Angelakopoulos N, De Luca S, Oliveira-Santos I, Ribeiro ILA, Bianchi I, Balla SB, Kis HC, Jiménez LG, Zolotenkova G, Yusof MYP, Selmanagić AH, Pandey H, Pereira PC, da Nóbrega JBM, Kalani H, Mieke SM, Kumagai A, Gulsahi A, Zelić K, Marinković N, Kelmendi J, Galić I, Vázquez IS, Spinas E, Velezmoro-Montes YW, Moukarzel M, Toledo JP, El-Bakary AAE, Cameriere R. Third molar maturity index (I_{3M}) assessment according to different geographical zones: a large multi-ethnic study sample. Int J Legal Med. 2023 Mar;137(2):403-425..

Epub 2022 Dec 15. PMID: 36520207.

The publisher's version is available at:
doi: 10.1007/s00414-022-02930-x

Third molar maturity index (I_{3M}) assessment according to different geographical zones: a large multi-ethnic study sample

Nikolaos Angelakopoulos[1,2] · Stefano De Luca[2,3] · Inês Oliveira-Santos[4] · Isabella Lima Arrais Ribeiro[5] · Ilenia Bianchi[6] · Sudheer B. Balla[2,7] · Hatice Cansu Kis[8] · Lourdes Gómez Jiménez[9] · Galina Zolotenkova[10] · Mohd Yusmiaidil Putera Yusof[11] · Aida Hadzić Selmanagić[12] · Hemlata Pandey[13] · Palmela C. Pereira[14] · Johnys Berton Medeiros da Nóbrega[15] · Hettiarachchi Kalani[16] · Sylvia M. Mieke[17] · Akiko Kumagai[18] ·
Ayse Gulsahi[2,19] · Ksenija Zelić[20] · Nemanja Marinković[21] · Jeta Kelmendi[22] · Ivan Galić[2,23] · Israel Soriano Vázquez[24] · Enrico Spinas[25] ·
Ymelda Wendy Velezmoro-Montes[26] · Maria Moukarzel[27] · Jorge Pinares Toledo[28,29] ·
Amal Abd El-Salam El-Bakary[30] · Roberto Cameriere[2]

Abstract

Identification of living undocumented individuals highlights the need for accurate, precise, and reproducible age estimation methods, especially in those cases involving minors. However, when their country of origin is unknown, or it can be only roughly estimated, it is extremely difficult to apply assessment policies, procedures, and practices that are accurate and child-sensitive. The main aim of this research is to optimize the correct classification of adults and minors by establishing new cut-off values for four different continents (Africa, America, Asia, and Europe). For this purpose, a vast sample of 10,701 orthopantomographs (OPTs) from four continents was evaluated. For determination and subsequent validation of the new third molar maturity index (I_{3M}) cut-off values by world regions, a cross-validation by holdout method was used and contingency tables (confusion matrices) were generated. The lower third molar maturity indexes, from both left and right side ($I_{3M}L$ and $I_{3M}R$) and the combination of both sides ($I_{3M}L\_I_{3M}R$) were calculated. The new cut-off values, that aim to differentiate between a minor and an adult, with more than 74.00% accuracy for all populations were as follows ($I_{3M}L$; $I_{3M}R$; $I_{3M}L\_I_{3M}R$, respectively): Africa = (0.10; 0.10; 0.10), America = (0.10; 0.09; 0.09), Asia = (0.15; 0.17; 0.14), and Europe = (0.09; 0.09; 0.09). The higher sensitivity (Se) was detected for the $I_{3M}L$ for male African people (91%) and the higher specificity (Sp) of all the parameters ($I_{3M}L$; $I_{3M}R$; $I_{3M}L\_I_{3M}R$) for Europeans both male and female (> 91%). The original cut-off value (0.08) is still useful, especially in discriminating individuals younger than 18 years old which is the goal of the forensic methods used for justice.

Keywords Forensic sciences · Dental age estimation · Age of majority · Third molar maturity index (I_{3M}) · Geographical zones · Population data

Abbreviations

Acc	Accuracy
ICC	Intraclass correlation coefficient
I_{3M}	Third molar maturity index
I_{3M}L	Left Third molar maturity index
I_{3M}R	Right Third molar maturity index
$I_{3M}L\_I_{3M}R$	Combined (left + right) third molar maturity index
LR +	Positive likelihood
LR −	Negative likelihood
OPTs	Orthopantomographs
PV +	Positive predictive value
PV −	Negative predictive value
Se	Sensitivity
Sp	Specificity

Introduction

Nowadays, the phenomena of migration, human trafficking, paedopornography, and underage delinquency are spread consistently across the globe [1–6]. As a consequence, the

---

✉ Stefano De Luca
stfndlc@gmail.com

Extended author information available on the last page of the article

identification of undocumented minors faces particular challenges, acknowledging the need to provide an additional legal protection to the most vulnerable ones.

It should not be overlooked that age assessment in living individuals evokes several procedures that aim to follow legal and civic guidelines [7] Thus, ethical and legislation concerning consent, cultural respect, safety, and well-being must always be present when processing the assessment [7]. Moreover, these are living individuals, often originating from precarious contexts, which demands the application of efficient, innocuous, and non-invasive methods. Also, it is well established that undocumented individuals arriving at different borders must first be screened to determine their country of origin and estimate their legal age. Nevertheless, sometimes, only an estimated geographic origin can be assessed, particularly because many cases do not follow the legally required steps of the immigration process. According to Dhamo et al. (2018), both geographical and genetic ancestry differently influence the dental development [8].

This raises another issue complementary to the underlying need for accurate, precise, and reproducible age estimation techniques, which is for these techniques to be applicable to a wide variety of population groups with different ancestral backgrounds.

The use of teeth as a reliable biological marker for children is widely described [9, 10]. Also, the use of third molars has been explored to be a good age marker at the 18-year threshold, discriminating between juvenile and adult status [11, 12]. Among several approaches [5], the third molar maturity index ($I_{3\mathrm{M}}$) was introduced by Cameriere et al. [13]. Based on the relationship between age and the normalized measures of the third molar's open apices, this method applies a cut-off value of 0.08 to assign an individual as a juvenile or adult [13]. Since its introduction, several authors have tested and validated this method in various populations [12, 14–43].

A recent study depicted and validated the use of both left ($I_{3\mathrm{M}}L$) and right ($I_{3\mathrm{M}}R$) lower third molar, in cases of legal inquiry on the 18-year-old threshold, and demonstrated that the accuracies attained for I_{3M} on both left and right sides were high and did not differ significantly [44].

Despite the fact that numerous studies have validated the third molar maturity index method ($I_{3\mathrm{M}}$) for different geographical populations, some missing pieces or insufficient information in the research literature were detected. More specifically, dental characteristics vary due to both geographical (climatic, nutritionally, latitude) [8, 45] and genetic factors [8]. So, the need for a continuously better performance of this method and a better understanding of the effects of geography and genetics on dental development established this research aimed at optimizing the correct classification of adults and minors in cases of unknown geographic origin. Thus, this study established new cut-off values for four different regions (Africa, America, Asia, and Europe) according to possible influences on the third molar maturity index ($I_{3\mathrm{M}}$) of the different geographical populations belonging to the same ancestral group.

Materials and methods

Sample

Extensive population data from globally distributed datasets and a total of 10,701 orthopantomographs (OPTs) from different countries were evaluated. The sample distribution was performed by classifying the different geographical populations into four continental groups, not based on a racial characterization of the sample, but simply on geographical distribution (1631 subjects from Africa; 1781 from America; 3919 from Asia; and 3770 from Europe). This study fused data from previously published papers and unpublished OPTs data included subjects from Mexico, Argentina, Indonesia, Malaysia, Sri Lanka, and Bosnia. Sample distribution, divided by country, world region, and published or unpublished dataset is shown in detail in Table 1.

The data collection followed the protocol first described by Cameriere and colleagues [13]. Only the lower left ($I_{3\mathrm{M}}L$) and right ($I_{3\mathrm{M}}R$), third molars were analyzed for this study. The sample selection criteria were as follows: clear OPTs with all the permanent teeth present, including mandibular left and right third molars, were included. Cases where only one of the mandibular left and right third molars were available (e.g., hypodontia, unilateral lower third molar agenesis, early extraction, or forced removal of the tooth) were excluded from the study. For the same reason, the sample size of our included data does not correspond to the sample size of the original individual studies.

The presence of any visible dental and bone pathology (e.g., large carious lesions or endodontic treatments), children with any systemic diseases or endocrine anomalies (e.g., dysmorphology, abnormally short roots), and subjects with previous root canal treatment in the lower permanent teeth were excluded from the study. Heavily rotated and impacted third molar with no visible roots were also excluded from this study.

No identification data were collected, and images were saved with high resolution in the JPEG file format, and automatically anonymized before the selection process. All data were recorded in an Excel file and the columns indicated as follows: continent, subject's identification number, sex, date of birth, and date of OPTs.

Table 1 Distribution of frequencies of individuals included in study and division for training phase for determination of the new cutoffs and for test of the new cutoffs, for region of world

Region	Country	Sample size	Real age classification	Cutoff determination phase (training)		Test of new cutoffs	
Africa	All	1631	Adult ($n = 761$)	1088	508	543	253
			Minor ($n = 870$)		580		290
	Black South Angelakopoulos et al. (2018)	742	Adult ($n = 289$)	495	193	247	96
			Minor ($n = 453$)		302		151
	Botswana Cavric et al. (2016)	779	Adult ($n = 398$)	519	266	260	132
			Minor ($n = 381$)		253		128
	Egypt El-Bakary et al. (2019)	110	Adult ($n = 74$)	74	50	36	24
			Minor ($n = 36$)		24		12
America	All	1781	Adult ($n = 933$)	1188	620	593	313
			Minor ($n = 848$)		568		280
	Brazil Deitos et al. (2015)	372	Adult ($n = 200$)	249	134	123	66
			Minor ($n = 172$)		115		57
	Mexico (Unpublished data)	105	Adult ($n = 30$)	70	20	35	10
			Minor ($n = 75$)		50		25
	Peru Quispe et al. (2017)	210	Adult ($n = 86$)	140	58	70	28
			Minor ($n = 124$)		82		42
	Dominican Republic Gómez Jiménez et al. (2019)	174	Adult ($n = 92$)	118	62	56	30
			Minor ($n = 82$)		56		26
	Chile Cameriere et al. (2018)	593	Adult ($n = 317$)	397	212	196	105
			Minor ($n = 276$)		185		91
	Argentina (Unpublished data)	327	Adult ($n = 208$)	219	139	108	69
			Minor ($n = 119$)		80		39
Asia	All	3919	Adult ($n = 2346$)	2617	1566	1302	780
			Minor ($n = 1573$)		1051		522
	India Balla et al. (2017)	358	Adult ($n = 264$)	239	176	119	88
			Minor ($n = 94$)		63		31
	Indonesia (Unpublished data)	426	Adult ($n = 295$)	285	197	141	98
			Minor ($n = 131$)		88		43
	Japan Kumagai et al. (2019)	271	Adult ($n = 188$)	182	126	89	62
			Minor ($n = 83$)		56		27
	Lebanon Moukarzel et al. (2020)	554	Adult ($n = 269$)	370	180	184	89
			Minor ($n = 285$)		190		95
	Malaysia (Unpublished data)	558	Adult ($n = 366$)	372	244	186	122
			Minor ($n = 192$)		128		64
	Sri Lanka (Unpublished data)	1026	Adult ($n = 554$)	684	369	342	185
			Minor ($n = 472$)		315		157
	Turkey Gulsahi et al. (2014)	726	Adult ($n = 410$)	485	274	241	136
			Minor ($n = 316$)		211		105

Intra- and inter-observer variabilities

Three forensic odontologists with varying levels of experience performed the observer error analysis. Repeated observations from the first author (NA) were used to determine intra-observer agreement, while inter-observer analysis was based on comparisons with those of two other observers (IG and LGJ). For this purpose, 100 OPTs from the new unpublished data were randomly selected one month after the initial scoring to calculate the agreement's percentage for both intra- and inter-observer test. During the process, the observers were categorically blinded from the chronological age and sex of each subject. Intraclass correlation coefficient (ICC) was applied to calculate intra- and inter-observer variability.

