0.14 to 0.22) | 0.28 (0.23 to 0.33) |
| **Bayes PTP** | | 0.72 (0.68 to 0.75) | 0.79 (0.75 to 0.82) | 0.88 (0.85 to 0.92) | 0.93 (0.89 to 0.96) | 0.96 (0.93 to 1.00) |

Note: J-index, Youden index; Positive PV, positive predictive value; Negative PV, negative predictive value; LR+, positive likelihood ratio; LR+, negative likelihood ratio; Bayes PTP, Bayes' post-test probability.

**Table 34.** Contingency table describing the discrimination performance of the test for different cut-off values of the third molar maturity index ($I_{3M}$)

| Test | Age males | | Total males | Age females | | Total females |
|---|---|---|---|---|---|---|
| | ≥18 | <18 | | ≥18 | <18 | |
| $I_{3M} < 0.02$ | 222 | 5 | 227 | 273 | 1 | 274 |
| $I_{3M} \geq 0.02$ | 82 | 273 | 355 | 116 | 322 | 438 |
| $I_{3M} < 0.04$ | 242 | 7 | 249 | 303 | 2 | 305 |
| $I_{3M} \geq 0.04$ | 62 | 271 | 333 | 86 | 321 | 407 |
| $I_{3M} < 0.06$ | 258 | 13 | 271 | 323 | 9 | 332 |
| $I_{3M} \geq 0.06$ | 46 | 265 | 311 | 66 | 314 | 380 |
| $I_{3M} < 0.08$ | 269 | 18 | 287 | 343 | 12 | 355 |
| $I_{3M} \geq 0.08$ | 35 | 260 | 295 | 46 | 311 | 357 |
| $I_{3M} < 0.10$ | 275 | 21 | 296 | 348 | 15 | 363 |
| $I_{3M} \geq 0.10$ | 29 | 257 | 286 | 41 | 308 | 349 |
| $I_{3M} < 0.12$ | 285 | 36 | 321 | 355 | 30 | 385 |
| $I_{3M} \geq 0.12$ | 19 | 242 | 261 | 34 | 293 | 327 |
| $I_{3M} < 0.14$ | 289 | 48 | 337 | 364 | 46 | 410 |
| $I_{3M} \geq 0.14$ | 15 | 230 | 245 | 25 | 277 | 302 |
| Total | 304 | 278 | 582 | 389 | 323 | 712 |

**Table 35.** The quantities derived from 2-by-2 contingency tables (95% confidence interval) for the test of age of majority in black Africans from Botswana when different values of the third molar maturity index ($I_{3M}$) were used to discriminate between those who are 18 years of age and older or under 18 years of age in males.

