Int J Legal Med (2016) 130:1109–1120
DOI 10.1007/s00414-016-1344-1

ORIGINAL ARTICLE

Third molar maturity index (I_{3M}) for assessing age of majority in a black African population in Botswana

Jelena Cavrić[1] · Ivan Galić[2] · Marin Vodanović[3] · Hrvoje Brkić[4] · Jelena Gregov[3] · Serena Viva[5] · Laura Rey[6] · Roberto Cameriere[6]

Received: 14 August 2015 / Accepted: 17 February 2016 / Published online: 14 March 2016

Abstract Assessment of legal age, also known as age of majority, is a controversial issue as there are few body biomarkers or evidence during late adolescence differentiating a subject from being a minor or adult. The third molar was recognized as a suitable site for age examination in late adolescence. We analyzed the development of the left mandibular third molar by the third molar maturity index (I_{3M}) and a specific cut-off value of $I_{3M}=0.08$, established by Cameriere et al. in 2008 and used it for discriminating between minors and adult black Africans from Gaborone, Botswana. A final sample of panoramic radiographs (OPTs) of 1294 people (582 males and 712 females) aged between 13 and 23 years was evaluated. The real age decreased as I_{3M} gradually increased. There was no statistically significant difference in the third molar development evaluated using I_{3M} between males and females ($p>0.05$) across different I_{3M} classes. Results of 2×2 contingency tables for different cut-off values indicated that $I_{3M}=0.08$ was useful in discriminating between adults and minors. Precisely, for $I_{3M}=0.08$, the values of accuracy or overall fraction of correctly classified were 0.91 in males with a 95 % confidence interval (95 % CI) of 0.88 to 0.93 and 0.92 (95 % CI, 0.90 to 0.93) in females. Values of sensitivity of the test or the proportion of participants being 18 years and older were 0.88 (95 % CI, 0.87 to 0.90) in males and 0.88 (95 % CI, 0.90 to 0.93) in females, while values of specificity or proportion of individuals younger than 18 who have I_{3M} <0.08 were 0.94 (95 % CI, 0.91 to 0.96) in males and 0.96 (95 % CI, 0.94 to 0.98) in females. Positive predictive values of the test, where

Jelena Cavrić and Ivan Galić contributed equally to this work.

✉ Ivan Galić
igalic@mefst.hr

Jelena Cavrić
jelena.cavric@gmail.com

Marin Vodanović
vodanovic@sfzg.hr

Hrvoje Brkić
brkic@sfzg.hr

Jelena Gregov
jelena.galic@gmail.com

Serena Viva
serenaviva@hotmail.it

Laura Rey
reylau@live.com

Roberto Cameriere
roberto.cameriere@unimc.it

1 Henry M. Goldman School of Dental Medicine, Boston University, 100 E Newton St, Boston, MA 02118, USA

2 Departments of Research in Biomedicine and Health and Dental Medicine, University of Split School of Medicine, Šoltanska 2, Split, 21000, Croatia

3 Department of Dental Anthropology, School of Dental Medicine, University of Zagreb, Gundulićeva 5, 10000 Zagreb, Croatia

4 School of Dental Medicine, University of Zagreb, Gundulićeva 5, 10000 Zagreb, Croatia

5 Dipartimento di Civilta e Forme del Sapere, University of Pisa, Via L. Galvani 1, I-56126 Pisa, Italy

6 AgEstimation Project, Institute of Legal Medicine, University of Macerata, Macerata, Italy

the participants whose I_{3M} <0.08 were adults, were 0.94 (95 % CI 0.91 to 0.96) in males and 0.97 (95 % CI, 0.94 to 0.98) in females, while negative predictive values of the test, where the participants whose I_{3M} was ≥0.08 were minors, were 0.88 (95 % CI 0.85 to 0.90) in males and 0.97 (95 % CI, 0.94 to 0.98) in females. The likelihood ratios of the positive test (LR+) were 13.67 (95 % CI, 9.21 to 21.02) in males and 23.73 (95 % CI, 14.20 to 42.28) in females, while likelihood ratios of the negative test (LR−) were 0.12 (95 % CI 0.10 to 0.16) in males and 0.12 (95 % CI, 0.11 to 0.15) in females. Bayes post-test probabilities, *p*, were 0.94 (95 % CI 0.90 to 0.98) in males and 0.97 (95 %CI, 0.93 to 1.00) in females. These results indicate with high accuracy that I_{3M} may be a useful alternative method in legal and forensic practice to discriminate individuals of black African origin who are around the legal adult age of 18 years in Botswana. Further studies should address the usefulness of this method and specific cut-off for different adolescent populations.

Keywords Botswana · Legal medicine · Third molar maturity index · Accompanied minor · Age estimation · Age of majority

Introduction

Whether a person is tried in court as a minor or adult, it can have very different consequences, especially in the Republic of Botswana, where capital punishment is still practiced in some circumstances [1]. This means that a reliable method for age estimation is critical in cases of unknown or withheld age [1]. To accurately determine one's age, legal documents such as drivers' licenses, passports, or identity cards are needed. Documents such as birth certificate or passport could be lost or repossessed such as in the case of displacement, refugees, asylum seeking, or during the war [2]. In the case when identification documents are missing, a full medical examination, including physical examination with additional skeletal and dental radiography, is necessary [2]. The estimation of mental maturity commonly overestimates the age since it is assumed that juveniles are mentally more mature than their age [2]. In situations where an individual does not wish to reveal their age or suspected tampering of documents has occurred, other available documented records and medical exams assessing age are compared to give a better idea on the actual age [2–4]. In 2010, Botswana reduced the classification of age of majority from a previous 21 to 18 years [5]. Juveniles in Botswana between the ages of 14–17 years who have been found guilty of misconduct stand trial in the Juvenile Courts while adults, 18 years and above, are tried in the High Court [6]. Some children and adolescents born in Botswana, especially in more isolated districts, might not have had their date of birth recorded in medical or hospital charts. The indigenous tribes of the Central and Ghanzi districts (the Basarwa) often are not born in proper healthcare facilities but out in the bush [7]. Commonly, children in Botswana do not seek legal documents (passports) since it is quite rare that they have travelled out of the country until they have turned 18 years (legal age) or 16 years to obtain a National identity card or Omang. Birth records have only recently become digitalized, thus those that were born as early as the 90s might have lost their records and might not have a means of tracking them. Through the implementation of integrated patient information management system (IPMS) in 2010 in the government hospitals and clinics countrywide, there has been an improvement in the computerization and registration of a patient's details such as birth and death records. However, the system still faces regular issues such as effective utilization of human resources [8]. Also, due to the impact of HIV/AIDS, many children have been left orphaned and have entered orphanages without adequate documents or birth records [9]. According to the Children's Act, 2009 [10], a child refers to any person who is below the age of 18, while 14 years was set as age of criminal responsibility. The Marriage Act in Section 14 [11] strictly states that no person below the age of 18 may marry while the Penal Code recognizes 14 years in males and 16 years of age in females as the age of consent for a sexual relationship [12].