Statistical analysis

For determination and subsequent validation of the new cut-off values by world regions, a cross-validation by holdout method was used. Initially, the database was divided into two databases. The first one (consisting of two-thirds of the sample) was for the training phase and determination of the best cut-off values for optimizing the correct classification of adults and minors using the I_{3M} method developed by Cameriere et al. [13], in each region of the world. The other

Table 1 (continued)

Region	Country	Sample size	Real age classification	Cutoff determination phase (training)		Test of new cutoffs	
Europe	All	3770	Adult ($n = 2380$)	2,516	1588	1254	792
			Minor ($n = 1390$)		928		462
	Bosnia (Unpublished data)	640	Adult ($n = 277$)	427	185	213	92
			Minor ($n = 363$)		242		121
	Italy De Luca et al. (2016) [35] Cameriere et al. (2014) [39]	447	Adult ($n = 246$)	298	164	149	82
			Minor ($n = 201$)		134		67
	Kossovo Kelmendi et al. (2018)	973	Adult ($n = 803$)	649	535	324	268
			Minor ($n = 170$)		114		56
	Montenegro Antunovic et al. (2018)	597	Adult ($n = 371$)	399	248	198	123
			Minor ($n = 226$)		151		75
	Portugal Albernaz Neves et al. (2020)	244	Adult ($n = 115$)	163	77	81	38
			Minor ($n = 129$)		86		43
	Russia Scandoni et al. (2020)	570	Adult ($n = 350$)	380	233	190	117
			Minor ($n = 220$)		147		73
	Serbia Zelic et al. (2016)	299	Adult ($n = 218$)	200	146	99	72
			Minor ($n = 81$)		54		27

database (one-third of the sample) was used in the test phase of the newly determined cut-off values.

All data were analyzed descriptively and inferentially, and the frequencies of cases per country in each of the regions were also described. After the division and random selection of the sample for training and testing of the new cut-off values for all analyses, the real age was classified as a binary variable, divided into adults (18.00 years or older) and minors (12–17.99 years old). The normality distribution for both the $I_{3M}L$ and $I_{3M}R$ was evaluated by the Kolmogorov–Smirnov test ($p < 0.05$). The $I_{3M}L$ and $I_{3M}R$ were compared according to the categories of age (adult or minor) according to each continent using Wilcoxon signed rank test and its respective parametric-paired sample *t*-test, in order to evaluate possible differences between the measurements.

Sequentially, the cases selected for training were analyzed using binary logistic regression and, from the optimal values in sensitivity, specificity, and accuracy, the best cut-off values for age classification were selected according to each of the estimators ($I_{3M}L$, $I_{3M}R$, or the mean of $I_{3M}L$ and $I_{3M}R$). The sensitivity indicates the I_{3M} ability to correctly identify individuals who are 18 years or older (I_{3M}<0.08). On the other hand, specificity is the ability to discriminate individuals younger than 18 years old (I_{3M}≥0.08). The values for Se, Sp, PV+, PV−, and Acc were calculated using the "value (100%)" and the LR−the "value (1%)."

After determining the best cut-off values in the first phase of the study, each subject of the second database (selected for testing the new cut-off values) was classified as minor or adult according to the respective cut-off value determined for each region of the world and predictor variable. Values of the variables of interest ($I_{3M}L$, $I_{3M}R$, or the mean of $I_{3M}L$ and $I_{3M}R$) below the determined cut-off values were classified as corresponding to adults, and values equal to or above the cut-off values were classified as corresponding to minors.

Finally, contingency tables (confusion matrices) were generated, and the respective predictive values of sensitivity and specificity (Se and Sp, including 95% CI), positive and negative predictive values (PV + and PV − , including 95% CI), positive and negative likelihood ratio (LR + and LR − , including 95% CI), and accuracy (Acc) of estimates were determined. The LR + shows how much the probability of being an adult is increased, given a positive test result. The LR − indicates how much the probability of being adult decreases, given a negative test result.

All the analyses were performed using the R software (version 3.6.3, R Core Team, R Foundation for Statistical Computing, Vienna, Austria) and SPSS software (version 26.0, Armonk, IBM Corporation, New York, USA). The threshold of significance was set at 5%.

Results

Regarding the measurement process, the intra-observer agreement values were 0.991 (95% CI: 0.985–0.994) and 0.955 (95% CI: 0.947–0.972), for the left and right mandibular third molars, respectively. The values for inter-observer agreement were 0.897 (95% CI: 0.881–0.957) and 0.931

(95% CI: 0.915–0.949), for the left and right mandibular third molars, respectively.

In Table 1, it is possible to observe the global geographical distribution of the sample included in this study and it is divided into the different phases of the analysis (phase of determination and phase of test for the new cut-off points). All the individuals are classified according to their real chronological age as adults or minors.

In Table 2, chronological age of the individuals and their real classifications are represented globally and divided by country and regions. The values of $I_{3M}L$ and $I_{3M}R$ are also compared, confirming that, for most countries, in different regions of the world, the measurements of $I_{3M}L$ do not differ from those of $I_{3M}R$ and, when this difference was verified ($p < 0.05$), it was of insignificant to small magnitude, especially for European adults (Italians and Bosnians).

Table 3 presents the data for the diagnoses of the logistic regression analysis in the prediction of age classification in adults and minors from the parameters of $I_{3M}L$, $I_{3M}R$, and for the mean cut-off values ($I_{3M}L$ and $I_{3M}R$) obtained from the different populations. Based on these parameters, the best cut-off values were defined for classification in each of the studied regions of the world. The results obtained in the training phase selected revealed very similar cut-off values among African, American, and European populations, while they showed a deviation based on the Asian population.

Table 4 describes the quantitative and predictive values obtained from testing the new cut-off values according to sex and country groups. The new cut-off values, applied to the test sample, generated the values of sensitivity (Se), specificity (Sp), positive predictive value (PV +), negative predictive value (PV −), positive likelihood (LR +), negative likelihood (LR −), and accuracy (Acc). Accuracies (Acc) > 74% were obtained for all world regions without significant differences between $I_{3M}L$, $I_{3M}R$, or $I_{3M}L\_I_{3M}R$ (Table 4).

It is possible to observe in Table 4 that the positive and negative predictive values were all above 70%, both for adults and minors, respectively, except for the Asian population. In the case of Asians, it is observed that the general reliability for a correct classification of minors is a lower than in the other regions especially due to female samples of all $I_{3M}L$, $I_{3M}R$, or $I_{3M}L\_I_{3M}R$ (Table 4).

In fact, based on the LR + , the prediction proposed for all populations in the present study has a moderate to high potential to increase the chances of correctly classifying an individual as an adult (> 80.00% for all the three predictive parameters — $I_{3M}L$, $I_{3M}R$, and $I_{3M}L\_\mathrm{I}_{3M}\mathrm{R}$ — in each population), and, based on the values observed for LR−, the prediction proposed in the present study has a small to moderate potential to reduce the chances of misclassifying someone who is minor as an adult (> 70% for all the three predictive parameters — $I_{3M}L$, $I_{3M}R$, and $I_{3M}L\_I_{3M}R$ — in African, American, and European populations) (Table 4). According to sex, the new cut-off estimates show a prediction accuracy higher for males than for females in practically all inferences (Table 4). The difference in accuracy between genders ranged from 0.49% (America; $I_{3M}L$ and $\_I_{3M}R$) to 4.48% (Asia; $I_{3M}L$). The only exception is the American female population with an accuracy of 2.25% higher than American males considering the $I_{3M}L$ predictive parameter (Table 4).

Table 5 show quantities and predictive values for classification of subjects based on $I_{3M}L$, $I_{3M}R$, and $I_{3M}L$ and $\_I_{3M}R$ measurements for cut-off of 0.08 (gold standard) for the same samples to compare with the new cut-off estimates.

Meanwhile, the negative predictive values for the Asian sample are lower than for other populations for all three I_{3M} predictors (about 65.00% to 68.00%). Table 6 illustrates the accuracy values obtained by specifically applying the new cut-off to each Asian country in the testing phase. The results obtained show a low level of accuracy for all three predictors only for the Malaysian population: the new I_{3M} cut-off seems to correctly classify only 50.00% of the subjects, unlike all other Asian populations where the accuracy of the new index is above 80.00% (Table 6). Further research should be performed to assess the influence of geography as the main factor in shaping the variation of the cut-off values' diversity across the Asian region.

Table 7 summarizes the main findings from previous studies on original cut-off ($I_{3M} = 0.08$), including details on continent, author and year, country of subjects origin, sensitivity (Se), specificity (Sp), and accuracy (Acc) data according to sex with 95% confidence interval.

Discussion

Nowadays, while there is not a homogeneous approach to age assessment of the living across the globe, dental development is generally highlighted as one of the steps to follow to achieve a fair age estimation. The third molar has been one of the indicators used throughout the years, since it encompasses the important threshold between minor and adult [13, 46, 47].

In the original study, Cameriere et al. [13] reported a cut-off value of $I_{3M} = 0.08$ (sensitivity = 70% and specificity = 98%) to differentiate between minors and adults. Several researchers tested and validated the $I_{3M} = 0.08$ cut-off value on different populations, obtaining promising results [48].

Santiago et al. [48] reported a high specificity of I_{3M} tool in correctly classifying minors. This outcome is key in forensic science because if a minor is wrongly processed as an adult, it would violate his/her rights.

So far, the I_{3M} has not only been tested in various populations worldwide [12, 14–43], but a recently multi-ethnic study has also shown that there is no significant variability between the development of left and right mandibular third molars in the same subject [44]. Therefore, the $I_{3M}L$ and

Table 2 Characterization of study sample and comparison between measurements of $I_{3M}L$ and $I_{3M}R$

Region	Country	Sample size	Real age classification	Real age Mean (±DP) (Range)	$I_{3M}L$ Median (p25; p75)	$I_{3M}R$ Median (p25; p75)	p	Effect size
Africa	All	1631	Adult ($n=761$)	20.26 (±1.60) (18.00 to 24.88)	0.02 (0.00; 0.07)	0.01 (0.00; 0.07)	0.353	-
			Minor ($n=870$)	16.00 (±1.07) (14.00 to 17.98)	0.25 (0.14; 0.41)	0.24 (0.14; 0.40)	0.002	0.03
	Black South	742	Adult ($n=289$)	20.23 (±1.60)	0.00 (0.00;0.00)	0.00 (0.00; 0.00)	0.507	-
			Minor ($n=453$)	15.82 (±1.09)	0.21 (0.13; 0.38)	0.22 (0.12; 0.38)	0.244	-
	Botswana	779	Adult ($n=398$)	20.19 (±1.53)	0.04 (0.00; 0.09)	0.05 (0.00; 0.09)	0.651	-
			Minor ($n=381$)	16.18 (±1.05)	0.30 (0.19; 0.43)	0.28 (0.16; 0.41)	0.000	0.08
	Egypt	110	Adult ($n=74$)	20.77 (±1.88)	0.03 (0.01; 0.05)	0.02 (0.01; 0.04)	0.002	0.28
			Minor ($n=36$)	16.41 (±0.61)	0.26 (0.16; 0.40)	0.23 (0.13; 0.39)	0.010	0.11
America	All	1.781	Adult ($n=408$)	20.39 (±1.61) (18.00 to 24.96)	0.01 (0.00; 0.07)	0.01 (0.00; 0.06)	0.949	-
			Minor ($n=453$)	15.84 (±1.13) (12.32 to 17.99)	0.32 (0.15; 0.60)	0.30 (0.16; 0.60)	0.939	-
	Brazil	372	Adult ($n=200$)	20.92 (±1.73)	0.02 (0.00; 0.08)	0.03 (0.00; 0.07)	0.883	-
			Minor ($n=172$)	15.88 (±1.10)	0.29 (0.16; 0.47)	0.30 (0.15; 0.57)	0.095	-
	Mexico	105	Adult ($n=30$)	20.16 (±1.61)	0.07 (0.03; 0.12)	0.08 (0.05; 0.13)	0.280	-
			Minor ($n=75$)	16.19 (±0.97)	0.30 0.13; 0.41)	0.29 (0.15; 0.49)	0.438	-
	Peru	210	Adult ($n=86$)	19.27 (±0.70)	0.04 (0.00; 0.07)	0.04 (0.00; 0.07)	0.628	-
			Minor ($n=124$)	15.54 (±1.15)	0.47 (0.16; 0.70)	0.37 (0.18; 0.66)	0.093	-
	Dominican Republic	174	Adult ($n=92$)	20.38 (±1.42)	0.00 (0.00; 0.00)	0.00 (0.00; 0.00)	0.019	0.01
			Minor ($n=82$)	15.87 (±1.18)	0.28 (0.14; 0.66)	0.27 (0.14; 0.63)	0.409	-
	Chile	593	Adult ($n=317$)	20.97 (±2.00)	0.04 (0.00; 0.12)	0.04 (0.00; 0.13)	0.403	-
			Minor ($n=276$)	16.06 (±1.16)	0.34 (0.19; 0.52)	0.32 (0.19; 0.47)	0.621	-
	Argentina	327	Adult ($n=208$)	20.90 (±1.97)	0.02 (0.01; 0.03)	0.02 (0.01; 0.03)	0.957	-
			Minor ($n=119$)	15.47 (±1.14)	0.56 (0.18; 0.80)	0.55 (0.20; 0.80)	0.016	0.01
Asia	All	3,919	Adult ($n=2346$)	20.69 (±1.63) (18.00 to 24.90)	0.05 (0.00; 0.16)	0.04 (0.00; 0.16)	0.018	0.06
			Minor ($n=1573$)	15.98 (±1.17) (13.10 to 17.98)	0.38 (0.21; 0.70)	0.38 (0.20; 0.70)	0.960	-
	India	358	Adult ($n=264$)	20.90 (±1.69)	0.00 (0.00; 0.11)	0.00 (0.00; 0.09)	0.460	-
			Minor ($n=94$)	16.86 (±0.58)	0.40 (0.24; 0.68)	0.42 (0.26; 0.64)	0.604	-
	Indonesia	426	Adult ($n=295$)	20.71 (±1.86)	0.00 (0.00; 0.13)	0.00 (0.00; 0.13)	0.297	-
			Minor ($n=131$)	16.63 (±0.65)	0.34 (0.18; 0.62)	0.34 (0.20; 0.59)	0.186	-
	Japan	271	Adult ($n=188$)	21.50 (±1.95)	0.00 (0.00; 0.00)	0.00 (0.00; 0.00)	0.001	0.01
			Minor ($n=83$)	16.10 (±1.03)	0.41 (0.17; 0.62)	0.33 (0.15; 0.57)	0.000	0.18
	Lebanon	554	Adult ($n=269$)	20.60 (±1.67)	0.04 (0.00; 0.12)	0.04 (0.00; 0.13)	0.892	-
			Minor ($n=285$)	15.70 (±1.21)	0.54 (0.25; 0.95)	0.53 (0.28; 0.98)	0.000	0.01
	Malaysia	558	Adult ($n=366$)	20.63 (±1.32)	0.34 (0.08; 0.62)	0.28 (0.06; 0.55)	0.074	-
			Minor ($n=192$)	16.16 (±0.94)	0.33 (0.18; 0.54)	0.36 (0.18; 0.58)	0.771	-
	Sri Lanka	1,026	Adult ($n=554$)	20.70 (±1.65)	0.05 (0.00; 0.08)	0.05 (0.00; 0.08)	0.196	-
			Minor ($n=472$)	15.54 (±1.28)	0.42 (0.20; 0.78)	0.38 (0.17; 0.79)	0.861	-
	Turkey	726	Adult ($n=410$)	20.27 (±1.27)	0.05 (0.00; 0.22)	0.05 (0.00; 0.20)	0.465	-
			Minor ($n=316$)	16.45 (±0.96)	0.31 (0.20; 0.49)	0.32 (0.20; 0.52)	0.656	-