| Quantities | $I_{3M}$ | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0.02** | **0.04** | **0.06** | **0.08** | **0.10** | **0.12** | **0.14** |
| | **Males** | | | | | | |
| **Accuracy** | 0.85 (0.83 to 0.86) | 0.88 (0.86 to 0.90) | 0.90 (0.87 to 0.92) | 0.91 (0.88 to 0.93) | 0.91 (0.89 to 0.93) | 0.90 (0.88 to 0.93) | 0.89 (0.86 to 0.91) |
| **Sensitivity** | 0.73 (0.71 to 0.74) | 0.80 (0.77 to 0.81) | 0.85 (0.82 to 0.87) | 0.88 (0.86 to 0.90) | 0.91 (0.88 to 0.93) | 0.94 (0.91 to 0.96) | 0.95 (0.92 to 0.97) |
| **Specificity** | 0.98 (0.96 to 0.99) | 0.98/7 (0.95 to 0.99) | 0.95 (0.93 to 0.97) | 0.94 (0.91 to 0.96) | 0.92 (0.90 to 0.95) | 0.87 (0.84 to 0.89) | 0.83 (0.80 to 0.85) |
| **J-index** | 0.71 (0.67 to 0.73) | 0.77 (0.72 to 0.80) | 0.80 (0.75 to 0.84) | 0.82 (0.77 to 0.86) | 0.83 (0.77 to 0.87) | 0.81 (0.75 to 0.85) | 0.78 (0.72 to 0.82) |
| **Positive PV** | 0.98 (0.95 to 0.99) | 0.97 (0.94 to 0.99) | 0.95 (0.92 to 0.97) | 0.94 (0.91 to 0.96) | 0.93 (0.90 to 0.95) | 0.89 (0.86 to 0.91) | 0.86 (0.83 to 0.87) |
| **Negative PV** | 0.77 (0.75 to 0.78) | 0.81 (0.79 to 0.82) | 0.85 (0.83 to 0.87) | 0.88 (0.85 to 0.90) | 0.90 (0.87 to 0.92) | 0.93 (0.90 to 0.95) | 0.94(0.91 to 0.96) |
| **LR+** | 40.60 (17.13 to 110.08) | 31.61 (15.50 to 71.37) | 18.15 (11.08 to 31.50) | 13.67 (9.21 to 21.02) | 11.97 (8.44 to 17.36) | 7.24 (5.75 to 8.93) | 5.51 (4.59 to 6.38) |
| **LR-** | 0.27 (0.26 to 0.30) | 0.21 (0.19 to 0.24) | 0.16 (0.14 to 0.19) | 0.12 (0.10 to 0.16) | 0.10 (0.08 to 0.14 ) | 0.07 (0.05 to 0.11) | 0.06 (0.04 to 0.09) |
| **Bayes PTP** | 0.98 (0.94 to 1.00) | 0.97 (0.93 to 1.00) | 0.95 (0.91 to 0.99) | 0.94 (0.90 to 0.98) | 0.93 (0.89 to 0.97) | 0.89 (0.85 to 0.93) | 0.86 (0.82 to 0.90) |
| | **Females** | | | | | | |
| **Accuracy** | 0.84 (0.82 to 0.84) | 0.88 (0.86 to 0.88) | 0.89/90 (0.87 to 0.91) | 0.92 (0.90 to 0.93) | 0.92 (0.90 to 0.94) | 0.91 (0.89 to 0.93) | 0.90 (0.87 to 0.92) |
| **Sensitivity** | 0.70 (0.69 to 0.70) | 0.78 (0.76 to 0.78) | 0.83 (0.81 to 0.84) | 0.88 (0.86 to 0.89) | 0.89 (0.87 to 0.91) | 0.91 (0.89 to 0.93) | 0.94 (0.91 to 0.95) |
| **Specificity** | 1.00 (0.98 to 1.00) | 0.99 (0.98 to 1.00) | 0.97 (0.95 to 0.99) | 0.96 (0.94 to 0.98) | 0.95 (0.93 to 0.97) | 0.91 (0.88 to 0.93) | 0.86 (0.83 to 0.88) |
| **J-index** | 0.70 (0.67 to 0.70) | 0.77 (0.74 to 0.78) | 0.80 (0.76 to 0.83) | 0.84 (0.80 to 0.87) | 0.85 (0.80 to 0.88) | 0.82 (0.77 to 0.86) | 0.79 (0.74 to 0.83) |
| **Positive PV** | 1.00 (0.98 to 1.00) | 0.99 (0.98 to 1.00) | 0.97 (0.95 to 0.99) | 0.97 (0.94 to 0.98) | 0.96 (0.94 to 0.97) | 0.92 (0.90 to 0.94) | 0.89 (0.87 to 0.91) |
| **Negative PV** | 0.73 (0.72 to 0.74) | 0.79 (0.77 to 0.79) | 0.83 (0.81 to 0.84) | 0.87 (0.85 to 0.89) | 0.88 (0.86 to 0.90) | 0.90 (0.87 to 0.92) | 0.92 (0.89 to 0.94) |
| **LR+** | 226.68 (36.17 to 4354.82) | 125.80 (32.64 to 727.35) | 29.80 (16.00 to 60.07) | 23.73 (14.20 to 42.28) | 19.26 (12.34 to 31.59) | 9.83 (7.40 to 13.15) | 6.57 (5.37 to 7.92) |
| **LR-** | 0.30 (0.30 to 0.32) | 0.22 (0.22 to 0.24) | 0.17 (0.16 to 0.20) | 0.12 (0.11 to 0.15) | 0.11 (0.09 to 0.13) | 0.10 (0.07 to 0.13) | 0.07 (0.05 to 0.10) |
| **Bayes PTP** | 1.00 (0.96 to 1.00) | 0.99 (0.96 to 1.00) | 0.97 (0.94 to 1.00) | 0.97 (0.93 to 1.00) | 0.96 (0.92 to 0.99) | 0.92 (0.88 to 0.96) | 0.89 (0.85 to 0.92) |

Note: J-index, Youden index; Positive PV, positive predictive value; Negative PV, negative predictive value; LR+, positive likelihood ratio; LR+, negative likelihood ratio; Bayes PTP, Bayes' post-test probability.

# 6. DISCUSSION

## 6.1 Dental development of permanent teeth in the Botswana sample

This is the first study of the analysis of development and mineralization of all permanent teeth using OPTs of children and adolescents of black African origin in Botswana. The timing of mineralization of permanent teeth from the left side of the maxilla and mandible was descriptively evaluated according to the Demirjian method for the staging of tooth mineralization (57). The results demonstrated that the mean ages within which the Demirjian stages were reached are generally lower in Batswana females than males and that a significant difference was only found in a few teeth and their stages. A lower mean age in females indicates that the development of permanent mandibular dentition in females is slightly faster compared to males. The findings are in line with other previous studies (63, 89). If compared to mean ages within stages in the study by Liversidge et al. (63), which included data of mandibular teeth of children from Australia, Belgium, Canada, England, Finland, France, South Korea and Sweden, mandibular teeth in Batswana subjects are generally faster in maturation. There are very few studies that have evaluated the maxillary dentition and compared them to the mandibular (28). This is because of more unobstructed visibility and less superimposition of the roots and distortion of the teeth in the mandible. The mean age within the stages in this study was comparable with the results obtained by Lee et al. (89) on the Korean sample of participants aged 1–20 years. The results from a similar study by Feijoo et al. (28), which analyzed permanent teeth from all four quadrants in subjects between 2 and 16 years of age and calculated mean age of onset in specific stages, were not comparable to mean age within the stages calculated in this study (90). The Demirjian system explains in detail and describes the continuum of mineralization from the beginning of mineralization to apex closure in permanent teeth by using eight developmental stages (57). According to Lee et al. (89), methods based on fractioning more stages of the crown and root development result in less precision and more rigorous assessment required. Therefore, the Demirjian approach was recommended because of the good reproducibility of developmental stages and their thorough explanation (91). Excellent average Kappa scores of the intra-rater and inter-rater agreement showed a high concordance between observations, which is in line with many similar studies using the Demirjian stages (92, 93). Mandibular teeth showed some better Kappa values of the agreement for the developmental stages, which can be attributed to their clearer visibility and lower distortion on OPTs when compared to maxillary (57). Most approaches to dental age estimation differentiate variance in the time span of the first seven teeth, which generally mature between 12 and 14 years, and the third molars that could mature as late as 20–23 years of age (94). Most dental methods in children are based on evaluating the seven mandibular teeth, such as the methods by Demirjian et al. (57, 95), Haavikko (96) and Cameriere et al. (88), or on evaluating at least four mandibular teeth as suggested by Demirjian and Goldstein (95) or Haavikko (97).