Botswana and South Africa have a more stable economy when compared to most sub-Saharan African countries, with gross domestic product per capita even ten times greater than some of the poorest countries [13]. This enhances Botswana as a desired destination for settlement and immigration, whether legal or illegal [13]. For example, due to the economic and political instability in neighboring countries such as Zimbabwe, an estimated 40,000–100,000 Zimbabwean reside in Botswana, of which an estimated third are illegal immigrants [14]. These facts all show that different age estimation techniques other than relying on legal documents are necessary and even more so these days in Botswana, akin to countries who face similar challenges in estimating the age of individuals when necessary for certain legal, criminal, forensic, humanitarian, and social reasons [15–21].

According to the Study Group on Forensic Age Diagnostics of the German Society of Legal Medicine (AGFAD) and Forensic Anthropology Society of Europe (FASE), there is a general agreement about the suitable methods available for age estimation [22, 23]. Examination of the dentition consisting of recording dental status and radiologic examinations of the dentition are listed after physical examination, which encompassed assessment of the constitution, inspection of sexual maturation, and radiologic examination of the left hand [22]. It is assumed that radiographic evaluation of the dental system in particular in a growing individual is more reliable than their skeletal system as it is less influenced by the systemic factors [24]. The analysis of developing permanent dentition in children up to 16 years of age, excluding third molars, on panoramic radiographs (OPT) is particularly interesting and generally considered accurate, with an error of up to a year [25–28]. The third molars were previously evaluated and thought to be an unreliable indicator in age estimation due to its varying presence, malposition, time of initial formation, and wide age range of mineralization [29, 30]. However, many studies have shown that a great proportion of adolescents and adults, aged 13 to 25 years, can be studied for age estimation by evaluating the development of their mandibular third molar [29, 31–36]. The third molars are in fact the only teeth available for assessing legal age because other permanent teeth mostly finish development between 12 to 14 years of age [25, 37–39]. Some participants and an additional proportion of third molars are often excluded from studies due to a wide age range of initial formation, the highest level of agenesis among all teeth or having a variable initial formation and completed mineralization [29, 30]. However, third molars are the only teeth that can be studied in the entire range of development from a crypt and first traces of mineralization up to the closed apex by using cross-sectional radiographic material [29]. Some previous studies on the third molars have focused on their applicability in discriminating between those who are 18 years and older and those younger than 18 years [31, 32, 40–48]. Historically, most studies have focused on analysis of the third molar mineralization using x-rays where a continuous process of their development has been divided into a finite number of stages. Different staging systems, original or adopted, previously used for the other permanent teeth were used for this purpose [49–52]. For example, the Moorrees et al. [53] staging system (MSS) recognizes a total of 14 stages from initial mineralization to apex closure and discriminates root development of mandibular molars by using 7 stages (R_i to $A_{1/2}$) from initial root formation up to apex closure, while the Demirjian et al. [28] staging system (DSS) recognizes 8 stages (A-H) and uses only 3 stages (D to G) for grading the development of roots. Cameriere et al. [32] showed that proportions of measurements of open apices and heights of third molars in development known as the third molar maturity index (I_{3M}), better discriminate adults and minors as being 18 years and older or younger than 18 years than the final stages of DSS, G, and H calculated on the Italian participants.

The tested sample in this study was the black African adolescents and adults in Botswana previously evaluated for the time of mineralization of permanent teeth using the Demirjian method [54]. There have not been previous studies on the usefulness of I_{3M} for discrimination between black adults and minors, in sub-Saharan Africa nor in other overseas countries where blacks represent a significant population.

The aim of this study was to evaluate the cut-off value of $I_{3M}=0.08$, previously recommended by Cameriere et al. [32] for discriminating black Africans from Botswana as adults or minors.

Materials and Methods

We analyzed OPTs of adolescents and adults of African origin from Botswana, aged between 13–23 years, who from 2001 until 2015 attended two private orthodontic practices in Gaborone, the capital city of Botswana, for diagnostic and treatment purposes. Also, we found no evidence of the development of permanent teeth in individuals over than 23 years. Socioeconomic status or belonging to specific ethnic groups or tribes was not evaluated among the participants. Patient data was recorded in an excel file, including date of birth, date the x-ray was taken, assigned patient number matching to patients x-ray, gender, and belonging to black Africans. All OPTs were recorded in JPG format and the ImageJ software, version 1.48v [56], was used to examine the images. OPTs without accompanying subject's full dental records, lack of birth date, and date when the OPTs were taken, as well as those OPTs of children with proven hereditary or systematic illnesses, malnutrition, severe destruction, extraction, or hypodontia of permanent teeth, and where the third molars were missing, were excluded from the study [54].

Additional criteria for the exclusion were OPTs with any pathologic processes or radiographic distortions obstructing clear visibility of the lower left third molar. Chronological age of each participant was calculated and converted into decimal ages. In total, 1294 OPTs of participants aged 13 to 23 (582 males and 712 females) were evaluated (Table 1).

The left lower third molar was assessed using the I_{3M} by the first author (JC) without knowledge of the date of birth of subjects in order to avoid bias during measuring of specific projections of third molars on OPT, as proposed by Cameriere et al. [32]. Briefly, I_{3M} is a ratio of sum of projections of open apices in multi-rooted teeth or apex width in single-rooted

Table 1 Panoramic radiographs from Botswana, according to sex and age where numbers in parenthesis represent samples with closed apices of the left third mandibular molar ($I_{3M}=0.00$)

Age (years)	Males	Females	Total
13	49	59	108
14	59	56	115
15	48	69	117
16	69	71	140
17	53 (4)	68 (1)	121 (5)
18	45 (9)	69 (20)	114 (29)
19	50 (33)	74 (42)	124 (75)
20	47 (37)	57 (42)	104 (79)
21	60 (48)	64 (52)	124 (100)
22	55 (52)	64 (57)	119 (109)
23	47 (41)	61 (56)	108 (97)
Total	582 (224)	712 (270)	1294 (494)

teeth and a tooth length of the mandibular third molar during growth [32]. If third molars were found with entirely closed roots, then $I_{3M}=0.00$ was recorded. The cut off value of $I_{3M}=0.08$ and additional cut-off values close to $I_{3M}=0.08$ were tested to discriminate adults (≥18 years) and minors (<18 years).