$I_{3M}R$ present the same reliability in terms of being used as a parameter for estimating age in African, European, American, and Asiatic populations [44], confirmed by the results showed in Table 2 of the study applying a partially different and largest sample.

In some of these populations, the I_{3M} method was reliable and highly accurate in discriminating whether a person is younger or older than 18 years [48]. However, systematic search of its application on a diverse array of populations has generated some discrepant results [48].

Table 2 (continued)

Region	Country	Sample size	Real age classification	Real age Mean (±DP) (Range)	$I_{3M}L$ Median (p25; p75)	$I_{3M}R$ Median (p25; p75)	p	Effect size
Europe	All	3,770	Adult (n = 2380)	21.01 (±1.88) (18.00 to 24.99)	0.00 (0.00; 0.07)	0.00 (0.00; 0.06)	0.000	0.15
			Minor (n = 1390)	15.94 (±1.16) (14.00 to 17.98)	0.35 (0.18; 0.65)	0.35 (0.17; 0.64)	0.079	-
	Bosnia	640	Adult (n = 277)	19.99 (±1.99)	0.06 (0.02; 0.17)	0.06 (0.00; 0.15)	0.032	0.01
			Minor (n = 363)	15.57 (±1.08)	0.34 (0.13; 0.81)	0.21 (0.10; 0.82)	0.531	-
	Italy	447	Adult (n = 246)	20.47 (±1.68)	0.02 (0.00; 0.05)	0.00 (0.00; 0.05)	0.021	0.38
			Minor (n = 201)	15.99 (±1.03)	0.33 (0.13; 0.57)	0.35 (0.10; 0.68)	0.996	-
	Kossovo	973	Adult (n = 803)	21.25 (±1.75)	0.00 (0.00; 0.08)	0.00 (0.00; 0.06)	0.000	0.14
			Minor (n = 170)	16.99 (±0.56)	0.37 (0.24; 0.60)	0.37 (0.25; 0.58)	0.065	-
	Montenegro	597	Adult (n = 371)	21.36 (±1.94)	0.00 (0.00; 0.03)	0.00 (0.00; 0.03)	0.916	-
			Minor (n = 226)	15.90 (±1.19)	0.48 (0.25; 0.95)	0.50 (0.22; 0.92)	0.144	-
	Portugal	244	Adult (n = 115)	20.82 (±1.71)	0.00 (0.00; 0.02)	0.00 (0.00; 0.02)	0.766	-
			Minor (n = 129)	15.69 (±1.19)	0.33 (0.17; 0.65)	0.36 (0.14; 0.77)	0.926	-
	Russia	570	Adult (n = 350)	21.22 (±1.81)	0.03 (0.00; 0.06)	0.02 (0.00; 0.04)	0.000	0.19
			Minor (n = 220)	15.50 (±1.14)	0.42 (0.21; 0.76)	0.45 (0.22; 0.74)	0.070	-
	Serbia	299	Adult (n = 218)	21.19 (±1,98)	0.03 (0.00; 0.10)	0.03 (0.00; 0.09)	0.168	-
			Minor (n = 81)	17.00 (±0.61)	0.21 (0.10; 0.38)	0.21 (0.11; 0.38)	0.652	-

Wilcoxon signed rank test. Effect size = Cohen's *d*. Significance level = 5%

$I_{3M}L$ left third molar, $I_{3M}R$ right third molar, *SD* standard deviation, *p25* percentile 25, *p75* percentile 75

Another study showed discrepancies in assessment accuracy, when they compared data on third molar mineralization among different ethnicities [46]. In other words, ethnic differences might exist and may influence different cut-off values, meaning that they cannot be safely applied to non-tested populations. As such, it is necessary to test, validate, revise, and adjust such dental age estimation methods in samples from diverse origins.

In this paper, this issue was addressed by exploring possible geographical and ethnic influences on Cameriere's third molar maturity index $I_{3M} = 0.08$ [13] and by establishing new cutoff values validated on different populations included in four continents (Africa, America, Asia, and Europe) according to the definition of ancestry by Dhamo et al. [8]. This research is focused on discovering whether any different trends are detectible in dental maturations on a geographical scale and within different populations in the same geographic zones.

Thevissen et al. [47] have found not differences in third molar development between nine different populations; on the contrary, another ethnic comparative study [46] conducted on German, Japanese, and South African populations found that the Japanese people reached third molars Demirjian D-F stages [49] 1–2 years earlier than the German subjects but the South African people about 1–2 years later than Germans. Examples of different trends in dental maturation and third molar mineralization stages among populations of the same country but in different locations were revealed in Chinese samples [50–52]. Furthermore, a study that investigated and compared the chronology of third molar development among African American and white American populations showed that African Americans mature faster [53]. In fact, third molar maturation discrepancies between different populations have been reported by numerous authors [54–60] but this could be explained by a heterogeneous age cohorts' distribution among samples rather than for the difference between populations according to the greater reliability of age estimation methods for early maturation stages rather than for the late ones [61, 62].

Despite the significance of Cameriere's $I_{3M} = 0.08$ cut-off value for all the different populations around the world, none of the data obtained from different origins has shown a variability high enough to invalidate the established cut-off value of $I_{3M} = 0.08$ [21, 26, 33], so far, except small corrections to the original cut-off value [23, 63]. In particular, in Polish population, the best performances were obtained

Table 3 Parameters verified for the better cutoff in age classification for each region of the world, according each one of predictor ($I_{3M}L$, $I_{3M}R$, and mean of $I_{3M}L$ and $I_{3M}R$)

Region	Parameters	Predictors		
		$I_{3M}L$	$I_{3M}R$	$I_{3M}L\_I_{3M}R$
Africa ($n = 1.088$)	Sensitivity	81.20%	81.30%	84.20%
	Specificity	86.90%	84.60%	83.90%
	Accuracy	89.80%	89.30%	90.20%
	Cutoff	**0.10**	**0.10**	**0.10**
America ($n = 1.188$)	Sensitivity	80.80%	80.20%	80.0%
	Specificity	87.60%	89.20%	89.50%
	Accuracy	90.20%	90.80%	91.10%
	Cutoff	**0.10**	**0.09**	**0.09**
Asia ($n = 2.617$)	Sensitivity	72.80%	75.60%	70.90%
	Specificity	84.10%	81.00%	85.80%
	Accuracy	84.10%	84.30%	84.80%
	Cutoff	**0.15**	**0.17**	**0.14**
Europe ($n = 2.516$)	Sensitivity	79.00%	80.90%	80.00%
	Specificity	93.00%	93.60%	93.90%
	Accuracy	91.90%	92.40%	92.70%
	Cutof	f**0.09**	**0.09**	**0.09**

Logistic regression. Significance level = 5%

$I_{3M}L$ left third molar, $I_{3M}R$ right third molar, $I_{3M}L\_I_{3M}R$ arithmetic mean of $I_{3M}L$ and $I_{3M}R$ measurements

using a cut-off value of $I_{3M} = 0.07$ [23] and of $I_{3M} = 0.10$ for Chinese people. When researchers around the world apply the I_{3M} cut-off value and draw conclusions based on similar values in sensitivity and specificity, it does not mean that its application in non-studied populations will return the same results. For this reason,, new I_{3M} cut-off values were generated for each of the studied world regions, despite suggestions [48] that it would not be highly influenced by population differences.

The new cut-off values, that aim to differentiate between a minor and an adult, with more than 74.00% accuracy for all populations were as follows ($I_{3M}L$; $I_{3M}R$; $I_{3M}L\_I_{3M}R$, respectively): Africa = (0.10; 0.10; 0.10), America = (0.10; 0.09; 0.09), Asia = (0.15; 0.17; 0.14), and Europe = (0.09; 0.09; 0.09). The higher sensitivity (Se) was detected for the $I_{3M}L$ for male African people (91%) and the higher specificity (Sp) of all the parameters ($I_{3M}L$; $I_{3M}R$; $I_{3M}L\_I_{3M}R$) for Europeans both male and female (> 91%).

African region

Previous studies on African populations that tested the I_{3M} cutoff value have been conducted separately on Libyan [30], Egyptian [42], Black South African [12], and Black African Botswanan [33] samples as summarized in Table 7. The results from these studies [12, 30, 33, 42] seem to be in agreement with the outcomes of the application of the new single cut-off value of 0.10 obtained from the overall African regions tested. In fact, after using it on the test sample, the new Se and Sp were found to be 86.95% and 85.86%, respectively (Table 4), taking into account the $I_{3M}L$, while the precision (Acc) was around 85% (similar to the results obtained by combining both $I_{3M}L$ and $I_{3M}R$; Table 4). Even if the Se and Sp values of the new cut-off are slightly lower than previous ones, the classification accuracy obtained is high considering the amplitude and heterogeneity of the analyzed African sample. It is also better than the single sensitivity obtained for Egyptian females (73%) in a much smaller selected sample [42] (Tables 4 and 7). Therefore, this confirms that although it is possible to detect an internal variability among different populations of the same area (e.g., Egypt compared to black Africans), this is not sufficiently high to invalidate the original cut-off value using the Cameriere method ($I_{3M} = 0.08$) [13], nor the new one (0.10).

American region

Some studies previously conducted using Cameriere's I_{3M} cut-off value on American populations showed a variability of results in terms of specificity and sensitivity (Table 7). The most promising outcome was related to the Colombian samples [34] contrary to Deitos et al. [37]. In terms of this study, it was not possible to collect a sample of the Colombian population, but the new cut-off value for the American geographical region was adjusted to 0.09 when considering the mean of $I_{3M}L$ and $I_{3M}R$ and the $I_{3M}R$, but 0.10 for the $I_{3M}L$. When applying the new mean of $I_{3M}L$ and $I_{3M}R$ cut-off 0.09 to the test sample for this population, the sensitivity was 77.63% and the specificity was 88.21% (Table 4). This is exactly in accordance with the results previously reported by Deitos et al. [37] for the Brazilian population with the cut-off at 0.08. Furthermore, the results of Se and Sp for the cut-off of 0.09 for the $I_{3M}R$ and the cut-off of 0.10 for the $I_{3M}L$ show slightly better results for the right third molar than for the left (Se 77.95%-Sp 87.14% and Se 77.95%-Sp 87.14%, respectively). This seems to confirm that there is no significant variability between left and right predictors when applying the I_{3M} index for the adult age assessment within the American population, whether the original of cut-off of 0.08 and the new cut-off values [44].