The third molars were studied separately or excluded in most studies on permanent teeth (70, 90, 98-101). They have wider variability in the timing of initial formation and finished mineralization as well as having the most dispersed age range of formation and the highest level of agenesis (90). In the age range when most of the first seven permanent teeth complete their mineralization, between 12 and 14 years of age, only the crown formation of the third molars could be completed (99, 102-105). However, the third molars are the only teeth that can be studied in the entire developmental range of stages from cross-sectional radiographic material (90). In our sample, there was no statistically significant difference in mean ages at most of the developmental stages between maxillary and mandibular third molars. A statistically significant difference in mean ages was found only in the maxilla at D and F stages of maxillary third molars without a particular pattern. Mandibular third molars similarly develop in both sexes. A study by Liversidge et al. (90), on the third molar mineralization on African–Zulu and Nguni populations and Cape coloureds (people from the western cape of mixed race) from Southern Africa, showed that African females on average develop earlier than African males for almost all stages of third molar formation and Cape females develop earlier than Cape males for crown stage formation, although few of these comparisons were significant. Contrary to these findings from Southern Africa, most previous studies on mandibular third molars reported earlier mean age within the stage in males compared to females for most stages (90). According to Kasper et al. (106), the usefulness of the third molar stages alone for age prediction is low; however, when combined with other skeletal and dental information, a more narrow range of predicted age is possible. Separate studies on the third molar development have been carried out by the American Board of Forensic Odontologists (ABFO study) by Mincer et al. (74) on American Caucasians. Kasper et al. (106) compared the results from ABFO studies to Texan Hispanics. Texan Hispanic third molar development was 8–18 months earlier when compared to the sample from ABFO studies. In American Caucasians, maxillary third molar formation was slightly advanced over mandibular, and root formation occurred earlier in males than females (74). In Texan Hispanics, all stages of development for the maxilla and mandible showed the mean ages for males to be less than females mean ages (106). Also, the mean ages in the maxilla at each developmental stage were smaller than in the mandible in both sexes, with the exceptions of stage C in females and stage D in males (106).

### 6.2 Dental age estimation methods using seven permanent mandibular teeth in children

Information and insight into the time of development of permanent teeth have importance in different fields. In clinical medicine, particularly in pediatrics, it can be used as an indicator of maturity or for showing improvement or side effects of a specific therapy (107, 108). In clinical dentistry, such as orthodontics, it can be used in combination with other skeletal methods to compare different patterns or to estimate the exact time of starting or finishing an intervention (109-111). Dental age estimation procedures are most commonly used to compare with other biological systems of the human body in relation to legal matters and criminal investigations when the date of birth of an individual is not known (112). Previous studies showed that there is no difference in the dental development between the left and right side of the dental arches, so analysis of only the left side of both jaws is representative for target populations (28, 57, 89, 97). There are two main approaches to the evaluation of dental development of permanent teeth: (1) evaluation of eruption and (2) evaluation of mineralization on panoramic radiographs (113, 114). Eruption is mostly influenced by dental development and mineralization, but also by local factors including alveolar space, previous local trauma of deciduous teeth, environmental and nutritional variations (54). Methods using OPTs are more appropriate because it is possible to evaluate the development of the whole dentition, pre- and post-eruption, in contrast to the insight into the number of teeth that are located in the mouth. Most approaches to dental age estimation differentiate variance in the time span of the first seven teeth, which generally mature between 12 and 14 years of age.

The second part of this study evaluated the accuracy of three age estimation methods, the first two based on the development of seven permanent mandibular teeth based on Demirjian scoring system and the 3$^{rd}$ method by measuring projections of open apices and length of teeth and counting the number of teeth with complete mineralization (57, 88). The sample size and age distribution were conditioned by the available images that were collected from two orthodontic practices and represent the distribution of patients being treated there. At the time of collection, these were the only two orthodontic practices in the city of Gaborone, and it was not possible to provide a sufficient number of images for an even distribution across age groups (115, 116). The accuracy and absolute accuracy or mean prediction error of the Demirjian, Willems, and Cameriere methods were tested in this study. The best accuracy or the smallest difference between dental and chronological age in males was seen in the Cameriere method, -0.11 years, followed by the Willems method, 0.58 years, and the least accurate was the Demirjian method, an overestimation of 1.68 years. In females, the best accuracy was seen in the Willems method, -0.1, followed by the Cameriere method, -0.33 years, and the least accurate was also the Demirjian method (1973), with an overestimation of 0.72 years. Most of the previous studies of the Demirjian method from 1973 showed a mean overestimation of dental age when tested on different samples (102, 112, 117). Our results of the overestimation of dental age are generally in line with most previous studies (112). Yan et al. (118) selected 26 studies for meta-analysis of the Demirjian method with a total of 11,499 children and showed a mean overestimation of 0.35 years in males and 0.39 in females. Additional analysis by origin showed that in males, dental age was lesser overestimated in Asians, 0.28 years, than in Caucasians, 0.38 years. In females, dental age was overestimated by 0.24 years in Asians and 0.52 years in Caucasians (118). A systematic review and meta-analysis by Jayaraman et al. (119) identified 274 studies which used the French-Canadian data set of Demirjian for dental age estimation, published between 1973 and December 2011, where 34 studies were appropriate for qualitative analysis, and 12 studies for quantitative assessment and meta-analysis (119). The Demirjian dataset overestimated the age of males by 0.60 years (-0.23 years to +3.04 years) and females by 0.65 years (-0.10 years to +2.82 years) (119).