Statistical analysis

A single observer performed measurements of root apices and tooth lengths and calculated I_{3M}. Intra-class correlation coefficient (ICC) of I_{3M} was used for intra-rater and inter-rater agreement between the same and the second observer (IG), respectively. Fifty randomly selected OPTs were reexamined 1 month after initial examination for this purpose. Scatter plot and box plot graphs and tables were used to show relationships between chronological age and different I_{3M} indexes for both genders.

Overall effectiveness of I_{3M} was evaluated by plotting the receiver operating characteristic (ROC) curve [32]. The value of the area under the ROC curve shows the accuracy of the test or how well the test separates the participants being tested into adults or minors. An area of 1 represents a perfect test while an area of 0.5 represents a worthless test [57]. To test the performance of specific cut-off value of I_{3M}, the results were summarized in a single table which consists of 2 × 2 contingency tables. Generally, the single 2 × 2 table displayed the number of participants who have I_{3M} <0.08 and are 18 years and older (known as true positives), then participants with I_{3M} <0.08 who are younger than 18 years (known as false positives), followed by those with I_{3M} ≥0.08 who are 18 years and older (known as false negatives) and those with I_{3M} ≥0.08 who are younger than 18 years (known as true negatives). Values of the test were presented with 95 % confidence interval (95 % CI).

Measurements of accurately classified individuals (*AC*), the sensitivity of the test (p_1), the proportion of subjects equal to 18 years or more together with its specificity (p_2), the proportion of individuals younger than 18 years were evaluated. A single statistic that captures performance of a diagnostic test is Youden's index (*J-index*), a function of p_1 and p_2 [58]. Positive predictive value (*PPV*) are tests that look at out of the positive tests of how many were truly positive and correctly classified; negative predictive value (*NPV*) tests look at out of the negative tests of how many were truly negative and classified correctly [59]. 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 selected positive for the age of majority (true positive) divided by the probability that an individual younger than 18 selected positive for the age of majority (false positive). *LR*− is equivalent to the probability that an individual 18 and older selected negative for the age of majority (false negative) divided by the probability that an individual younger than 18 selected negative for the age of majority (true negative). *LR*+ greater than 1 increases the probability of being an adult and the smaller (*LR*+) decreases the probability [31]. In this study, the likelihood ratios summarize how many times more or less likely adults are to have I_{3M} <0.08 than minors and minors are to have I_{3M} ≥0.08 than majors [31, 60]. Values of likelihood ratios above 10 and below 0.1 are considered to provide strong evidence to accept or rule out assessment in most situations [60].

The Bayes post-test probability (*p*) of being 18 years or older (i.e., the proportion of individuals who are 18 years or older with I_{3M} <0.08) may help to discriminate between those who are or are not aged 18 years or more [32]. According to Bayes' theorem, *p* may be written as

$$P = \frac{p_1 p_0}{p_1 p_0 + (1 - p_2)(1 - p_0)} \tag{1}$$

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 the 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 additional 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 I_{3M} values. Results of *AC*, p_1, p_2, *J-index*, *PPV*, *NPV*, *LR*+, *LR*− as well as *p* for different cut-off values of I_{3M} were also presented.

Results

The intra-rater and inter-rater agreements of I_{3M} between the same and two different observers respectively were almost perfect. In detail, ICCs of I_{3M} were 0.99 (95 % CI 0.98 to 0.99) and 0.98 (95 % CI 0.97 to 0.99) for the intra-rater and inter-rater agreement, respectively. Real age gradually decreased as I_{3M} increased across I_{3M} classes in males and females, respectively (Fig. 1). Mean ages were not statistically significantly different between males and females across all I_{3M} ranges ($p > 0.05$) (Table 2).

The tested sample was evaluated separately for both sexes for ROC curve and cut-off values of I_{3M} according to Cameriere et al. [32] (Fig. 2).

The areas under the ROC curve were 0.96 (95 % CI, 0.95 to 0.98) in males and 0.97 (95 % CI, 0.96 to 0.98) in females,

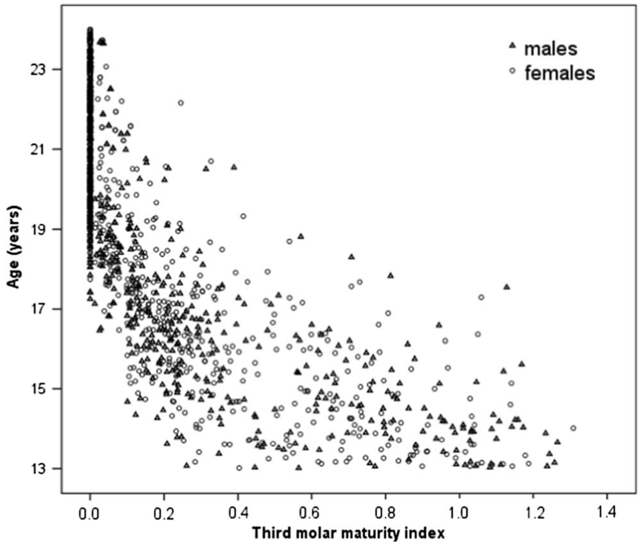

Fig. 1 Scatterplot of the relationship between chronological age and third molar maturity index of open apices of mandibular left third molar in black African males and females from Botswana

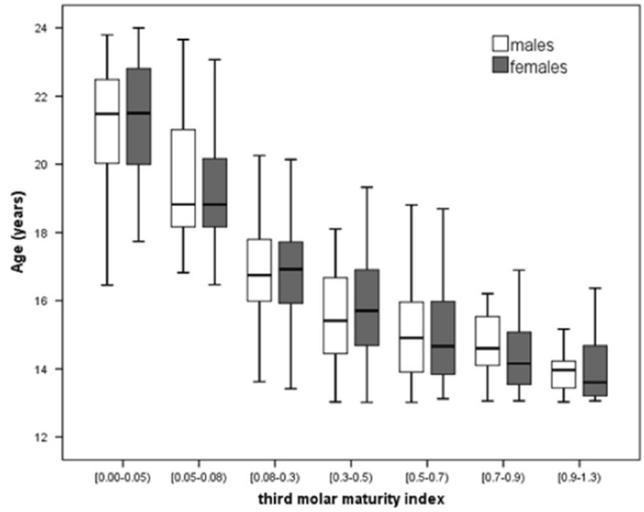

Fig. 2 Boxplot of the relationship between chronological age and third molar maturity index of open apices of the mandibular left third molar in black African males and females from Botswana. Boxplot shows medians and inter-quartile ranges while *whiskers* are lines extending from the box to maximum and minimum ages, excluding outliers

which indicated excellent performance of the method, according to Swets (Fig. 3) [57]. The results of cut-off value $I_{3M}=0.08$, to discriminate between adults and minors, were presented in 2 × 2 contingency tables (Table 3 for males and Table 4 for females), and the derived values of the test were presented in Table 5 for males and Table 6 for females.