European region

Several population-specific criteria papers were published for the I_{3M} on European populations (Table 7). From a medico-legal and forensic perspective, the better specificity or proportion of accurately selected minors is even more

Table 4 Quantities and predictive values for classification of subjects based on $I_{3M}L$, $I_{3M}R$, and $I_{3M}L\_I_{3M}R$ measurements for validation of the new cutoff points for region of world

Sample	Continent	($I_{3M}L$)	Real		Se 95% CI	Sp 95% CI	PV + 95% CI	PV − 95% CI	LR + 95% CI	LR − 95% CI	Acc 95% CI
			Adult	Minor							
General	Africa (*n* = 543)	Adult	220	41	86.95% (84.12–89.78)	85.86% (82.93–88.79)	84.29% (81.23–87.35)	88.29% (85.59–90.99)	6.14 (5.73–6.55)	0.15 (0.11–0.18)	86.37% (83.48–89.26)
		Minor	33	249							
	America (*n* = 593)	Adult	244	36	77.95% (74.61–81.29)	87.14% (84.45–89.83)	87.14% (84.45–89.83)	77.95% (74.61–81.29)	6.06 (5.67–6.45)	0.25 (0.21–0.28)	82.29% (82.08–88.08)
		Minor	69	244							
	Asia (*n* = 1.302)	Adult	562	72	72.05% (69.61–74.49)	86.20% (84.30–88.07)	88.64% (86.92–90.36)	67.36% (64.81–69.91)	5.22 (4.94–5.49)	0.32 (0.29–0.34)	77.72% (75.46–79.98)
		Minor	218	450							
	Europe (*n* = 1.254)	Adult	634	29	80.05% (77.84–82.26)	93.72% (92.38–95.06)	95.62% (94.49–96.75)	73.26% (70.81–75.71)	12.74 (12.54–13.84)	0.21 (0.18–0.23)	85.08% 83.11–87.05)
		Minor	158	433							
Female	Africa (*n* = 418)	Adult	176	30	85.85% (82.51–89.19)	85.92% (82.59–89.25)	85.44% (82.06–88.82)	86.32% (83.03–89.61)	6.09 (5.62–6.56)	0.16 (0.12–0.20)	85.89% (82.55–89.23)
		Minor	29	183							
	America (*n* = 293)	Adult	114	17	76.51% (72.45–80.57)	88.19% (84.49–91.89)	87.02% (83.17–90.87)	78.40% (73.69–83.11)	6.48 (5.93–7.03)	0.26 (0.21–0.31)	82.25% (77.87–86.63)
		Minor	35	127							
	Asia (*n* = 744)	Adult	303	35	67.63% (64.27–70.99)	88.18% (85.86–90.50)	89.64% (87.45–91.83)	64.29% (60.85–67.73)	5.71 (5.35–6.07)	0.36 (0.33–0.39)	75.81% (72.73–78.89)
		Minor	145	261							
	Europe (*n* = 719)	Adult	355	11	77.51% (74.46–80.56)	95.79% (94.32–97.26)	96.99% (95.74–98.24)	70.82% (67.50–74.14)	18.39 (18.15–18.63)	0.23 (0.20–0.26)	84.14% (81.47–86.81)
		Minor	103	250							
Male	Africa (*n* = 125)	Adult	43	12	91.49% (86.60–96.38)	84.62% (78.30–90.94)	78.18% (70.94–85.42)	94.29% (90.22–98.36)	5.94 (5. 08–6.80)	0.10 (0.05–0.15)	87.20% (81.34–93.06)
		Minor	4	66							
	America (*n* = 300)	Adult	125	21	76.22% (71.40–81.04)	84.56% (80.47–88.65)	85.62% (81.65–89.59)	74.68% (69.76–79.60)	4.93 (4.36–5.50)	0.28 (0.23–0.33)	80.00% (75.47–84.53)
		Minor	39	115							
	Asia (*n* = 558)	Adult	259	39	78.01% (74.57–81.45)	82.74% (79.60–85.88)	86.91% (84.11–89.71)	71.92% (68.19–75.65)	4.52 (4.11–4.93)	0.26 (0.22–0.30)	79.93% (76.61–83.25)
		Minor	73	187							
	Europe (*n* = 535)	Adult	277	18	82.93% (79.74–86.12)	91.04% (88.62–93.46)	93.90% (91.87–95.93)	76.25% (72.64–79.86)	9.26 (9.04–9.48)	0.18 (0.15–0.21)	85.98% (83.04–88.92)
		Minor	57	183							
		($I_{3M}R$)									

Table 4 (continued)

Sample	Continent	($I_{3\mathrm{M}}L$)	Real		Se 95% CI	Sp 95% CI	PV+ 95% CI	PV− 95% CI	LR+ 95% CI	LR− 95% CI	Acc 95% CI
			Adult	Minor							
General	Africa (n = 543)	Adult	210	47	83.00% (79.84–86.16)	83.79% (80.69–86.89)	81.71% (78.46–84.96)	84.96% (81.95–87.97)	5.12 (4.70–5.54)	0.20 (0.16–0.23)	83.42% (80.29–86.55)
		Minor	43	243							
	America (n = 593)	Adult	252	27	80.51% (77.32–83.70)	90.35% (87.97–92.73)	90.32% (87.94–92.70)	80.57% (77.39–83.75)	8.34 (8.04–8.64)	0.21 (0.17–0.24)	85.16% (82.17–88.15)
		Minor	61	253							
	Asia (n = 1.302)	Adult	578	86	74.10% (71.72–76.48)	83.52% (81.50–85.54)	87.04% (85.22–88.86)	68.33% (65.80–70.86)	4.49 (4.22–4.76)	0.31 (0.28–0.33)	77.88% (75.63–80.13)
		Minor	202	436							
	Europe (n = 1.254)	Adult	647	35	81.70% (79.56–83.84)	92.42% (90.96–93.88)	94.86% (93.64–96.08)	74.65% (72.24–77.06)	10.77 (10.68–10.86)	0.19 (0.16–0.21)	85.64% (83.70–87.58)
		Minor	145	427							
Female	Africa (n = 418)	Adult	167	35	81.46% (77.73–85.19)	83.57% (80.02–87.12)	82.67% (79.04–86.30)	82.41% (78.76–86.06)	4.95 (4.47–5.43)	0.22 (0.18–0.26)	82.54% (78.90–86.18)
		Minor	38	178							
	America (n = 293)	Adult	114	14	76.51% (71.66–81.36)	90.28% (86.89–93.67)	89.06% (85.49–92.63)	78.79% (74.11–83.47)	7.86 (7.39–8.33)	0.26 (0.21–0.31)	83.28% (79.01–87.55)
		Minor	35	130							
	Asia (n = 744)	Adult	312	44	69.64% (66.34–72.94)	85.14% (82.58–87.70)	87.64% (85.28–90.00)	64.95% (61.52–68.38)	4.68 (4.32–5.04)	0.35 (0.32–0.38)	75.81% (72.73–78.89)
		Minor	136	252							
	Europe (n = 719)	Adult	357	17	77.95% (74.92–80.98)	93.49% (91.69–95.29)	95.45% (93.93–96.97)	70.72% (67.39–74.05)	11.96 (11.91–12.01)	0.23 (0.20–0.26)	83.59% (80.88–86.30)
		Minor	101	244							

Table 4 (continued)

Sample	Continent	($I_{3M}L$)	Real		Se 95% CI	Sp 95% CI	PV+ 95% CI	PV− 95% CI	LR+ 95% CI	LR− 95% CI	Acc 95% CI
			Adult	Minor							
Male	Africa ($n=125$)	Adult	42	13	89.36% (83.95–94.77)	83.33% (76.80–89.86)	76.36% (68.91–83.81)	92.86% (88.35–97.37)	5.36 (4.49–6.23)	0.12 (0.06–0.18)	85.60% (79.45–91.75)
		Minor	5	65							
	America ($n=300$)	Adult	133	15	81.60% (77.22–85.98)	89.05% (85.52–92.58)	89.86% (86.44–93.28)	80.26% (75.76–84.76)	7.45 (6.96–7.94)	0.20 (0.15–0.25)	85.00% (80.96–89.04)
		Minor	30	122							
	Asia ($n=558$)	Adult	266	44	80.12% (76.89–83.35)	80.53% (77.33–83.73)	85.81% (82.99–88.63)	73.39% (69.82–76.96)	4.11 (3.70–4.52)	0.24 (0.20–0.28)	80.29% (77.07–83.51)
		Minor	66	182							
	Europe ($n=535$)	Adult	288	18	86.23% (83.31–89.15)	91.04% (88.62–93.46)	94.12% (92.13–96.11)	79.91% (76.51–83.31)	9.62 (9.46–9.78)	0.15 (0.12–0.18)	88.04% (85.29–90.79)
		Minor	46	183							
		($I_{3M}L\_I_{3M}R$)									
General	Africa ($n=543$)	Adult	215	43	84.98% (81.97–87.99)	85.17% (82.18–88.16)	83.33% (80.20–86.46)	86.66% (83.80–89.52)	5.73 (5.31–6.15)	0.17 (0.13–0.20)	85.08% (82.08–88.08)
		Minor	38	247							
	America ($n=593$)	Adult	243	33	77.63% (74.28–80.98)	88.21% (85.61–90.81)	88.04% (85.43–90.65)	77.91% (77.57–81.25)	6.58 (5.20–6.96)	0.25 (0.21–0.28)	82.63% (79.44–85.82)
		Minor	70	247							
	Asia ($n=1.302$)	Adult	542	64	69.48% (66.98–71.98)	87.73% (85.95–89.51)	89.43% (87.76–91.10)	65.80% (63.22–68.38)	5.66 (5.39–5.93)	0.34 (0.31–0.36)	76.80% (74.51–79.09)
		Minor	238	458							
	Europe ($n=1.254$)	Adult	640	31	80.80% (78.62–82.98)	93.29% (91.91–94.67)	95.38% (94.22–96.54)	73.92% (71.49–76.35)	12.04 (11.85–12.23)	0.20 (0.17–0.22)	85.40% (83.45–87.35)
		Minor	152	431							

Table 4 (continued)

Sample	Continent	($I_{3M}L$)	Real		Se 95% CI	Sp 95% CI	PV+ 95% CI	PV− 95% CI	LR+ 95% CI	LR− 95% CI	Acc 95% CI
			Adult	Minor							
Female	Africa (n = 418)	Adult	172	32	83.90% (80.38–87.42)	84.98% (81.55–88.41)	84.31% (80.82–87.80)	84.58% (81.12–88.04)	5.58 (5.10–6.06)	0.18 (0.14–0.22)	84.45% (80.98–87.92)
		Minor	33	181							
	America (n = 293)	Adult	112	17	75.17% (70.22–80.12)	88.11% (84.40–91.82)	86.82% (82.95–90.69)	77.30% (72.50–82.10)	6.32 (5.77–6.87)	0.28 (0.23–0.33)	81.51% (77.06–85.96)
		Minor	37	126							
	Asia (n = 744)	Adult	291	30	64.96% (61.53–68.39)	89.86% (87.69–92.03)	90.65% (88.56–92.74)	62.88% (59.41–66.35)	6.40 (6.06–6.74)	0.39 (0.35–0.43)	74.87% (71.75–77.99)
		Minor	157	266							
	Europe (n = 719)	Adult	358	15	78.17% (75.15–81.19)	94.25% (92.55–95.95)	95.98% (94.54–97.42)	71.10% (67.79–74.41)	13.60 (13.43–13.77)	0.23 (0.20–0.26)	84.01% (81.33–86.69)
		Minor	100	246							
Male	Africa (n = 125)	Adult	42	12	89.36% (83.95–94.77)	84.62% (78.30–90.94)	77.78% (70.49–85.07)	92.96% (88.48–97.44)	5.80 (4.93–6.67)	0.12 (0.06–0.18)	86.40% (80.39–92.41)
		Minor	5	66							
	America (n = 300)	Adult	126	17	77.30% (72.56–82.04)	87.59% (83.86–91.32)	88.11% (84.45–91.77)	76.43% (71.63–81.23)	6.22 (5.67–6.77)	0.25 (0.20–0.30)	82.00% (77.65–86.35)
		Minor	37	120							
	Asia (n = 558)	Adult	251	36	75.60% (72.04–79.16)	84.07% (81.03–87.11)	87.46% (84.71–90.21)	70.11 (66.31–73.91)	4.74 (4.33–5.15)	0.29 (0.25–0.33)	79.03% (75.65–82.41)
		Minor	81	190							
	Europe (n = 535)	Adult	280	16	83.83% (80.71–86.95)	92.04% (89.75–94.33)	94.59% (92.67–96.51)	77.41% (73.87–80.95)	10.53 (10.34–10.72)	0.17 (0.14–0.20)	86.92% (84.06–89.78)
		Minor	54	185							