Differences between dental and chronological age showed an overestimation in most studies, except in Venezuelan Amerindians (120). The drawback of these two meta-analyses was that they did not separately analyze the Demirjian methods from 1973 and 1976 as they used a different dataset, including sample size and distribution, for scoring dental maturity (57, 95). Willems et al. (64) improved and simplified the Demirjian method on seven teeth based on the sample of Belgian Caucasians and by using weighted analysis of the variance of each specific tooth in dental maturity score. Comparative studies between different Demirjian and Willems methods showed that the Willems accuracy was superior to the Demirjian (102, 117, 121, 122). Sehrawat and Singh (123) evaluated the Willems method for dental age estimation from 15 studies in a meta-analysis. It was found that the Willems method overestimates the age of children to a comparatively lesser extent, 0.04 years in males and 0.02 years in females.

Cameriere et al. (88) presented a linear regression formula for age estimation based on the relationship between age and measurement of open apices in teeth in 455 Italian children, aged 5 to 15 years. The correlations between age and open apices in teeth were significant and negative. Furthermore, gender and the number of teeth with the apical end of the root canals entirely closed showed a significant correlation with chronological age. In the following year, a European formula based on the sample of 2654 OPTs of children from Croatia, Germany, Kosovo, Italy, Slovenia, Spain, and the United Kingdom was published (65). In this study, the European formula was tested using the Cameriere method from 2007. The mean results of the Cameriere method of -0.11 years in males and -0.33 years in females in our Botswana children are generally in line with previous studies (104, 124-127). Gulsahi et al. (124) tested the European formula on 573 Turkish children aged 8 to 15 years, and the mean underestimation was -0.47 years in males and -0.24 years in females. Another Turkish study by Ozveren et al. (125) compared the Cameriere and Willems methods on the sample of 636 children between 6 and 15 years of age and reported the mean underestimation for Cameriere by -0.18 years in males and -0.37 years in females, while the Willems method overestimated by 0.52 years in males and 0.30 years in females. Galić et al. (104) compared the Willems, Haavikko and Cameriere methods on 1089 Bosnia-Herzegovina children 6 to 13 years of age, where the Cameriere method underestimated by -0.02 years for males and overestimated the mean age by 0.09 years for females, while the Willems method overestimated the mean age by 0.42 years in males and 0.24 years in females. A study from Serbia by Marinković et al. (127) on 423 children 5 to 15 years of age compared the European formula to the Willems method. The authors reported the mean underestimation of -0.38 years for the Cameriere method in both sexes and overestimation of 0.63 years in males and 0.58 years in females. The obtained results from different studies indicate that the Cameriere European formula is useful for age estimation in children and comparable to the Willems method, and both are widely accepted as standard and improved methods for age estimation in children in comparison to the Demirjian method. The Botswana results showed the highest error or underestimation in the oldest children, older than 13 years of age, with the most significant error in the oldest age group. The results of the Cameriere method may be contributed to specific factors, which are the linear regression formula used for age calculation and the specific size and sample distribution. A linear regression model, as we used in this study, overestimates dental age in the youngest participants and underestimate it in the oldest, which is a common limitation of those methods for age estimation based on linear regression models (128). Liversidge (99) indicated that at the age of 13 years, only a few individuals would not have finished maturation of their second molars, so this is excepted from the sample. The fraction of children with unfinished maturation of the second molar detectable on OPTs and available for measurement significantly decreases (102). Ambarkova et al. (102) reported individuals with complete maturation of the second molars at the age of 12 years primarily, while at 13 years, 41% males and 84% females had complete mineralization of the second mandibular molar. Both the particular regression model and the proportion of individuals with delayed maturation may contribute to the underestimation of dental age in the 14-years-old group. The mean values of DA-CA depend on the sample size, participant distribution among age groups, and age range, so if more participants are distributed in older ages, the mean age of DA-CA will be underestimated. The results of MAE show the mean absolute difference between DA and CA and may better present the accuracy of the method (129, 130). The results of MAE in Botswana sample of 0.91 years, 0.94 years and 1.36 years in males and 0.81 years, 0.95 years and 0.96 years in females for the Willems, Cameriere and Demirjian methods respectively indicated the best performance of the Willems method for age estimation in Botswana children. The obtained values of accuracy are worse than reported by Galić et al. in the Bosnian-Herzegovinian study; MAE for the Cameriere method was 0.55 years for males and 0.53 years for females, and for the Willems method 0.67 years for males and 0.69 years for females. Another study on the sample from Italy, Spain and Croatia showed MAE of 1.01 years for the Demirjian, 0.93 years for the Willems and 0.50 years for the Cameriere methods in males and 1.13 years for the Demirjian, 0.93 years for the Willems and 0.48 years for the Cameriere methods in females (122). Rivera et al. (131), in a study on 526 Colombian children 6 to 14 years of age, showed a considerable absolute accuracy of the European formula; the mean overestimation by 0.08 years and MAE 0.57 years in males and underestimation by -0.25 years and MAE 0.57 years in females. According to findings on the Botswana sample, the Willems method showed the smallest difference between dental age and chronological age and may be recommended for age estimation if all seven teeth are available. In our study, the repeated measures ANOVA verified the statistically significant differences of mean DA-CA among tested methods. DA-CA was statistically significantly different among all three methods and between each other in both sexes. The repeated measures ANOVA of MAE among all three methods also showed a statistically significant difference, while posthoc pairwise comparison showed that MAE of the Demirjian method was statistically significantly different to the Willems and the Cameriere methods in males and the Willems method was statistically significantly different to the Demirjian and the Cameriere methods in females. There are different ways to quantify the age estimation methods, including the lack of bias, mean or median absolute difference, as well as a high percentage of age within six months (129). Liversidge et al. (99, 129) indicated that the most important is the smallest mean absolute error. In this study, both DA-CA and MAE was reported, as recommended by Liversidge [43]. The best performance, according to MAE, also showed the Willems method with MAE of 0.61 years in males and 0.64 years in females. Consequently, in this research, the effectiveness of the six methods was compared regarding a mean absolute error between the estimated and actual age, and the number of age estimates that were either < ±1 year considered as accurate from actual age, otherwise > ±1 year were considered as inaccurate (132). The age error of up to 1.0 year is considered accurate in forensic anthropology in most circumstances (133). The mean age error across different age groups in this study just partly accomplish these criteria.