In total, 1183 of the 1294 participants were accurately classified which indicates close association between the age of majority and that the test was positive or $I_{3M}<0.08$. In males, the overall fraction of accurately classified participants or *AC* was 0.91 (95 % CI, 0.88 to 0.93). Value of p_1 or proportion of correctly classified participants being 18 years and older was 0.88 (95 % CI, 0.86 to 0.90), while p_2 or proportion of correctly classified participants younger than 18 years was 0.94 (95 % CI, 0.91 to 0.96). *PPV* of the test, where the participants whose $I_{3M}<0.08$ were adults, was 0.94 (95 % CI, 0.91 to 0.96) while *NPV* of the test, where the participants whose $I_{3M}\geq0.08$ 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 5). The *LR*+ was 13.67 (95 % CI, 9.21 to 21.02) while the *LR*− was 0.12 (95 % CI, 0.10 to 0.16). Estimated post-test probability *p* was 0.94 (95 % CI 0.90 to 0.98).

In females, *AC* was 0.92 (95 % CI, 0.90 to 0.93), p_1 and p_2 were 0.88 (95 % CI, 0.86 to 0.89) and 0.96 (95 % CI, 0.94 to 0.98), respectively. *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 6), like in males. *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 estimated post-test probability *p* was 0.97 (95 % CI 0.93 to 1.00).

The results of discrimination between adults and minors by using additional cut-off values of I_{3M} (0.02 to 0.14) were also

Table 2 Summary statistics of chronological age according to sex and third molar maturity index (I_{3M}) classes

Cp	Males								Females									
I_{3M}	*N*	Mean	Sd	Min	Q_1	Med	Q_3	Max	*N*	Mean	Sd	Min	Q_1	Med	Q_3	Max	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	−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	0.54(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	0.17(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	−0.64(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	0.56(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	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	−0.08(69)	0.939

N number of individuals, *Mean* mean age within I_{3M} class, *Sd* standard deviation of mean age, *Min* minimum value, Q_1 first quartile, *Med* median, Q_3 third quartile, *Max* maximum age, *t* independent samples test, *df* degrees of freedom. Significance set at <0.05

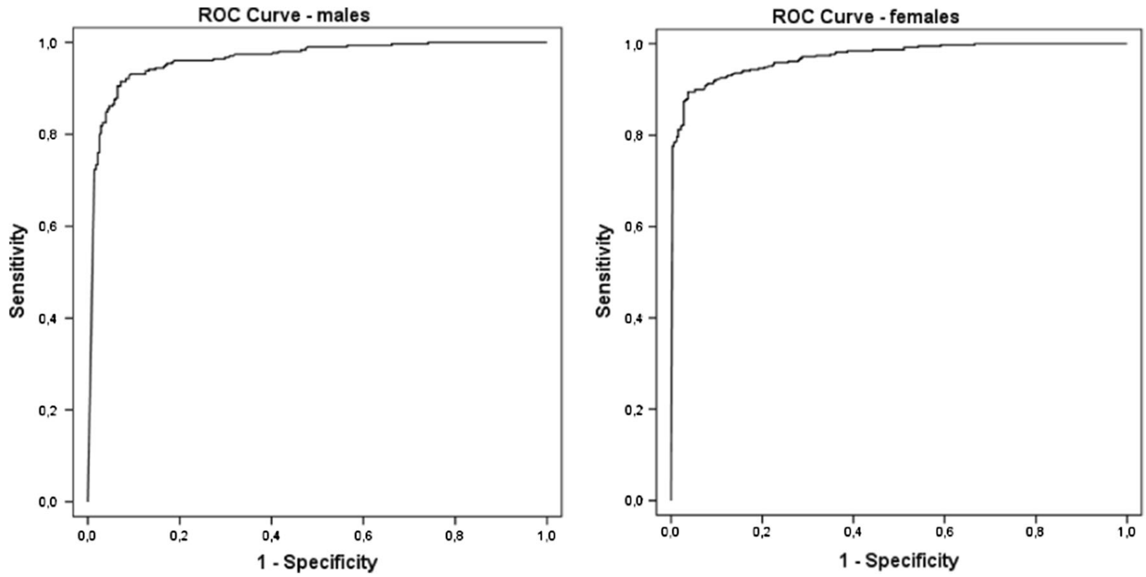

Fig. 3 Receiver operating characteristic (ROC) curves for third molar maturity index of the tooth 38 for predicting age of ≥18 years and <18 years in black Africans males and females from Botswana

presented in 2 × 2 contingency tables in Table 3 for males and Table 4 for females, and derived values of the tests were also presented in Tables 5 and 6. Tables 5 and 6 also showed that cut-off value of $I_{3M} = 0.10$ has similar *AC* and better *J-index* and appears more accurate. However, it is less appropriate for practical use as the obtained p_2 and Bayes' post-test probability p are less than for cut-off value of $I_{3M} = 0.08$ for both sexes. Specifically, in medicolegal matters of whether an individual in question is an adult or minor, it is more important that discrimination test shows a low proportion of false positive subjects (minors who are selected as adults) or higher specificity, p_2. From this point of view, the cut-off value of $I_{3M} = 0.08$, which represents better p_2 and Bayes' post-test probability p, may be recommended for discriminating 18 years and above or under in black Africans in Botswana.

Discussion

The third molars are the only permanent teeth in development during the specific age span from late adolescence to early adulthood that may be useful for age estimation and discrimination between adults and minors [33, 36]. Mineralization of the third molars can be studied by conventional radiography,

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

Test	Age		Total
	≥18	<18	
$I_{3M} < 0.02$	222	5	227
$I_{3M} \geq 0.02$	82	273	355
$I_{3M} < 0.04$	242	7	249
$I_{3M} \geq 0.04$	62	271	333
$I_{3M} < 0.06$	258	13	271
$I_{3M} \geq 0.06$	46	265	311
$I_{3M} < 0.08$	269	18	287
$I_{3M} \geq 0.08$	35	260	295
$I_{3M} < 0.10$	275	21	296
$I_{3M} \geq 0.10$	29	257	286
$I_{3M} < 0.12$	285	36	321
$I_{3M} \geq 0.12$	19	242	261
$I_{3M} < 0.14$	289	48	337
$I_{3M} \geq 0.14$	15	230	245
Total	304	278	582

Table 4 Contingency table describing discrimination performance of the test for different cut-off values of third molar maturity index (I_{3M}) in females