$I_{3M}L$ left third molar, $I_{3M}R$ right third molar, $I_{3M}L\_I_{3M}R$ arithmetic mean of $I_{3M}L$ and $I_{3M}R$ measurements, *Se* sensitivity, *Sp* specificity, *PV+* positive predictive value, *PV−* negative predictive value, *LR+* positive likelihood, *LR−* negative likelihood

Table 5 Quantities and predictive values for classification of subjects based on $I_{3M}L$, $I_{3M}R$, and $I_{3M}L\_I_{3M}R$ measurements for cut-off of 0.08 for region of world

Sample	Continent	($I_{3M}L$)	Real		Se	Sp	PV+	PV-	LR+	LR-	Acc
			Adult	**Minor**	95% CI	95% CI	95% CI	95% CI	95% CI	95% CI	95% CI
General	Africa (n = 543)	Adult	206	37	81.74% (78.49–84.99)	87.28% (84.48–90.08)	84.77% (81.75–87.79)	84.66% (81.63–87.69)	6.42 (6.02–6.82)	0.20 (0.17–0.23)	84.71% (81.68–87.74)
		Minor	46	254							
	America (n = 593)	Adult	233	31	74.67% (71.17–78.17)	88.96% (86.44–91.48)	88.25% (85.66–90.84)	75.98% (72.54–79.42)	6.76 (6.38–7.14)	0.28 (0.24–0.32)	81.45% (78.32–84.58)
		Minor	79	250							
	Asia (n = 1.302)	Adult	455	31	58.63% (55.95–61.31)	94.09% (92.81–95.37)	93.62% (92.29–94.95)	60.61% (57.96–63.26)	9.92 (9.87–9.97)	0.43 (0.40–0.46)	72.88% (70.47–75.29)
		Minor	321	494							
	Europe (n = 1.254)	Adult	616	23	77.48% (5.17–79.79)	94.98% (93.77–96.19)	96.40% (95.37–97.43)	70.89% (68.38–73.40)	15.43 (15.27–15.59)	0.23 (0.21–0.25)	83.89% (81.86–85.92)
		Minor	179	436							
Female	Africa (n = 418)	Adult	164	26	80.00% (76.17–83.83)	87.79% (85.44–90.14)	86.32% (83.03–89.61)	82.02% (78.34–85.70)	6.55 (6.09–7.01)	0.22 (0.18–0.26)	83.97% (80.45–87.49)
		Minor	41	187							
	America (n = 293)	Adult	110	14	73.83% (68.80–78.86)	90.28% (86.89–93.67)	88.71% (85.09–92.33)	76.92% (72.10–81.74)	7.59 (7.10–8.08)	0.28 (0.23–0.33)	81.91% (77.50–86.32)
		Minor	39	130							
	Asia (n = 744)	Adult	239	14	53.35% (47.64–59.06)	95.27% (93.72–96.82)	94.47% (92.83–96.11)	57.43% (53.88–60.98)	11.27 (11.06–11.48)	0.48 (0.44–0.52)	70.03% (66.74–73.32)
		Minor	209	282							
	Europe (n = 719)	Adult	346	10	75.55% (72.41–78.69)	96.17% (94.77–97.57)	97.19% (95.98–98.40)	69.15% (65.77–72.53)	19.71 (19.54–19.88)	0.25 (0.22–0.28)	83.03% (80.29–85.77)
		Minor	112	251							
Male	Africa (n = 125)	Adult	42	11	89.36% (83.95–94.77)	85.90% (79.80–92.00)	79.25% (72.14–86.36)	93.06% (88.60–97.52)	6.33 (5.49–7.17)	0.12 (0.06–0.18)	87.20% (81.34–93.06)
		Minor	5	67							
	America (n = 300)	Adult	123	17	75.46% (70.59–80.33)	87.86% (84.16–91.56)	87.86% (84.16–91.56)	75.00% (70.10–79.90)	6.08 (5.53–6.63)	0.28 (0.23–0.33)	81.00% (76.56–85.44)
		Minor	40	120							
	Asia (n = 558)	Adult	218	18	65.66% (61.72–69.60)	92.04% (89.79–94.29)	92.37% (90.17–94.57)	64.60% (60.63–68.57)	8.24 (7.92–8.56)	0.37 (0.33–0.41)	76.34% (72.81–79.87)
		Minor	114	208							
	Europe (n = 535)	Adult	268	15	80.24% (76.87–83.61)	92.54% (90.31–94.77)	94.70% (92.80–96.60)	73.81% (70.08–77.54)	10.75 (10.61–10.89)	0.21 (0.18–0.24)	84.86% (81.82–87.90)
		Minor	66	186							
		($I_{3M}R$)									
General	Africa (n = 543)	Adult	189	40	75.00% (71.35–78.65)	86.25% (83.35–89.15)	82.53% (79.33–85.73)	79.93% (76.56–83.30)	5.45 (5.03–5.87)	0.28 (0.24–0.32)	81.03% (77.73–84.33)
		Minor	63	251							
	America (n = 593)	Adult	238	28	76.28% (72.86–79.70)	90.03% (87.62–92.44)	89.47% (87.00–91.94)	77.37% (74.00–80.74)	5.54 (5.14–5.94)	0.26 (0.22–0.30)	83.00% (79.98–86.02)
		Minor	74	253							
	Asia (n = 1.302)	Adult	453	28	58.30% (55.62–60.98)	94.66% (93.44–95.88)	94.17% (92.90–95.44)	60.53% (57.87–63.19)	10.91 (10.86–10.96)	0.44 (0.41–0.47)	72.96% (70.55–75.37)
		Minor	324	497							
	Europe (n = 1.254)	Adult	622	21	78.23% (75.95–80.51)	95.42% (94.26–96.58)	96.73% (95.75–97.71)	71.68% (69.19–74.17)	17.08 (16.86–17.30)	0.22 (0.20–0.24)	84.53% (82.53–86.53)
		Minor	173	438							

Table 5 (continued)

Sample	Continent	($I_{3M}L$)	Real		Se 95% CI	Sp 95% CI	PV + 95% CI	PV- 95% CI	LR + 95% CI	LR- 95% CI	Acc 95% CI
			Adult	**Minor**							
Female	Africa (*n* = 418)	Adult	148	27	72.20% (67.91–76.49)	87.32% (84.13–90.51)	84.57% (81.11–88.03)	76.54% (72.48–80.60)	5.69 (5.22–6.16)	0.31 (0.27–0.35)	79.90% (76.06–83.74)
		Minor	57	186							
	America (*n* = 293)	Adult	112	13	75.17% (70.22–80.12)	90.97% (87.69–94.25)	89.60% (86.10–93.10)	77.98% (73.24–82.72)	8.32 (7.96–8.68)	0.27 (0.22–0.32)	82.94% (78.63–87.25)
		Minor	37	131							
	Asia (*n* = 744)	Adult	237	12	52.90% (49.31–56.49)	95.95% (94.53–97.37)	95.18% (93.64–96.72)	57.37% (53.82–60.92)	13.04 (12.84–13.24)	0.49 (0.45–0.53)	70.03% (66.74–73.32)
		Minor	211	284							
	Europe (*n* = 719)	Adult	342	10	74.67% (71.49–77.85)	96.17% (94.77–97.57)	97.16% (95.95–98.37)	68.39% (64.99–71.79)	19.48 (19.25–19.71)	0.26 (0.23–0.29)	82.48% (79.70–85.26)
		Minor	116	251							
Male	Africa (*n* = 125)	Adult	41	13	87.23% (81.38–93.08)	83.33% (76.80–89.86)	75.93% (68.44–83.42)	91.55% (86.67–96.43)	5.23 (4.35–6.11)	0.15 (0.09–0.21)	84.80% (78.51–91.09)
		Minor	6	65							
	America (*n* = 300)	Adult	126	15	77.30% (72.56–82.04)	89.05% (85.52–92.58)	89.36% (85.87–92.85)	76.73% (71.95–81.51)	7.06 (6.54–7.58)	0.25 (0.20–0.30)	82.67% (78.39–86.95)
		Minor	37	122							
	Asia (*n* = 558)	Adult	218	16	65.66% (61.72–69.60)	92.92% (90.79–95.05)	93.16% (91.07–95.25)	64.81% (60.85–68.77)	9.27 (9.05–9.49)	0.36 (0.32–0.40)	76.70% (73.19–80.21)
		Minor	114	210							
	Europe (*n* = 535)	Adult	278	13	83.23% (80.06–86.40)	93.53% (91.45–95.61)	95.53% (93.78–97.28)	77.05% (73.49–80.61)	12.86 12.75–12.97)	0.17 (0.14–0.20)	87.10% (84.26–89.94)
		Minor	56	188							
		($I_{3M}L$_$I_{3M}R$)									
General	Africa (*n* = 543)	Adult	206	34	80.46% (77.12–83.80)	88.15% (85.43–90.87)	85.83% (82.90–88.76)	83.49% (80.37–86.61)	6.78 (6.39–7.17)	0.22 (0.19–0.25)	84.53% (81.49–87.57)
		Minor	50	253							
	America (*n* = 593)	Adult	235	30	75.32% (71.85–78.79)	89.32% (86.83–91.81)	88.67% (86.12–91.22)	76.52% (73.11–79.93)	7.05 (6.68–7.42)	0.27 (0.23–0.31)	81.95% (78.85–85.05)
		Minor	77	251							
	Asia (*n* = 1.302)	Adult	454	29	58.35% (55.67–61.03)	94.46% (93.22–95.70)	93.99% (92.70–95.28)	60.43% (57.77–63.09)	10.53 (10.41–10.65)	0.44 (0.41–0.47)	72.88% (70.47–75.29)
		Minor	324	495							
	Europe (*n* = 1.254)	Adult	620	21	77.98% (75.59–80.19)	95.42% (94.26–96.58)	96.72% (95.73–97.71)	71.45% (68.95–73.95)	17.02 (16.79–17.25)	0.23 (0.21–0.25)	84.37% (82.36–86.38)
		Minor	175	438							
Female	Africa (*n* = 125)	Adult	164	22	80.00% (72.99–87.01)	89.67% (84.33–95.01)	88.17% (82.51–93.83)	82.33% (75.64–89.02)	7.74 (7.01–8.47)	0.22 (0.15–0.29)	84.93% (78.66–91.20)
		Minor	41	191							
	America (*n* = 293)	Adult	110	14	73.83% (68.80–78.86)	90.28% (86.89–93.67)	88.71% (85.09–92.33)	76.92% (72.10–81.74)	7.59 (7.10–8.08)	0.28 (0.23–0.33)	81.91% (77.50–86.32)
		Minor	39	130							
	Asia (*n* = 744)	Adult	239	14	53.35% (49.77–56.93)	95.27% (93.74–96.80)	94.47% (92.83–96.11)	57.43% (53.88–60.98)	11.27 (11.06–11.48)	0.48 (0.44–0.52)	70.03% (66.74–73.32)
		Minor	209	282							
	Europe (*n* = 719)	Adult	345	9	75.33% (72.18–78.48)	96.55% (95.22–7.88)	97.46% (96.31–98.61)	69.04% (65.66–72.42)	21.84 (21.70–21.98)	0.25 (0.22–0.28)	83.03% (80.29–85.77)
		Minor	113	252							

Table 5 (continued)

Sample	Continent	($I_{3M}L$)	Real		Se 95% CI	Sp 95% CI	PV+ 95% CI	PV- 95% CI	LR+ 95% CI	LR- 95% CI	Acc 95% CI
			Adult	**Minor**							
Male	Africa (*n* = 125)	Adult	42	12	89.36% (83.95–94.77)	84.62% (78.30–90.94)	77.78% (70.49–85.07)	92.96% (88.48–97.44)	5.80 (4.93–6.67)	0.12 (0.06–0.18)	86.40% (80.39–92.41)
		Minor	5	66							
	America (*n* = 300)	Adult	125	16	76.69% (71.91–81.47)	88.32% (84.69–91.95)	88.65% (85.06–92.24)	76.10% (71.27–80.93)	6.56 (6.02–7.10)	0.26 (0.21–0.31)	82.00% (77.65–86.35)
		Minor	38	121							
	Asia (*n* = 558)	Adult	217	16	65.36% (61.41–69.31)	92.92% (90.79–95.05)	93.13% (91.03–95.23)	64.62% (60.65–68.59)	9.23 (9.01–9.45)	0.37 (0.33–0.41)	76.52% (73.00–80.04)
		Minor	115	210							
	Europe (*n* = 535)	Adult	273	14	81.74% (78.47–85.01)	93.03% (90.87–95.19)	95.12% (93.29–96.95)	75.40% (71.75–79.05)	11.73 (11.58–11.88)	0.19 (0.16–0.22)	85.98% (83.04–88.92)
		Minor	61	187							