### 6.3 Dental age estimation methods using mandibular third molars

In late adolescence up until early adulthood, the third molars are the only permanent teeth that are still developing that may be useful for age estimation and discrimination between majors and minors (100, 101). Using conventional radiography, one can study the mineralization of third molars from the initial crypt stage, which can start from 5 to 12 years of age, up to the apex closure, which can happen as late as 25 years of age (70, 134, 135). An open apex was not found in any participants 24 years or older in the Botswana sample, so they were excluded from additional analysis. This upper age limit of their development was also reported in other studies on different ethnic groups from England and South Africa (84, 90). The mean measured values of $I_{3M}$ and total performances of the discrimination test will be affected by older participants whose apices have closed, so they should be excluded from the study that was performed (84). To avoid peaking at mean age, the number of available participants across the age range can ensure consistency over the entire range (84).

The more advanced phases of the development and final maturation of the roots of third molars were identified as a developmental span, which is useful for discrimination between majors and minors (67, 84). Lower third molar development in participants in this study was completed between 17 to 23 years of age. This indicates that people from this population with evidence of apex closure of their third molars should be recognized as having reached 17 years of age at least. No statistically significant difference was observed in the maturation rate of lower third molars across the different $I_{3M}$ ranges between black African males and females from Botswana. Previous European studies on $I_{3M}$ for discriminating majors and minors (67, 136) are in line with these findings. In the Brazilian study, most of the $I_{3M}$ ranges found early mineralization and significant sexual dimorphism in males, except for the $I_{3M}$ range from 0.7 to 0.9 (137). In the Croatian study, dimorphism was also found for $I_{3M}$, ranging from 0.00 to 0.3 (138) and also in the Albanian sample, ranging from 0.04 to 0.08 (139). In general, most studies showed male precedence in the development of third molars (140-142). Comparative studies indicate greater sexual dimorphism of third molars in black populations than in other ancestries (140, 143).

A comparison of the linear regression formulas of different third molar registration methods showed the best performance by the Demirjian3M for estimating dental age. The best performance was evident by the smallest RMSE of 1.66 years in males and 1.68 years in females as well as the smallest MAE on the test sample, 1.32 years in males, and 1.40 years in females. The worst performance of the $R^2$ of the Cameriere$I_{3M}$ method is evident if compared to the Köhler3M, the mean difference of 147 days in males and 119 days in females. The difference between the real age and the predicted age as a linear function of the true age was evaluated on the test sample for the Demirjian3M, Köhler3M, and Cameriere$I_{3M}$ methods. A comparison of the boxplots and reported quantifications of DA-CA in an age cohort shows the most significant error using the Cameriere$I_{3M}$ registration method. The same boxplot shows that young individuals are generally overestimated up to 20 years of age, while older individuals were underestimated, especially using Cameriere$I_{3M}$ in both sexes. According to Thevissen et al. (80), the Cameriere method of registering dental development is a different approach to registering tooth development, and it registers continuous variables. The Cameriere technique is, in principle, measuring changes of apical pulp widths of developing third molars (80). Thevissen et al. (80), also noted that measures and related ratios used to register molar development, incorporate the variance in tooth size between individuals, which could explain the lowest $R^2$ and highest RMSE values if compared with the other two methods of third molar staging registration techniques. The use of a staging technique should mainly depend on the number of stages available in the developmental period of interest. Generally, the more stages used by the method, the less precise the classification. The error made by a misclassification of a stage will ponder much higher in age prediction than the one obtained by choosing a technique promising slightly better age predictions (80).

As seen in the results, all three tested methods showed a mean age of prediction or error of over ±1 year of age, which is considered inaccurate in forensic anthropology in most circumstances. A linear regression model, as used in this study, may overestimate the dental age in the youngest participants and underestimate it in the oldest (128). This is an ordinary limitation of methods for age estimation based on linear regression models and may be improved by different data processing, such as Bayesian prediction models (100, 110). The extended age prediction intervals based on third molar development could be reduced by combining with age-related skeletal evidence (134).