Test	Age		Total
	≥18	<18	
$I_{3M} < 0.02$	273	1	274
$I_{3M} \geq 0.02$	116	322	438
$I_{3M} < 0.04$	303	2	305
$I_{3M} \geq 0.04$	86	321	407
$I_{3M} < 0.06$	323	9	332
$I_{3M} \geq 0.06$	66	314	380
$I_{3M} < 0.08$	343	12	355
$I_{3M} \geq 0.08$	46	311	357
$I_{3M} < 0.10$	348	15	363
$I_{3M} \geq 0.10$	41	308	349
$I_{3M} < 0.12$	355	30	385
$I_{3M} \geq 0.12$	34	293	327
$I_{3M} < 0.14$	364	46	410
$I_{3M} \geq 0.14$	25	277	302
Total	389	323	712

Table 5 The quantities derived from 2-by-2 contingency tables (95 % confidence interval) of test of age of majority in black Africans from Botswana when different values of 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

	I_{3M}						
	0.02	0.04	0.06	0.08	0.10	0.12	0.14
Quantities							
AC	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)
p_1	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)
p_2	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)
PPV	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)
NPV	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)

AC accurate classification, p_1 sensitivity, p_2 specificity, *J-index* Youden index, *PPV* positive predictive value, *NPV* negative predictive value, *LR+* positive likelihood ratio, *LR−*, negative likelihood ratio, *Bayes PTP* Bayes post-test probability

from the initial crypt stage, which may start from 5 to 12 years, to apex closure, which can happen as late as 25 years [30, 62, 63]. No apex was found to still be open in any of the participants 24 years and older in the current study, thus participants 24 years and older were excluded from additional analysis. This upper age limit of development was also reported in other studies on different ethnic groups from England and South Africa [29, 31]. Older subjects whose root apices have closed affect mean measured values of I_{3M} and total performances of the discrimination test so they should be excluded from the study, which was performed [31]. The number of available participants across the age range may ensure consistency over the entire range and avoiding peaking at mean age [31].

The more advanced phases of development and final maturation of roots of third molars were identified as a developmental span useful for discrimination between adults and minors [31, 32]. The development of lower third molars in participants in this study was completed between 17 to 23 years of age. This indicates that persons from this population with evidence of apex closure of their third molars should be recognized as at least 17 years of age. No statistical significant difference in maturation tempo of lower third molars across the different I_{3M} ranges was found between males and females in African blacks from Botswana. This is in line with some previous studies from Europe on I_{3M} for discriminating adults and minors [32, 45]. The significant sexual dimorphism and early mineralization in males was found for the most I_{3M} ranges in the Brazilian study, except for the I_{3M} range from 0.7 to 0.9 [44]. Similar dimorphism was found for the I_{3M} range from 0.00 to 0.3 in the Croatian study [49] and from 0.04 to 0.08 in the Albanian study [46]. Generally, most studies showed male precedence of development of third molars [64–66]. Comparative studies indicate greater sexual dimorphism of third molars in the black population than in other ancestries [64, 67].

This cut-off value of $I_{3M} = 0.08$ showed the best specificity and Bayes' post-test probability. Accurate classification of this cut-off, with less than 10 % incorrect classification, is even better than 17 % previously reported by Cameriere et al. [32] on Italian sample, or 36 and 26 % when early and late root stages reported by Liversidge and Marsden [31] on Whites and Bangladeshi sample from the United Kingdom were evaluated.

Also the results of ROC in this study are comparable to values of 0.90 for MSS, from Liversidge and Marsden [31], 0.72 from Garamendi et al. [68], 0.85 from Thevissen et al. [36], 0.83 , 0.86, 0.90 from Martin-de las Heras et al. [40] for DSS. The results of the sensitivity measure how well the selected cut-off value of the I_{3M} classifies those who are 18 years and older, and the specificity measures how well it classifies those who are younger than 18 years [31, 32, 40]. Values of specificity of 0.94 and 0.96 in males and females respectively with a sensitivity of 0.88 are better than for any MSS cut-off

Table 6 The quantities derived from 2-by-2 contingency tables (95 % confidence interval) of test of age of majority in black Africans from Botswana when different values of 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 females

Quantities	I_{3M} 0.02	0.04	0.06	0.08	0.10	0.12	0.14
AC	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)
p_1	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)
p_2	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)
PPV	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)
NPV	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)

AC accurate classification, p_1 sensitivity, p_2 specificity, *J-index* Youden index, *PPV* positive predictive value, *NPV* negative predictive value, *LR+* positive likelihood ratio, *LR−*, negative likelihood ratio, *Bayes PTP* Bayes post-test probability

stage from study by Liversidge and Marsden [31] or DSS stage from study on two Spanish and Magrebian populations by Martin-de las Heras et al. [40]. Our results are also comparable to findings from the previous studies on I_{3M} in different populations [32, 43, 45–47].

From a forensic point of view, discrimination performance of the test should show better specificity or fewer minors being classified as adults [32, 40]. Practically this means that besides better total discrimination performance of the cut-off value than for $I_{3M}=0.10$, for forensic purposes, the value of $I_{3M}=0.08$ showed high confidence for properly selecting minors for persons of a totally different geographical, ethnic, and socioeconomic backgrounds as a previously tested specific cut-off value was not established.

In a legal and forensic context, this means that it is ethically safer to underestimate age than to overestimate age due to constitutional and judicial implications when involving a possible adult. These findings also indicate the similarity among tested populations in the tempo of late root development and final apex closure of third molars [32]. Previous studies showed that dentition in African children tend to develop faster than in Caucasian or Asian children, including a shift in the timing of initiation [29]. Thus it is important to assess the third molars of the sample population and to compare them to published literature from other populations.

The results of *PPV* of 0.94 and 0.97 in males and females, respectively, the probability that the individual with I_{3M} <0.08 is an adult, is comparable to results of mature apices in previous studies that used staging of the third molars [31]. Liverside and Mardsen [31] reported a value of 0.95 and listed values from the previous studies from 0.88 to 1.00. Positive likelihood ratios assess the potential utility of a diagnostic test and how likely it is that the individual in question is properly classified. It is an appropriate measure at an individual level and for the selected cut-off value of $I_{3M}=0.08$ was 13.67 for males and even better for females [31]. This means that an individual of I_{3M} <0.08 is over 13 times more likely to be correctly classified as an adult than a minor. On the other hand, negative likelihood ratio at the same cut-off point means that an individual with an I_{3M} ≥0.08 is over eight times more likely to be correctly classified as a minor in both sexes. These results showed 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 good prediction of the probability of majority, while higher cut-off values showed lower LRs−, good at predicting minority. Observations were similar if different cut-offs when MSS or DSS stages were evaluated [31, 40]. The same cut-off value of I_{3M} also showed appropriateness when tested in other populations [43–47].