$I_{3M}L$ left third molar, $I_{3M}R$ right third molar, $I_{3M}L\_I_{3M}R$ arithmetic mean of $I_{3M}L$ and $I_{3M}R$ measurements, *Se* sensitivity, *Sp* specificity; *PV*+positive predictive value, *PV*− negative predictive value, *LR* + positive likelihood, *LR* − negative likelihood

Table 6 Cutoff for Asian countries

Country	I3M_L Cutoff/Acc	I3M_R Cutoff/Acc	I3M_L_I3M_R Cutoff/Acc
India	0.09/91.50%	0.11/92.10%	0.09/92.2%
Indonesia	0.15/84.30%	0.17/84.40%	0.19/85.10%
Japan	0.10/89.80%	0.09/90.20%	0.09/90.10%
Lebanon	0.19/91.80%	0.24/92.20%	0.23/92.10%
Malaysia	0.21/51.40%	0.19/56.20%	0.16/53.10%
Sri Lanka	0.18/92.20%	0.14/88.70%	0.14/92.70%
Turkey	0.13/82.40%	0.11/82.70%	0.11/83.00%

Acc accuracy

critical for the protection of any individual without a birth certificate when examining those who claim to be under 18 years of age. The new cut-off obtained from this study (0.09 for all 3 predictors) showed an Sp greater than 90% and a PPV − greater than 73% (Table 4). This confirms that in terms of the European population, the I_{3M} cut-off is a reliable method to reduce the risk of misclassifying minor subjects [13].

Asian region

On the other hand, the data from the Asian region could be described as very interesting (Table 4). Previous analyses on Asian population data (Table 7) have revealed identical trends to other worldwide datasets. The two different, new cut-off values for $I_{3M}L$ and $I_{3M}R$ in the Asian sample were 0.17 and 0.15, respectively, with the mean being 0.14. They are in accordance with previous works that have indicated a complex geographical history among Southeast Asians and, to a lesser extent, among East/Northeast Asians [64]. All these works fully agreed that when certain dental characteristics (such as morphological and morphometrical) are analyzed, the impact of environmental factors should be considered as well as how such factors may influence phenotypic features. This is particularly the case among Northeast Asians when assessing if environmental influences on phenotypic variation are able to erase the effects of genetic control [64]. When applying the new cut-off of $I_{3M}L$ and $I_{3M}R$ [0.15 and 0.17] to the test sample for this population, the sensitivity was 72.05% and 74.10% and the specificity 86.20% and 83.52%, respectively (Table 4) showing a better Se performance for males than for females (with a discrepancy of 10% for $I_{3M}L$ Se and of 10.48% for $I_{3M}R$ Se). In fact, only the Asian population presented a lower negative predictive value than all the other populations (Table 4) and this means that the correct classification power of minors using the new I_{3M} cut-off is consistently less accurate than in the other regions especially for females but better than the original at 0.08 ($I_{3M}L$ Se = 53.35% in female and $I_{3M}L$ Se = 58.63% in both sexes, Table 5).

Table 7 Summarize of sample references and previous research

Continent	Author and year	Country of subjects' origin	Sensitivity (Se)	Specificity (Sp)	Percentage of individuals correctly classified accuracy
African region	Angelakopoulos et al. (2018) [11]	**Black South Africa**	Overall males and females 80%	Overall males and females 95%	Overall males and females 90%
	Cavrić et al. (2016) [32]	**Black Botswana**	Males 88% (95% CI 86–90%); Females 0.88% (95%CI 86–89%)	Males 94% (95% CI 91–96%); Females 96% (95% CI 94–98%)	Males 91% (95% CI 88–93%); Females 92% (95%CI 90–93%)
	Dardouri et al. (2016) [29]	**Libya**	Males, 90.9%; Females, 90.6%	Males, 100%; Females, 100%	Males 95.1% (95% CI 91.5–98.7%); Females 94.5% (95%CI 90.9–98.1%)
	El-Bakary et al. (2019) [41]	**Egypt**	Males 95% (95% CI 89–100%); Females 73% (95% CI 64–82%)	Males 100% Females 97% (95% CI 92–100%)	Males 97% (95% CI 93–100%) Females 82% (95% CI 76–88%)
American region	Gómez Jiménez et al. (2019) [16]	**Dominican Republic**	Males 94% (95% CI 88–97%) Females 99% (95% CI 96–99%)	Males 99% (95% CI 95–99%) Females 92% (95% CI 86–95%)	Males 96% (95% CI: 93–98%) Females 96% (95% CI: 93–97%)
	Nóbrega et al. (2019) [18]	**Northeastern Brazil**	Males 81.1% Females 68.5%	Males 84.0% Females 89.0%	Males 84.2% (95%CI: 78.1–90.3%) Females 78.8% (95%CI: 72.4–85.1%)
	Cameriere et al. (2018) [24]	**Chile**	Overall males and females 70.5%	Overall males and females 88.4%	Overall males and females 83%
	Quispe Lizarbe et al. (2017) [28]	**Peru**	Males 96%; Females 84%	Males 96%; Females 95%	Males 96%; Females 90%
	De Luca et a. (2016) [33]	**Colombia**	Males 91.7% (95% CI 85.1–96.8%); Females 95.1% (95% CI 87.1–95.0%)	Males 90.6% (95% CI 82.1–97.8%); Females 93.8% (95% CI 87.1–98.8%)	Males 91.2%; Females 94.4%
	Deitos et al. (2015) [36]	**Brazil**	Overall 77.4% (95% CI 71.3–82.7%) (males 87.6%; females 86.5%)	Overall 86.2% (95% CI 80.9–90.4%) (males 87.0%; females 67.2%)	Overall males 87.3%; Overall females 76.1%

This result has partially contradicted the expectations of the pre-existing literature [65], suggesting the existence of a difference in the development of the third molar between the Asian population and other geographical areas. By analyzing the levels of accuracy obtained with the new cut-offs in each population studied for the Asian sample (Table 6), it was highlighted that the difference in the results derives from a clear variability between the populations of the same Asian area and not between different geographical areas. For example, the Lebanese population showed a higher new cut-off than when the original Cameriere's method was used (0.19 for $I_{3M}L$, 0.24 for $I_{3M}R$, and 0.23 for $I_{3M}L$ and $I_{3M}R$; Table 6). This allows, with the same interval of confidence, an accuracy which almost surpasses the estimated post-test probabilities reported in the specific literature [41]. In contrast, the Malaysian sample has the greatest variability compared to the cut-off of the original method (0.21 for $I_{3M}L$, 0.19 $I_{3M}R$, and 0.16 for $I_{3M}L$-$I_{3M}R$) with the lowest accuracy levels at the same 95% confidence interval, i.e., 51.40%, 56.20%, and 53.10%, respectively (Table 6). This level of accuracy would certainly not be acceptable from a legal point of view. Furthermore, no previous studies have tested the I_{3M} index on a referenced Malaysian population. In particular, some authors validated Cameriere's method of estimating age but only on growing subjects up to 15–16 years [66–68], obtaining excellent results in terms of precision and accuracy but not evaluating the maturation stages and the reliability of the third molar for the age of majority. Other studies on the Malaysian population showed a wide variability regarding the development of the lower third molars in the population of Malaysia [69, 70]. Johan et al. (2012) found that 71.1% of the variance in outcomes was explained by sex and developmental stage of the right, as well as left, mandibular third molar, which developed earlier in males than in females

Table 7 (continued)

Continent	Author and year	Country of subjects' origin	Sensitivity (Se)	Specificity (Sp)	Percentage of individuals correctly classified accuracy
European region	Scandoni et al. (2020) [14]	**Russia**	Males 96% Females 93%	Males 98% Females 98%	Not available
	Albernaz Neves et al. (2020) [15]	**Portugal**	Males (94.9%; 95% CI 93.3–96.4%) Females (87.5%; 95% CI 85.2–89.8%)	Males (93.5%; 95% CI 91.8–95.3%) Females (95.7%; 95% CI 94.3–97.1%)	Males 94% (95% CI 92.4–95.7%) Females 91.2% (95% CI 89.9–93.8%)
	Tafrount et al. (2019) [20]	**Southern France**	Males 87.1% (95% CI 80.4–83.4%) Females 81.3% (95% CI 75–84.5%)	Males 95.3% (95% CI 89.8–98.3%) Females 96.2% (95% CI 91.3–97%)	Males 91.6% (95% CI 87.1–90.8%) Females 89.7% (95%CI 84.2–92.5%)
	Spinas et al. (2018) [21]	**Sardinia, Italy**	Males 85% (95% CI 78– 91%) Females 79% (95% CI 71–85%)	Males 91% (95% CI 82–96%) Females 100% (95% CI, 92–100%)	Males 87% (95% CI 82–91%) Females 84% (95% CI, 0.78– 0.89%)
	Różyło-Kalinowska et al. (2018) [22]	**Poland**	Males 86.2% (95% CI 82.8–89.6%) Females 82.6% (95% CI 78.4– 86.7%)	Males 91.2% (95% CI 86.7% to 95.8%) Females 93% (95% CI, 88.3–97.7%)	Males 87.6% (95% CI 84.8–90.3%) Females 85.3% (95% CI 82–88.6%)
	Antunovic et al. (2018) [23]	**Montenegro**	Males 92% (95% CI 88–96%) Females 82% (95% CI 79–84%)	Males 94% (95% CI 90–98%) Females 96% (95% CI 0.93–0.98)	Males 93% (95% CI 90–96%) Females 89% (95% CI 85–91%)
	Kelmendi et al. (2018) [25]	**Kosovo**	Males 96.2% (95% CI 92.5–97.8%) Females 82.6% (95% CI 78.7–83.4%)	Males 97.6% (95% CI 92.9–99.5%) Females 99.1% (95% CI 95.3– 100%)	Males 96.8 (95% CI 92.6–98.5%) Females 90.9% (95% CI 87–91.7%)
	Boyacıoğlu Doğru et al. (2018) [26]	**Netherlands**	Males 84% (95% CI 78.9–86.6%) Females 72.7% (95%CI, 67.6–75.0%)	Males 95.0% (95% CI, 88.7–98.3%) Females 96.3% (95%CI, 90.0–99.0%)	Males 88.9% (95% CI 83.3–91.8%) Females 83.3 (95% CI 77.7–85.8%)
	Zelic et al. (2016) [34]	**Serbia**	Males 96% (95% CI 93–98%); Females 86% (95% CI 83–87%)	Males 94% (95% CI 90–98%); Females 98% (95% CI 94–99)	Males 95% (95% CI 92–98%); Females 91% (95%CI 87–92%)
	De Luca et al. (2016) [35]	**Italy**	Overall 86.6% (95% CI 80.8–91.1%)	Overall 95.7% (95% CI 92.1–98.0- %)	Overall 91.4%
	Cameriere et al. (2014) [39]	**Italy**	Overall 84.1% (95% CI 76.7–89.9%)	Overall 92.5% (95% CI 87.0–96.2- %)	Overall 88.5%
	Galić et al. (2015) [37]	**Croatia**	Males 91.2% (95% CI 88.7–93.1%); Females 84.3% (95% CI 80.6–87.5%)	Males 91.9% (95% CI 88.8–94.3-%); Females 95.4% (95% CI 92.5–97.5%)	Males 91.5% (95% CI 89.0–93.5%); Females 88.8% (95%CI 86.3–90.9%)
	Cameriere et al. (2014) [38]	**Albania**	Males 94.1% (95% CI 87.6–97.8%); Females 75.4% (95%CI 68.1–78.8%)	Males, 90.9% (95% CI 84.2–94.7%); Females 96.6% (95% CI 91.1–99.1%)	Males 92.5% (95% CI 85.9–96.2%); Females 87.5% (95% CI 81.2–90.4%)

Table 7 (continued)