Although third molars are not reliable in accurately estimating an individual's age, research to date suggests their possible application in assessing the likelihood of reaching adulthood. An age span of development of third molars and evidence of the use of different third molar registration techniques for discriminating adults from minors in different populations pointed to the need to examine the sample from Botswana for the same purpose (144-149). Three different registration methods of development of third molars showed a high potential of application of each method. The values of quantities of both staging methods indicate the usefulness of only the last stages of mineralization, the best performance to discriminate adults from minors were for stage H in males and stage G in females for the Demirjian method and stage A½ for the Köhler method. Some better performance of the specificity, a mandatory performance of the test to accurately select minors and to avoid selecting them in the adult group, could be achieved only if the Demirjian stage H in females and the Köhler stage Ac are used for the test. The Cameriere $I_{3M}$ <0.08 cut-off value indicated the best specificity and Bayes' post-test probability. With this cut-off value, accurate classification is achieved, with less than 10% incorrect classification, and these findings are better than 17% previously reported by Cameriere et al. in the original study (67) on the Italian sample, or 36% and 26% when early and late root stages were reported by Liversidge and Marsden (84) on white and Bangladeshi samples from the United Kingdom. Results of ROC in this study are also comparable to values of 0.90 for MSS from Liversidge and Marsden (84), 0.72 from Garamendi et al. (150), 0.85 from Thevissen et al. (100), 0.83, 0.86, 0.90 from Martin-de-las-Heras et al. (151) for the Demirjian stages. Sensitivity results measure how well the cut-off value of $I_{3M}$ or the specific stages classify those who are 18 years and older and the specificity measures how well it classifies those who are younger than 18 years (67, 84, 151). The specificity values, 0.94 and 0.96 in males and females respectively with a sensitivity of 0.88, are better than for any Moorrees cut-off stage from the study by Liversidge and Marsden (84) or from the Demirjian stage study on two Spanish and Magrebian populations by Martin-de-las-Heras et al. (151). The results obtained are also comparable to findings from the previous studies on $I_{3M}$ in different populations (67, 136, 138, 139, 152).

The discrimination performance of the test, from a forensic science point of view, should show better specificity or as few minors being classified as majors (67, 151) as possible. This means in theory that other than the better total discrimination performance of the cut-off value of $I_{3M}$ <0.10, f, the value of $I_{3M}$ <0.08, for forensic purposes, indicated high confidence for properly selecting minors in children and young adults of a totally different geographical, ethnic and socio-economic background, since previously tested specific cut-off values were not established.

In practice, this means that it is ethically better to underestimate age than to overestimate age, from a forensic and legal point of view, due to constitutional and judicial implications when involving a possible major (adult). These findings also show the similarity among the so far tested populations in the tempo of late root development and final closure of the apex of third molars (67). Liversidge et al. have also noted that the dentition in other African children tends to develop faster than in Caucasian or Asian children, including a shift in the timing of initiation of third molar development (90). This is why it is important to assess the sample population's third molars and to compare them to previously published literature from other populations of similar geographic locations and populations from all across the world.

The results of PPV were 0.94 and 0.97 in males and females, respectively. The probability that an individual with an $I_{3M}$ <0.08 is a major is comparable to results obtained in evaluating mature apices in previous studies that used the staging system of the third molars (84). Liversidge and Mardsen (84) reported a value of 0.95 and listed that the values from the previous studies were 0.88 to 1.00. Positive likelihood ratios (LR+) assess the potential use of a diagnostic test, assessing how likely an individual in question is appropriately classified. It is an appropriate measure at an individual level, and for the selected cut-off value of $I_{3M}$ <0.08, it was 13.67 for males, and an even better result in females (84). This means that an individual with an $I_{3M}$ <0.08 is over 13 times more likely to be correctly classified as a major than a minor. Similarly, the negative likelihood ratio (LR-) at the same cut-off point means that an individual with an $I_{3M} \geq 0.08$ is over 8 times more likely to be correctly classified as a minor in both sexes. These results indicate that the selected cut-off value of $I_{3M}<0.08$ was the most appropriate because it has balanced values of the positive and negative likelihood ratios, also meaning high LR+ and poorer LH-. Lower specific cut-off values of $I_{3M}$ had good LR+ but poorer LR-, meaning that there was a good prediction of the probability of majority while higher cut-off values showed lower LRs-, good at predicting minority. Observations were similar when different cut-offs of MSS or DSS stages were evaluated (84, 151). The same cut-off values of $I_{3M}$ also indicated appropriateness when they were tested in other populations (136-139, 152).