Our findings for intra-rater and inter-rater agreements for I_{3M} were excellent and comparable to those studies using staging systems [31]. I_{3M} depends strictly on the measuring of specific projection points of third molars on OPT which is not the case for staging of the development. According to Liversidge and Marsden [31], reproducibility was better if fewer intermediate stages of mineralization of the third molars were used when compared to the systems that had more stages. Dhanjal et al. [69] showed that DSS showed better agreement than methods which use more stages if the test–re-test was performed by the same and different observers which is in agreement with Liversidge and Marsden [31] who also showed better kappa values for Demirjian than for Moorrees stages. The problem in assigning the stage arises when the tooth in development has reached somewhere in-between the 2 available stages of development. Demirjian suggested assigning lower stages if tooth development appears between stages [28]. Misclassification of the stage of mineralization of third molars leads to the great differences in the estimated age and may misclassify adults and minors. For discrimination between adults and minors, evidence of the final, stage H, completion or stages A–D using DSS can indicate that the investigated person is an adult or minor, respectively [48]. Liversidge and Marsden [31] showed adapted maturity data of halfway between mean age entering DSS tooth stage by difference between mean age for stage F are 16.9 years and 17.3 and for stage G are 18.4 years and 19.5 years in males and females, respectively. Even a 95 % CI of lower stage E includes an age of 18 years which discriminates adults and minors.

There are a few studies on third molar development on blacks in Africa or abroad [31, 48, 54, 70]. The early studies on the third molars in blacks for prediction of 18 years were done in 1993 by Mincer et al. [48]. Their evaluated sample consisted only 19 % of American blacks [48]. They reported no difference between blacks and whites in terms of time of tooth development. However, the authors states that the lack of difference may be due to the small number of blacks in the study, only one fifth, and the uneven distribution of the sample [48]. Their findings showed that the third molar could not be used as highly accurate age indicator and that more than 90 % showed DSS stage H were 18 years or older. The questionable accuracy of this approach was addressed to the fact that there are only four stages that describe the development of the roots from the initial appearance to the stage before closure of the apex [48]. All this takes place in a wide age range and stages do not correspond to the uniform time span in development [48].

A small but statistically significant improvement was seen in age prediction if the corresponding maxillary and mandibular molars were added to the prediction model [48]. This is of little practical usage according to Mincer et al. [48] because of small reduction in average residual of 0.1 years which indicates that a single, mandibular tooth can give sufficient and relevant information for age prediction for this purpose. Mincer et al. [48] also presented empirical probabilities on being an adult (18 years) based on specific DSS staging of third molars; however, this data was only for whites because of the small and uneven sample size of black participants. It is not possible to compare our results to DSS or MSS except in the applicability of a particular stage as a cut-off for discriminating between adults and minors. Cameriere et al. [32] showed that if stage H was changed with stage G of DSS, the estimated probability that the individual is an adult is decreased from 0.98 to 0.94. The values of sensitivity of stages H and G were 0.58 and 0.75, respectively, while values of specificity were 0.98 and 0.90, respectively. Also, the proposed value of $I_{3M}=0.08$ showed 17 % incorrect classification, with a better specificity, which is ethically mandatory for legal and criminal purposes [31, 32, 68]. Samples from different populations were tested and results showed similar incorrect classification and discrimination ability of the purposed cut-off value of I_{3M} [45–47]. Mineralization of the third molars in blacks and its usefulness for discrimination between adults and minors was also studied by Olze et al. [71], Blankenship et al. [70], Mincer et al. [48], Harris and McKee [72], and Liversidge et al. [29].

According to our search of the literature, this is the first radiographic study on the usefulness of I_{3M} for discriminating between adults and minors in black African children from Botswana or elsewhere in sub-Saharan Africa. This great geographic region with a population of over 920 million in 2007 showed significant overpopulation problems and an annual high growth rate of 2.3 %, with projection of growth to 1300 billion up to the year 2050 [73]. The region displays the greatest diversity in languages, cultures, religions, and huge social and economic disparities. This creates room for illegal migration in the region and Botswana together with South Africa and Namibia are indicated as potential countries for immigration [13]. Also from this region, 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 [43, 45, 74]. Many of these individuals may have no reliable documents or even registered births [45]. Immigration policies and legislation, including penal and criminal law in different countries, recognized one or more age limits and cut-offs [31, 43, 75]. The legal adult age or age of majority was set at 18 years of age in many countries and recently in Botswana [31, 76]. Different rates of correct classification of persons in investigation may be sufficient for the legal civil and criminal proceedings [41, 77]. According to Corradi et al. [41], 51 % of correct classification may be sufficient for civil cases with more probable than not evidence while very high levels, at 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 the different medicolegal purposes, there are general agreements in guidelines and procedures to estimate someone's age, as proposed by the AGFAD or FASE [44]. From this point of view and according to our findings, specific cut-off value of $I_{3M} = 0.08$ may be used as a reliable method within confidence interval for discriminating adults and minors in black Africans from Botswana.

In conclusion, the results of this study, carried out on a previously unexamined sample of specific geographic and ethnical origin, confirm the validity of the cut-off value of $I_{3M} = 0.08$ for discriminating between adults and minors. This finding is useful in daily medicolegal practice, where a qualified forensic examiner must decide on or classify the age of the investigated person as adult or minor. This 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 of I_{3M} with the best possible accuracy and likelihood ratio for discrimination between the age of majority or minority with acceptable small incorrect classification, regardless of race and socioeconomic standing. Considering that the findings are in correspondence with other populations, it should be justifiable to use in medicolegal practice.

Compliance with ethical standards The study was conducted in accordance to the ethical standards laid down by the Declaration of Helsinki [55]. The approval for the study was granted by the Human Research and Development Committee (HRDC) of the Ministry of Health in Botswana and by the Ethical Council of the School of Dental Medicine at the University of Zagreb. Jelena Cavrić and Ivan Galić equally contributed to this article.