Continent	Author and year	Country of subjects' origin	Sensitivity (Se)	Specificity (Sp)	Percentage of individuals correctly classified accuracy
Asian region	Khare et al. (2020) [17]	**Eastern China**	Males 91.9% (95%CI 87.1–96.7%) Females 75.0% (67.5% to 82.5%)	Males 91.9% (95%CI 87.1–96.7%) Females 100%	Males 92% (95%CI 88.4–95.5%) Females 85.8% (95% CI 81.3–90.4%)
	Chu et al. (2018) [63]	**Northern Chinese**	Males ≥ 18 years 87.1% (95% CI 82.9–91.4); Females 77.2% (95% CI 72–82.5%)	Males <18 years 95.6% (95% CI 92.6–98.6%); Females 98.4% (95% CI 96.6–100%)	Males 90.7% (95% CI 87.9%–93.5%); Females 87.6% (95% CI 84.5–90.8)
	Kumagai et al. (2019) [19]	**Japan**	Males 89% (95% CI: 83–95%); Females 84% (95% CI 77–92%)	Males 96% (95%CI: 90–100%); Females 93% (95% CI 85–100%)	Males 91% (95% CI: 87–96%) Females 87% (95% CI 81–93%)
	Balla et al. (2017) [27]	**South India**	Males 90.2% (95% CI 81.2–99.3%); Females 83.3% (95%CI 73.4–93.3%)	Males 95.1% (95% CI 89.7–100.0%); Females 98.3% (95%CI 95.1–100.0%)	Males 93.1% (95% CI 88.2–98.0%); Females 91.2% (95%CI 86.0–96.4%)
	Gulsahi et al. (2016) [31]	**Turkey**	Males 94.6% (95% CI 88.1–99.8%); Females 85.9% (95%CI 77.1–92.8%)	Males 100%; Females 100%	Males 97.6%; Females 92.7%
	Moukarzel et al. (2020) [40]	**Lebanon**	Males 63% (95% CI 56–70%); Females 61% (95% CI 53–69%)	Males 89% (95% CI: 83–93%); Females 97% (95% CI: 93–98%)	Males 75% (95% CI 70–79%) Females 79% (95% CI 74–83%)
	AlQahtani et al. (2017) [13]	**Saudi Arabia**	Males 52.3%; Females 51.3%	Males 100%; Females 97%	Males 75.6%; Females 72.4%

[69]. An additional study [71] confirmed that the Malaysian population also shows great variability in the manifestation of agenesis of the third molar and that the root development begins earlier in females up to 9 years old. However, the molar development of male children above 9 years old accelerates more quickly in central adolescence than in females.

Sex differences

Previous studies have already widely demonstrated that the rate of development varies according to sex, with a greater delay in females than in males for both $I_{3M}L$ and $I_{3M}R$ [44, 72]. However, sex differences in third molar development have been reported with contradictory results in studies of samples of different origins [14, 20, 37, 39]. Comparing the original cut-off in Table 5, the new cut-off estimates show and confirm a prediction accuracy higher for males than for females in practically all inferences. The difference in accuracy between genders ranged from 0.49% (America; $I_{3M}L$ and _I_{3M}R) to 4.48% (Asia; $I_{3M}L$) and the accuracy was only higher for American females (2.25% higher) for the $I_{3M}L$ estimate (Table 4).

Different genetic studies have confirmed the hypothesis that the majority of the variations exists within a population as a group composed of different subgroups rather than between large populations [73], including dental development variation [74]. For example, regarding the Demirjian method [49], Dhamo et al. [8] revealed differences in the timing of dental development within a multi-ethnic population. This seem to suggest that differences in dental development should be evaluated in samples of heterogeneous origin when using the original patterns (i.e., Demirjian stage-based approach). In sum, the results obtained in this research showed that although moderate geographical differences in the application of the new cut-off (0.08) were detected, the accuracy values increased only slightly when compared with the studies performed on the I_{3M} values in each specific population (Tables 5, 6, and 7). This confirms the usefulness of both the original and specific cut-off for obtaining population-specific values by having a very high classification capacity in both sexes.

According to the data, only population-specific studies have been carried out so far to test the validity of the original cut-off. However, the ethnic group considered in each different study was based on a relatively small sample size (about 200 or 300 subjects). In addition, it was analyzed independently from their original common geographic group (i.e., Africa in case of Botswana [33] or Asia in case of Lebanon [41]).

By contrast, in this study, the sample was investigated as a whole for each continent, considering the genetic ancestry unceasingly based on African, European, American, or Asian genetic content for each subject belonging to the general sample.

A similar study was performed in 2013 by Thevissen et al. [65], in which no evidence was detected for important differences in the degree of third molar development when comparing 14 country-specific populations. The major strength of this work is the inclusion of a huge number of subjects from a multi-ethnic and geographic-based prospective cohort design, with determined measurements of dental development (I_{3M}). In this case, the original and the new cut-off estimates were applied in a heterogeneous sample of 10,701 OPT's coming from 4 continents in order to investigate whether differences in third molar development between various geographic groups could be assessed.

Another strength of this research is the combined approach of both left and right side ($I_{3M}L$ and $I_{3M}R$), showing that there are no significant differences between $I_{3M}L$ and $I_{3M}R$ for most countries in the different regions of the world. Even when a discrepancy was detected, it was statistically insignificant (Table 2).

Finally, based on LR +, the results of this study presented a moderate to high potential to increase the chances of classifying an adult individual as an adult. Furthermore, LR − presented a small to moderate potential to reduce the chances of classifying a minor as a minor. Concerning the LR +, it is noteworthy that within the European groups, all the values were above 10, indicating for all the considered situations (i.e., use of $I_{3M}R$, $I_{3M}L$, or both indices, respectively), a large and often conclusive increase in the likelihood of legal age ($\geq$ 18 years). Other data of interest is related to the PPV values that, for all the groups, were very high, thus indicating a strong correlation with the specificity values. PPV +is the probability that a subject with a positive (18 years old) test result is indeed 18 years old or older. In accordance with the results, the more specific the test, the less likely an individual with a positive test will be minor and the greater the positive predictive value.

Limitations of this research

This research is not without limitations. The samples analyzed originated from urban areas in different countries, which have very distinct socio-economic environments. As such, this does not provide a fair representation of the socio-economic status of the global population. In addition, data from subjects presenting with systemic diseases and endocrine anomalies were excluded. Finally, there was some difficulty in collecting data from certain geographical areas: the retrospective selection of data influences the amount of data available and where they are obtained from.

No images were collected in some geographical areas such as North Africa or North America, or Oceania. Another limitation is the lack of some important population sample, such as Chinese sample for Asian population. The inclusion of a Chinese sample in this study would have provided the authors with further insight about how, in such a vast region, made up of a complex demographic history and migration routes, the patterns and rates of third molar development differ from those of other geographical groups. Furthermore, the lack of specific studies that validated I_{3M} index on the Malaysian population prevents comparison of the results obtained in the literature with the new cut-off. The lack of research on the variability of the third molar in relation to its radicular morphology rather than to its maturity stages according to Demirjian et al. [49] means that the interpretation of the results for the Malaysian sample cannot yet be confirmed.

A natural progression of this work is to assess the influence of geography as the main factor in shaping the variation of the cut-off values' diversity across the Asian area. For this purpose, and in accordance with the hypothesis that the majority of the variations exists within a population made of different subgroups, a larger and more diverse sample of Asian subjects should be analyzed in the same research study.

Conclusion

A long-debated question, whether the I_{3M} cut-off used by the forensic community to allow an accurate distinction of someone being a major or minor, may also be used in a global area such as a continent, and independently from the specific origin (i.e., his/her country) of a subject belonging to that area.

Since variations in the rate of dental development were detected across populations [75], all previous studies were limited to regional scales, analyzing mostly limited population samples. A study seeking to investigate this topic with a larger set of globally distributed population samples is still pending.

Acknowledgements The authors would like to thank Adriana Santos, Ana Rodrigues, and Rui Santos, researchers from the Portuguese Institution CEAUL, under FCT — Fundação para a Ciência e a Tecnologia and the project UIDB/00006/2020, and the Clinic of Dentistry at the Universidad Científica del Sur (Perú).

Funding Inês Oliveira-Santos is supported by a FCT-Fundação para a Ciência e Tecnologia grant (SFRH/BD/139158/2018).

This research study was conducted retrospectively from OPTs obtained for clinical purposes. The study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Data availability The data that support the findings of this study are available on request from the corresponding author, [SDL]. The data are not publicly available due to [restrictions e.g. their containing information that could compromise the privacy of research participants].

Declarations

Informed consent According to the journal's submission guidelines, an informed consent to participate in the study is not necessary for images such as x-rays to which the subjects had already consented to undergo and conserve for other reasons of a clinical nature.

Conflict of interest The authors declare no competing interests.

References

1. Garamendi PM, Landa MI (2003) Estimación forense de la edad en torno a 18 años. Revision bibliografica. Cuad Med Forense (31): 13–24. ISSN: 1988–611X
2. Cattaneo C, Ritz-Timme S, Gabriel P, Gibelli D, Giudici E, Popp P, Nohrden D, Assmann S, Schmitt R, Grandi M (2009) The difficult issue of age assessment on pedo-pornographic material. Forensic Sci Int 183(1–3):21–24. https://doi.org/10.1016/j.forsciint.2008.09.005
3. Balla SB, Chinni SS, Galic I, Alwala AM, Machani P, Cameriere R (2019) A cutoff value of third molar maturity index for indicating a minimum age of criminal responsibility: older or younger than 16 years? J Forensic Leg Med 65:108–112. https://doi.org/10.1016/j.jflm.2019.05.014
4. Cunha E, Baccino E, Martrille L, Ramsthaler F, Prieto J, Schuliar Y, Lynnerup N, Cattaneo C (2009) The problem of aging human remains and living individuals: a review. Forensic Sci Int 193(1–3):1–13. https://doi.org/10.1016/j.forsciint.2009.09.008
5. EASO. 2018. EASO Practical Guide on age assessment. 2nd edition. doi:https://doi.org/10.2847/23618732
6. Ritz-Timme S, Kaatsch HJ, Marré B, Reisinger W, Riepert T, Rösing FW, Rötzscher K, Schmeling A, Geserick G (2002) Empfehlungen für die Altersdiagnostik bei Lebenden im Rentenverfahren. Rechtsmedizin 12(4):193–194. https://doi.org/10.1007/s00194-002-0159-1
7. Franklin D, Karkhanis S, Flavel A, Collini F, De Luca S, Cameriere R (2016) Accuracy of a cutoff value based on the third molar index: validation in an Australian population. Forensic Sci Int 266:575-e1
8. Dhamo B, Kragt L, Grgic O, Vucic S, Medina-Gomez C, Rivadeneira F, Jaddoe V, Wolvius EB, Ongkosuwito EM (2018) Ancestry and dental development: a geographic and genetic perspective. Am J Phys Anthropol 165(2):299–308. https://doi.org/10.1002/ajpa.23351
9. Mughal AM, Hassan N, Ahmed A (2014) Bone age assessment methods: a critical review. Pak J Med Sci 30(1):211–215. doi:http://dx.doi.org/https://doi.org/10.12669/pjms.301.4295
10. Kim S, Lee YH, Noh YK, Park FC, Auh AS (2021) Age-group determination of living individuals using first molar images based on artificial intelligence. Sci Rep 11(1):1073. https://doi.org/10.1038/s41598-020-80182-8
11. 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. https://doi.org/10.1038/sj.bdj.2010.976
12. Angelakopoulos N, De Luca S, Velandia Palacio LA, Coccia E, Ferrante L, Cameriere R (2018) Third molar maturity index (I_{3M}) for assessing age of majority: study of a black South African sample. Int J Legal Med 132(5):1457–1464. https://doi.org/10.1007/s00414-018-1818-4
13. 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 122:493–497. https://doi.org/10.1007/s00414-008-0279-6
14. AlQahtani S, Kawthar AA, AlShalan A (2017) Third molar cutoff value in assessing the legal age of 18 in Saudi population. Forensic Sci Int 272:64–67
15. Scandoni R, Zolotenkova GV, Vanin S, Pigolkin YI, Cameriere R, (2020) Forensic validity of the third molar maturity index (I_{3M}) for age estimation in a Russian population. Biomed Res Int Article ID 6670590https://doi.org/10.1155/2020/6670590
16. Albernaz Neves J, Antunes-Ferreira N, Machado V, Botelho J, Proença L, Quintas A, Delgado AS, Mendes JJ, Cameriere R (2020) Validation of the third molar maturation index (I_{3M}) to assess the legal adult age in the Portuguese population. Sci Rep 10(1):18466. https://doi.org/10.1038/s41598-020-75324-x
17. Gómez Jiménez L, Velandia Palacio LA, De Luca S, Ramirez Vasquez Y, Corominas Capellán M, Cameriere R (2019) Validation of the third molar maturity index (I_{3M}): study of a Dominican Republic sample. J Forensic Odontostomatol 3(37):27–33
18. Khare P, Li J, Velandia Palacio LA, Galić I, Ferrante L, Cameriere R (2020) Validation of the third molar maturity index cutoff value of <008 for indicating legal age of 18 years in Eastern Chinese region. Leg Med (Tokyo) 42(101):645
19. Nóbrega J, Protasio A, Ribeiro I, Valença A, Santiago BM, Cameriere R (2019) Validation of the third molar maturation index to estimate the age of criminal responsibility in Northeastern Brazil. Forensic Sci Int 304:109917. https://doi.org/10.1016/j.forsciint.2019.109917
20. Kumagai A, Takahashi N, Palacio L, Giampieri A, Ferrante L, Cameriere R (2019) Accuracy of the third molar index cutoff value for estimating 18 years of age: validation in a Japanese samples. Leg Med (Tokyo) 38:5–9. https://doi.org/10.1016/j.legalmed.2019.03.001
21. Tafrount C, Galić I, Franchi A, Fanton L, Cameriere R (2019) Third molar maturity index for indicating the legal adult age in southeastern France. Forensic Sci Int 294:218.e1-218.e6. https://doi.org/10.1016/j.forsciint.2018.10.013
22. Spinas E, De Luca S, Lampis L, Velandia Palacio LA, Cameriere R (2018) Is the third molar maturity index (I_{3M}) useful for a genetic isolate population? Study of a Sardinian sample of children and young adults. Int J Legal Med 132(6):1787–1794. https://doi.org/10.1007/s00414-018-1933-2
23. Różyło-Kalinowska I, Kalinowski P, Kozek M, Galić I, Cameriere R (2018) Validity of the third molar maturity index I_{3M} for indicating the adult age in the Polish population. Forensic Sci Int 290:352.e1-352.e6. https://doi.org/10.1016/j.forsciint.2018.06.034
24. Antunovic M, Galic I, Zelic K, Nedeljkovic N, Lazic E, Djuric M, Cameriere R (2018) The third molars for indicating legal adult age in Montenegro. Leg Med (Tokyo) 33:55–61. https://doi.org/10.1016/j.legalmed.2018.05.006. (**Advance online publication**)
25. Cameriere R, Velandia Palacio LA, Pinares J, Bestetti F, Paba R, Coccia E, Ferrante L (2018) Assessment of second (I2M) and third (I_{3M}) molar indices for establishing 14 and 16 legal ages and validation of the Cameriere's I_{3M} cutoff for 18 years old in Chilean population. Forensic Sci Int 285:205.e1-205.e5. https://doi.org/10.1016/j.forsciint.2017.12.043
26. Kelmendi J, Cameriere R, Koçani F, Galić I, Mehmeti B, Vodanović M (2018) The third molar maturity index in indicating the legal adult age in Kosovar population. Int J Legal Med 132(4):1151–1159. https://doi.org/10.1007/s00414-017-1761-9