The findings for intra-rater and inter-rater agreements for $I_{3M}$ were excellent and comparable to those studies using the staging systems (84). $I_{3M}$ depends heavily on the accurate measuring of specific projection points of third molars on the OPTs, which is not the case for the staging systems. According to Liversidge and Marsden (84), the reproducibility of the test was better if there were fewer intermediate stages of mineralization of third molars when compared to the systems that had more stages. Dhanjal et al. (91) showed that the Demirjian system indicated better agreement than methods that use more stages for evaluation if the test re-test was performed by the same observers and different observers. This is in agreement with Liversidge and Marsden (84), who also found better kappa values for DSS that had fewer stages than for MSS. The issue in assigning the stages arises when the tooth in development has reached a point somewhere in between the two available developmental stages, and this is when Demirjian suggests assigning the lower stages if tooth development appears between stages (57). Mineralization stages of third molars that are misclassified lead to more significant differences in the estimated age and may misclassify majors and minors. For discrimination between majors and minors, evidence of the final stage H or completion of stages A–D, using the Demirjian stages, can indicate that the investigated person is a major or minor, respectively (74). Liversidge and Marsden (84) showed adapted maturity data for halfway between the mean age entering a Demirjian tooth stage as the difference between mean age for stage F being 16.9 years and 17.3 and for stage G being 18.4 years and 19.5 years in males and females respectively. Even a 95% CI of a lower stage E includes the age of 18 years, which discriminates majors and minors.

Few studies have been done on third molar development on blacks in Africa or abroad (74, 84, 115, 153). Mincer et al. did earlier studies on third molar development for the prediction of attainment of 18 years of age in 1993 (74). Their sample consisted of only 19% American blacks (74). The study reported no difference between blacks and whites in terms of the time of tooth development. However, the authors state that the lack of difference may be due to the humble sample of black African-Americans in the study, who made up only one fifth, and the uneven distribution of the sample (74). Their findings showed that the third molar could not be used as a highly accurate age indicator and that more than 90% showing Demirjian stage H of development were 18 years or older. The questionable accuracy of this method was addressed due to the fact that there are only four stages that describe the development of the roots from the initial appearance to the stage before the closure of the apex (74). This all takes place in a wide age range, and stages of development do not correspond to a uniform time span in development (74). A statistically significant but small improvement was observed in the prediction of age if the corresponding maxillary and mandibular molars were added to the prediction model (74). This was of little practical use, according to Mincer et al. (74), because of a small reduction in the mean error of 0.1 years, which implies that only one mandibular tooth can give sufficient information for age prediction for this purpose. Mincer et al. (74) also presented empirical probabilities on being a major (18 years) based on specific DSS staging of third molars; however, this data was only used for whites in the study because of the small and uneven sample size of black participants. Other than in the applicability of a particular stage as a cut-off for discriminating between majors and minors, it is not possible to compare the results to the Demirjian or Moorrees stages. Cameriere et al. (67) showed that if stage H was changed with stage G by Demirjian, an estimated probability that the individual is a major decreased from 0.98 to 0.94. Sensitivity values for stages H and G were 0.58 and 0.75, respectively, while specificity values were 0.98 and 0.90, respectively. Also, the proposed value of $I_{3M}$ <0.08 showed a 17% incorrect classification, with a better specificity, which is ethically more mandatory for legal and criminal purposes (67, 84, 150). Samples from different populations were tested and results showed similar incorrect classification and discrimination ability of the purposed cut-off value of $I_{3M}$ (136, 139, 152). Mineralization of third molars in blacks and its usefulness for discrimination between majors and minors was also studied by Olze et al. (154), Blankenship et al. (153), Mincer et al. (74), Harris and McKee (155) and Liversidge et al. (90).

According to a literature search, this is the first radiographic study on the usefulness of $I_{3M}$ in discriminating between majority and minority in black African children from Botswana or elsewhere in sub-Saharan Africa. This expansive geographic area had a regional population of over 920 million in 2007 and has already shown signs of significant overpopulation problems and an annual high growth rate of 2.3%, with a projection of growth of 1.300 billion up to the year 2050 (156). This area displays great diversity in languages, religions, cultures, and extensive social and economic disparities. This indicates the needs for illegal migrations in the region and Botswana, together with South Africa and Namibia, are indicated and desirable as potential countries for immigration (157). Also from these regions and further travel north, thousands of immigrants, including unaccompanied minors, transit through North Africa and over the Mediterranean to the closest European countries (EU), mostly Italy, Spain, and then to others (136, 138, 158). Many of these migrants may have no reliable documentation or even registered birth records, as seen with some indigenous populations in Botswana (60, 136). Legislation and immigration policies, including penal and criminal law in different countries, recognize two significant age limits, mostly being of adult age or a minor (84, 110, 138). In the Republic Botswana, whether a person is tried in court as a minor or an adult may have very different consequences because capital punishment (death sentence by hanging) is still practiced in some circumstances (159).

The legal adult age is set at 18 years in many countries, and recently in Botswana, it has been reduced to 18 years from the previous 21 years (84, 160). Different levels of correct classification of a person under investigation may be sufficient for legal civil and criminal proceedings (161, 162). According to Corradi et al. (161), only 51% correct classification can be sufficient for civil cases with more probable than not evidence. On the other hand, a very high level of least 90% was needed for criminal cases, which require beyond any reasonable doubt evidence. In the tested sample, the suggested cut-off value of $I_{3M} < 0.08$ meets both legal standards. For different medico-legal purposes, there are general agreements in guidelines and procedures to estimate someone's age, as proposed by AGFAD or FASE (137). From this point of view and according to the findings in the study, a specific cut-off value $I_{3M} < 0.08$ may be used as a reliable method, within the confidence interval, for discriminating majors and minors in black Africans from Botswana.

In conclusion, the results of this study, which was carried out on a previously unexamined sample of a specific geographic and ethnic origin, confirm the validity of the cut-off value of $I_{3M} < 0.08$ for discrimination between adults and minors. This finding is useful in daily medico-legal practice when a qualified forensic examiner must determine whether the subject being investigated is an adult or a minor. This is very important when there are no validated cut-off values available for the population of the subject at hand.