References

1. Novak A (2009) Guilty of murder with extenuating circumstances: transparency and the mandatory death penalty in Botswana. Boston Univ Int Law J 27:173–204
2. Thevissen PW, Kvaal SI, Willems G (2012) Ethics in age estimation of unaccompanied minors. J Forensic Odontostomatol 30(Suppl 1): 84–102
3. Cattaneo C, De Angelis D, Ruspa M, Gibelli D, Cameriere R, Grandi M (2008) How old am I? Age estimation in living adults: a case report. J Forensic Odontostomatol 26:39–43
4. Focardi M, Pinchi V, De Luca F, Norelli GA (2014) Age estimation for forensic purposes in Italy: ethical issues. Int J Legal Med 128: 515–22
5. Botswana e-laws. Age of maturity, Interpretation act, CAP 01:04 Section 49. In: Attorney Generals Chambers, editor.2013.
6. Cole R (2010) Juvenile offenders and the criminal justice system in Botswana: exploring the restorative approach. In: Maundeni T (ed) Thari ya bana—reflections on children in Botswana 2010. UNICEF Botswana and University of Botswana, Gaborone, pp 54–7
7. Ohenjo N, Willis R, Jackson D, Nettleton C, Good K, Mugarura B (2006) Health of Indigenous people in Africa. Lancet 367:1937–46
8. Seitio-Kgokgwe O, Gauld RDC, Hill PC, Barnett P (2014) Assessing performance of Botswana's public hospital system: the use of the World Health Organization health system performance assessment framework. Int J Health Policy Manag 3:179–89
9. Miller CM, Gruskin S, Subramanian SV, Rajaraman D, Heymann SJ (2006) Orphan care in Botswana's working households: growing responsibilities in the absence of adequate support. Am J Public Health 96:1429–35
10. Children's Act 2009 in Botswana. 2009.
11. Marriage Act Botswana. Section 14. 2001.
12. Penal Code in Botswana. 1964.
13. Campbell EK (2006) Reflections on illegal immigration in Botswana and South Africa. Afr Popul Stud 21:23–44
14. UN High Commissioner for Refugees. National and international responses to the Zimbabwean exodus: implications for the refugee protection regime. Geneva: UNHCR; 2009.
15. Solheim T (2003) The Scandinavian Star Ferry Disaster 1990. Experience and Recommendation for Records in Dental Practice. Acta Stomat Croat 37:292–3
16. Schmeling A, Reisinger W, Geserick G, Olze A (2008) Forensic age estimation of live adolescents and young adults. In: Tsokos M, editor. Humana Press, Forensic Pathology Reviews, pp 269–88
17. Schmeling A, Reisinger W, Geserick G, Olze A (2006) Age estimation of unaccompanied minors. Part I. General considerations. Forensic Sci Int 159(Suppl 1):S61–4
18. Schmeling A, Olze A, Reisinger W, Rosing FW, Geserick G (2003) Forensic age diagnostics of living individuals in criminal proceedings. Homo Internationale Zeitschrift fur die vergleichende Forschung am Menschen 54:162–9
19. Schmeling A, Olze A, Reisinger W, Hermann KG, Rossel U (2003) Age determination of an unknown body in early adulthood. Arch Kriminol 211:129–38
20. Schmeling A, Olze A, Reisinger W, Geserick G (2004) Forensic age diagnostics of living people undergoing criminal proceedings. Forensic Sci Int 144:243–5
21. Schmeling A, Olze A, Reisinger W, Geserick G (2001) Age estimation of living people undergoing criminal proceedings. Lancet 358:89–90
22. Schmeling A, Grundmann C, Fuhrmann A, Kaatsch HJ, Knell B, Ramsthaler F et al (2008) Criteria for age estimation in living individuals. Int J Legal Med 122:457–60
23. Baccino E. Forensic Anthropology Society of Europe (FASE), a subsection of the IALM, is 1 year old. International Journal of Legal Medicine. 2005;119:N1-N.
24. Feijoo G, Barberia E, De Nova J, Prieto JL (2012) 2012. Forensic Sci Int 214(213):e1–6
25. Ambarkova V, Galić I, Vodanović M, Bioćina-Lukenda D, Brkić H (2014) Dental age estimation using Demirjian and Willems methods: cross sectional study on children from the Former Yugoslav Republic of Macedonia. Forensic Sci Int 234(187):1–7
26. Maber M, Liversidge HM, Hector MP (2006) Accuracy of age estimation of radiographic methods using developing teeth. Forensic Sci Int 159(Suppl 1):S68–73
27. Galić I, Vodanovic M, Cameriere R, Nakaš E, Galić E, Selimović E et al (2011) Accuracy of Cameriere, Haavikko, and Willems radiographic methods on age estimation on Bosnian-Herzegovian children age groups 6–13. Int J Legal Med 125:315–21
28. Demirjian A, Goldstein H, Tanner JM (1973) A new system of dental age assessment. Hum Biol 45:211–27
29. Liversidge HM (2008) Timing of human mandibular third molar formation. Ann Hum Biol 35:294–321
30. Liversidge HM (2008) Predicting mandibular third molar agenesis from second molar formation. Acta Stomat Croat 42:311–7
31. Liversidge HM, Marsden PH (2010) Estimating age and the likelihood of having attained 18 years of age using mandibular third molars. Br Dent J 209:E13

32. Cameriere R, Ferrante L, De Angelis D, Scarpino F, Galli F (2008) The comparison between measurement of open apices of third molars and Demirjian stages to test chronological age of over 18 year olds in living subjects. Int J Legal Med 122:493–7
33. Thevissen P, Altalie S, Brkić H, Galić I, Fieuws S, Franco A et al (2013) Comparing 14 country-specific populations on third molars development: consequences for age predictions of individuals with different geographic and biological origin. J Forensic Odontostomatol 31:87–8
34. Thevissen PW, Alqerban A, Asaumi J, Kahveci F, Kaur J, Kim YK et al (2010) Human dental age estimation using third molar developmental stages: accuracy of age predictions not using country specific information. Forensic Sci Int 201:106–11
35. Thevissen PW, Fieuws S, Willems G (2010) Human third molars development: comparison of 9 country specific populations. Forensic Sci Int 201:102–5
36. Thevissen PW, Fieuws S, Willems G (2010) Human dental age estimation using third molar developmental stages: does a Bayesian approach outperform regression models to discriminate between juveniles and adults? Int J Legal Med 124:35–42
37. Liversidge HM (2008) Dental age revisted. In: Irish JD, Nelson GC (eds) Technique and application in dental anthropology. Cambridge University Press, Cambridge, pp 234–52
38. Galić I, Vodanović M, Janković S, Mihanović F, Nakaš E, Prohić S et al (2013) Dental age estimation on Bosnian-Herzegovinian children aged 6–14 years: evaluation of Chaillet's international maturity standards. J Forensic Leg Med 20:40–5
39. Cameriere R, Brkić H, Ermenc B, Ferrante L, Ovsenik M, Cingolani M (2008) The measurement of open apices of teeth to test chronological age of over 14-year olds in living subjects. Forensic Sci Int 174:217–21
40. Martin-de las Heras S, Garcia-Fortea P, Ortega A, Zodocovich S, Valenzuela A (2008) Third molar development according to chronological age in populations from Spanish and Magrebian origin. Forensic Sci Int 174:47–53
41. Corradi F, Pinchi V, Barsanti I, Manca R, Garatti S (2013) Optimal age classification of young individuals based on dental evidence in civil and criminal proceedings. Int J Legal Med 127:1157–64
42. Corradi F, Pinchi V, Barsanti I, Garatti S (2013) Probabilistic classification of age by third molar development: the use of soft evidence. J Forensic Sci 58:51–9
43. Galić I, Lauc T, Brkić H, Vodanović M, Galić E, Biazevic MG et al (2015) Cameriere's third molar maturity index in assessing age of majority. Forensic Sci Int 252(191):e1–5
44. Deitos AR, Costa C, Michel-Crosato E, Galić I, Cameriere R, Biazevic MG (2015) Age estimation among Brazilians: younger or older than 18? J Forensic Leg Med 33:111–5
45. De Luca S, Biagi R, Begnoni G, Farronato G, Cingolani M, Merelli V et al (2014) Accuracy of Cameriere's cut-off value for third molar in assessing 18 years of age. Forensic Sci Int 235(102):e1–6
46. Cameriere R, Santoro V, Roca R, Lozito P, Introna F, Cingolani M et al (2014) Assessment of legal adult age of 18 by measurement of open apices of the third molars: Study on the Albanian sample. Forensic Sci Int 245C(205):e1–e5
47. Cameriere R, Pacifici A, Viva S, Carbone D, Pacifici L, Polimeni A (2014) Adult or not? Accuracy of Cameriere's cut-off value for third molar in assessing 18 years of age for legal purposes. Minerva Stomatol 63:283–94
48. Mincer HH, Harris EF, Berryman HE (1993) The A.B.F.O. study of third molar development and its use as an estimator of chronological age. J Forensic Sci 38:379–90
49. Olze A, Bilang D, Schmidt S, Wernecke KD, Geserick G, Schmeling A (2005) Validation of common classification systems for assessing the mineralization of third molars. Int J Legal Med 119:22–6
50. Gleiser I, Hunt EE Jr (1955) The permanent mandibular first molar: its calcification, eruption and decay. Am J Phys Anthropol 13:253–83
51. Kohler S, Schmelzle R, Loitz C, Puschel K (1994) [Development of wisdom teeth as a criterion of age determination]. Ann Anat 176: 339–45
52. Thevissen PW, Fieuws S, Willems G (2013) Third molar development: evaluation of nine tooth development registration techniques for age estimations. J Forensic Sci 58:393–7
53. Moorrees CF, Fanning EA, Hunt EE Jr (1963) Age variation of formation stages for ten permanent teeth. J Dent Res 42:1490–502
54. Cavrić J, Vodanović M, Marušić A, Galić I (2016) Time of mineralization of permanent teeth in children and adolescents in Gaborone, Botswana. Ann Anat Anatomischer Anz 203:24–32
55. World Medical Association (2013) World medical association declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 310:2191–4
56. Rasband WS. Image J. Bethesda, Maryland,USA: U. S. National Institutes of Health; 1997–2013
57. Swets JA (1988) Measuring the accuracy of diagnostic systems. Science 240:1285–93
58. Schisterman EF, Perkins NJ, Liu A, Bondell H (2005) Optimal cut-point and its corresponding Youden Index to discriminate individuals using pooled blood samples. Epidemiol (Cambridge, Mass) 16: 73–81
59. Fletcher R, Fletcher S. Diagnosis. In: Fletcher R, Fletcher S, editors. Clinical epidemiology The essentials. Baltimore: Wolters, Kluwer, Lippincott, Williams & Wilkins; 2005. p. 35–58.
60. Deeks JJ, Altman DG (2004) Diagnostic tests 4: likelihood ratios. BMJ 329:168–9
61. Central Statistics Office in Botswana. Resident population on the January, 1st 2014. 2011.
62. Thevissen PW, Kaur J, Willems G (2012) Human age estimation combining third molar and skeletal development. Int J Legal Med 126:285–92
63. Nystrom ME, Ranta HM, Peltola JS, Kataja JM (2007) Timing of developmental stages in permanent mandibular teeth of Finns from birth to age 25. Acta Odontol Scand 65:36–43
64. Harris EF (2007) Mineralization of the mandibular third molar: a study of American blacks and whites. Am J Phys Anthropol 132: 98–109
65. Garn SM, Lewis AB, Polacheck DL (1959) Variability of tooth formation. J Dent Res 38:135–48
66. Garn SM, Lewis AB, Vicinus JH (1962) Third molar agenesis and reduction in the number of other teeth. J Dent Res 41:717
67. Lewis JM, Senn DR (2010) Dental age estimation utilizing third molar development: a review of principles, methods, and population studies used in the United States. Forensic Sci Int 201:79–83
68. Garamendi PM, Landa MI, Ballesteros J, Solano MA (2005) Reliability of the methods applied to assess age minority in living subjects around 18 years old. A survey on a Moroccan origin population. Forensic Sci Int 154:3–12
69. Dhanjal KS, Bhardwaj MK, Liversidge HM (2006) Reproducibility of radiographic stage assessment of third molars. Forensic Sci Int 159(Suppl 1):S74–7
70. Blankenship JA, Mincer HH, Anderson KM, Woods MA, Burton EL (2007) Third molar development in the estimation of chronologic age in American blacks as compared with whites. J Forensic Sci 52:428–33
71. Olze A, van Niekerk P, Schmidt S, Wernecke KD, Rosing FW, Geserick G et al (2006) Studies on the progress of third-molar mineralisation in a Black African population. Homo internationale Zeitschrift fur die vergleichende Forschung am Menschen 57:209–17

72. Harris EF, McKee JH (1990) Tooth mineralization standards for blacks and whites from the middle southern United States. J Forensic Sci 35:859–72
73. Haub C, Kaneda T (2014) 2014 world population data sheet. Population Reference Bureau, Washington
74. Nuzzolese E (2012) Missing people, migrants, identification and human rights. J Forensic Odontostomatol 30(Suppl 1):47–59
75. Cameriere R, Giuliodori A, Zampi M, Galic I, Cingolani M, Pagliara F et al (2015) Age estimation in children and young adolescents for forensic purposes using fourth cervical vertebra (C4). Int J Legal Med 129:347–55
76. Thevissen PW, Pittayapat P, Fieuws S, Willems G (2009) Estimating age of majority on third molars developmental stages in young adults from Thailand using a modified scoring technique. J Forensic Sci 54:428–32
77. Pinchi V, Norelli GA, Pradella F, Vitale G, Rugo D, Nieri M (2012) Comparison of the applicability of four odontological methods for age estimation of the 14 years legal threshold in a sample of Italian adolescents. J Forensic Odontostomatol 30:17–25