Finally, further aims of this study were to establish or validate the cut-off value of $I_{3M}$ with the best possible accuracy and likelihood ratio for discrimination between the age of majority or minority, with an acceptable small incorrect classification, regardless of race and socio-economic standing. Considering that the findings are in correspondence with results obtained from other populations, it should be justifiable for use in medico-legal practice.

# 7. CONCLUSIONS

1. In the sample of black African children and adolescents, the antagonistic teeth showed similar development, with a statistically significant difference in some stages for a few teeth. The advanced development of mandibular teeth was found for Demirjian stage G between the central incisors, stages E and G between lateral incisors and stage G between first molars. In contrast, significantly advanced development of maxillary teeth was found in stage G in second premolars in males. The significant difference in advanced development of mandibular teeth was only found in stage G between antagonists of both incisors, stage E of the second incisor, stage E in the second premolars and stage G in the first molars and advanced development of maxillary teeth was only found for stage G in the second premolar in females. The difference in mean age within stage between sexes in maxillary teeth was found at stage F for the central incisors, stage F for the second molars and stages D and F for the third molars, while in the mandibular teeth, it was found at stage G for the canines, stage E for the second premolars and stage F for the second molars.
2. The Cameriere method is the most accurate for estimating dental age in males followed by the Willems method, while the Willems method is the most accurate in females following Cameriere; therefore, one may encourage both methods to be used for age estimation in Batswana children.
3. The Demirjian method overestimated the dental age in both sexes and showed that it is not a suitable method for age estimation. This also shows that there is a need for establishing specific standards for Batswana children of black African origin.
4. Different age predicting results were found among linear regression models of the three mandibular third molars mineralization registration methods. The staging registration methods were advanced for age estimation if compared to methods of measuring open apices and heights of developing teeth. All three of the tested methods showed mean age prediction or error over ±1 year of age, which is considered inaccurate in forensic anthropology in most circumstances.
5. The results of this study carried out on a previously unexamined sample of mandibular third molars of a specific geographic and ethnical origin, confirm the validity of the cut-off value of $I_{3M}$ <0.08, the Köhler stage $A_{½}$ for both sexes and the Demirjian stage H in males and stage G in females for discriminating between Batswana young adults and

minors. This finding is useful in daily medico-legal practice, where a qualified forensic examiner must decide on or classify the age of the investigated person as an adult or minor. It is also particularly significant in Botswana, where capital punishment is still practiced, and an adult who commits a crime could be sentenced to death. Also, it is particularly important when there are no available validated cut-off values for the population of the subject in question. Finally, further goals of this study were to validate or establish the cut-off value for the Demirjian, Köhler stages, and $I_{3M}$ with the best possible accuracy and likelihood ratio for discrimination between the age of majority or minority with an acceptable small incorrect classification, regardless of race and socio-economic standing. Considering that the findings are in correspondence with other populations, it should be justifiable to use in medico-legal practice.

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# 9. BIOGRAPHY AND LIST OF PUBLISHED WORKS

Dr. Cavric was born in Belgrade, Serbia and moved to Botswana with her family in the early nineties. Upon completing the International Baccalaureate program at Westwood International School in Gaborone, Botswana in 2006, she decided to attend dental school in Europe and graduated as a Doctor of Dental Medicine from Charles University in Prague in 2012. She started her PhD studies at the University of Zagreb, School of Dental Medicine in 2012 and commenced work at the Princess Marina Hospital in Botswana in the same year. While working in Botswana, she collected data for her study. She then moved to the United States in 2015 and wished to continue practicing as a dentist, and in order to do so, she nostrificated her diploma and gained US dental licensure at Boston University's Goldman School of Dental Medicine, where she graduated with High Honors and OKU (Dental Honors Society) recognition in 2017. She is currently working as a dentist in a private practice in Phoenix, Arizona.

**LIST OF PUBLISHED WORKS**

**Scientific articles emerged from doctoral dissertation:**

1. **Cavrić, Jelena**; Galić, Ivan; Vodanović, Marin; Brkić, Hrvoje; Gregov, Jelena; Viva, Serena; Reu; Laura, Paula; Cameriere, Roberto. Third molar maturity index (I3M) for assessing age of majority in a black African population in Botswana // International journal of legal medicine, 130 (2016), 4; 1109-1120 doi:10.1007/s00414-016-1344-1. **Q1 Web of Science, Q1 Scopus**

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**Other paper:**

3. Latić-Dautović, Melina; Nakaš, Enita; Jelešković, Azra; **Cavrić, Jelena**; Galić, Ivan. Cameriere's European formula for age estimation: A study on the children in Bosnia and Herzegovina // South Eur J Orthod Dentofac Res, 4(2017), 2;26-30 doi: 10.5937/sejodr4-15528.

**International presentations:**

1. **Cavrić, Jelena**; Vodanović, Marin; Viva, S; Reu, LP; Cameriere, R; Galic, Ivan Third Molar Maturity Index (I3M) for Assessing Age of Majority in a Black African Population in Botswana // Proceedings of 68th Annual Scientific Meeting of American Academy of Forensic Sciences, Las Vegas, NV, February 22 - 27, 2016, page 664. Proceedings of 68th Annual Scientific Meeting of American Academy of Forensic Sciences, Las Vegas, NV, February 22 - 27, 2016, page 664. Las Vegas, SAD, 2016.

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