27. Boyacıoğlu Doğru H, Gulsahi A, Çehreli SB, Galić I, van der Stelt P, Cameriere R (2018) Age of majority assessment in Dutch individuals based on Cameriere's third molar maturity index. Forensic Sci Int 282:231.e1-231.e6. https://doi.org/10.1016/j.forsciint.2017.11.009
28. Balla SB, Galic I, Karunakar P, Vanin S, De Luca S, Cameriere R (2017) Validation of third molar maturity index (I_{3M}) for discrimination of juvenile/adult status in South Indian population. J Forensic Leg Med 49:2–7. https://doi.org/10.1016/j.jflm.2017.05.003
29. Quispe Lizarbe RJ, Solís Adrianzén C, Quezada-Márquez MM, Galić I, Cameriere R (2017) Demirjian's stages and Cameriere's third molar maturity index to estimate legal adult age in Peruvian population. Leg Med (Tokyo) 25:59–65. https://doi.org/10.1016/j.legalmed.2017.01.003
30. Dardouri A, Cameriere R, De Luca S, Vanin S (2016) Third molar maturity index by measurements of open apices in a Libyan sample of living subjects. Forensic Sci Int 267:230.e1-230.e6. https://doi.org/10.1016/j.forsciint.2016.07.020
31. Franklin D, Karkhanis S, Flavel A, Collini F, De Luca S, Cameriere R (2016) Accuracy of a cutoff value based on the third molar index: validation in an Australian population. Forensic Sci Int 266:575.e1-575.e6. https://doi.org/10.1016/j.forsciint.2016.06.032
32. Gulsahi A, De Luca S, Cehreli SB, Tirali RE, Cameriere R (2016) Accuracy of the third molar index for assessing the legal majority of 18 years in Turkish population. Forensic Sci Int 266:584.e1-584.e6. https://doi.org/10.1016/j.forsciint.2016.04.034
33. Cavrić J, Galić I, Vodanović M, Brkić H, Gregov J, Viva S, Rey L, Cameriere R (2016) Third molar maturity index (I_{3M}) for assessing age of majority in a black African population in Botswana. Int J Legal Med 130(4):1109–1120. https://doi.org/10.1007/s00414-016-1344-1
34. De Luca S, Aguilar L, Rivera M, Palacio LA, Riccomi G, Bestetti F, Cameriere R (2016) Accuracy of cutoff value by measurement of third molar index: study of a Colombian sample. Forensic Sci Int 261:160.e1-160.e1605. https://doi.org/10.1016/j.forsciint.2016.01.026
35. Zelic K, Galic I, Nedeljkovic N, Jakovljevic A, Milosevic O, Djuric M, Cameriere R (2016) Accuracy of Cameriere's third molar maturity index in assessing legal adulthood on Serbian population. Forensic Sci Int 259:127–132. https://doi.org/10.1016/j.forsciint.2015.12.032
36. De Luca S, Pacifici A, Pacifici L, Polimeni A, Fischetto SG, Velandia Palacio LA, Vanin S, Cameriere R (2016) Third molar development by measurements of open apices in an Italian sample of living subjects. J Forensic Leg Med 38:36–42. https://doi.org/10.1016/j.jflm.2015.11.007
37. 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 33:111–115. https://doi.org/10.1016/j.jflm.2015.04.016
38. Galić I, Lauc T, Brkić H, Vodanović M, Galić E, Biazevic MG, Brakus I, Badrov J, Cameriere R (2015) Cameriere's third molar maturity index in assessing age of majority. Forensic Sci Int 252:191.e1-191.e1915. https://doi.org/10.1016/j.forsciint.2015.04.030
39. Cameriere R, Santoro V, Roca R, Lozito P, Introna F, Cingolani M, Galić I, Ferrante L (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 245:205.e1-205.e2055. https://doi.org/10.1016/j.forsciint.2014.10.013
40. Cameriere R, Pacifici A, Viva S, Carbone D, Pacifici L, Polimeni A (2014) Adult or not? Accuracy of Cameriere's cutoff value for third molar in assessing 18 years of age for legal purposes. Minerva Stomatol 63(9):283–294
41. Moukarzel M, Angelakopoulos N, De Luca S, Velandia Palacio LA, Aquilanti L, Coccia E, Pigolkin YI, Cameriere R (2020) Validity assessment of the third molar maturity index (I_{3M}) in a Lebanese sample of adolescents and young adults. Aust J Forensic Sci. https://doi.org/10.1080/00450618.2020.1805008
42. El-Bakary A, El-Azab SM, Abou El Atta HM, Velandia Palacio LA, Cameriere R (2019) Accuracy of the cutoff value of the third molar maturity index: an Egyptian study. Egypt J Forensic Sci 9:52. https://doi.org/10.1186/s41935-019-0156-0
43. Ribier L, Saint-Martin P, Seignier M, Paré A, Brunereau L, Rérolle C (2020) Cameriere's third molar maturity index in assessing age of majority: a study of a French sample. Int J Legal Med 134(2):783–792. https://doi.org/10.1007/s00414-019-02123-z
44. Angelakopoulos N, Galić I, Balla SB, Kiş HC, Gómez Jiménez L, Zolotenkova G, Mohd Yusof M, Hadzić Selmanagić A, Pandey H, Palmela Pereira C, Nóbrega J, Hettiarachchi K, Mieke SM, Kumagai A, Gulsahi A, Zelić K, Marinković N, Kelmendi J, Bianchi I, Soriano Vázquez I, Cameriere R (2021) Comparison of the third molar maturity index (I_{3M}) between left and right lower third molars to assess the age of majority: a multi-ethnic study sample. Int J Legal Med 135(6):2423–2436. https://doi.org/10.1007/s00414-021-02656-2
45. Hanihara T, Ishida H (2005) Metric dental variation of major human populations. Am J Phys Anthropol Off Publ Am Assoc Phys Anthropologists 128(2):287–298
46. Olze A, Schmeling A, Taniguchi M, Maeda H, van Niekerk P, Wernecke KD, Geserick G (2004) Forensic age estimation in living subjects: the ethnic factor in wisdom tooth mineralization. Int J Legal Med 118(3):170–173. https://doi.org/10.1007/s00414-004-0434-7
47. Thevissen PW, Fieuws S, Willems G (2010) Human third molars development: comparison of 9 country specific populations. Forensic Sci Int 201:102–105
48. Santiago BM, Almeida L, Cavalcanti YW, Magno MB, Maia LC (2018) Accuracy of the third molar maturity index in assessing the legal age of 18 years: a systematic review and meta-analysis. Int J Legal Med 132(4):1167–1184. https://doi.org/10.1007/s00414-017-1766-4
49. Demirjian A, Goldstein H, Tanner JM (1973) A new system of dental age assessment. Hum Biol 45(2):211–227
50. Guo YC, Lin XW, Zhang WT, Yan CX, Pan F, Yan TL, Li JP, Chen T, Schmeling A, Zhou H (2015) Chronology of third molar mineralization in a northern Chinese population. Rechtsmedizin 25:34–39
51. Li G, Ren J, Zhao S, Liu Y, Li N, Wu W, Yuan S, Wang H (2012) Dental age estimation from the developmental stage of the third molars in western Chinese population. Forensic Sci Int 219:158–164
52. Liu Y, Geng K, Chu Y, Xu M, Zha L (2018) Third molar mineralization in relation to chronologic age estimation of the Han in central southern China. Int J Legal Med. https://doi.org/10.1007/s00414-018-1804-x
53. 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 52:428–43
54. Solari AC, Abramovitch K (2002) The accuracy and precision of third molar development as an indicator of chronological age in Hispanics. J Forensic Sci 47(3):531–5
55. Arany S, Iino M, Yoshioka N (2004) Radiographic survey of third molar development in relation to chronological age among Japanese juveniles. J Forensic Sci 49:534–8
56. Gunst K, Mesotten K, Carbonez A, Willems G (2003) Third molar root development in relation to chronological age: a large sample sized retrospective study. Forensic Sci Int 136(1–3):52–7

57. De Salvia A, Calzetta C, Orrico M, De Leo D (2004) Third mandibular molar radiological development as an indicator of chronological age in a European population. Forensic Sci Int 146:S9-12
58. Prieto JL, Barberia E, Ortega R, Magana C (2005) Evaluation of chronological age based on third molar development in the Spanish population. Int J Legal Med 119(6):349–54
59. Friedrich RE, Ulbricht C, von Maydell LA, Scheuer HA (2005) Identification of developmental stages of wisdom teeth on orthopantomograms of adolescents and young adults as an aid for forensic-odontological age-estimations: predictive values for the chronological age of 18 years. Arch Kriminol 216(3–4):73–88 (**[Article in German]**)
60. Chaillet N, Nystrom M, Demirjian A (2005) Comparison of dental maturity in children of different ethnic origins: international maturity curves for clinicians. J Forensic Sci 50(5):1164–74
61. Knell B, Ruhstaller P, Prieels F, Schmeling A (2009) Dental age diagnostics by means of radiographical evaluation of the growth stages of lower wisdom teeth. Int J Legal Med 123:465–469
62. Gelbrich B, Lessig R, Lehmann M, Dannhauer KH, Gelbrich G (2010) Age selection in reference samples. Rechtsmedizin 20:459–463
63. Chu G, Wang YH, Li MJ, Han MQ, Zhang ZY, Chen T, Zhou H, Guo YC (2018) Third molar maturity index (I_{3M}) for assessing age of majority in northern Chinese population. Int J Legal Med 132(6):1759–1768. https://doi.org/10.1007/s00414-018-1907-4
64. Hanihara T (2013) Geographic structure of dental variation in the major human populations of the world. Anthropol Perspect Tooth Morphol Genet, Evol Var 19:479–509. https://doi.org/10.1017/CBO9780511984464.019
65. Thevissen P, Altalie S, Brkic H, Galic I, Fieuws S, Franco A, Lee SS, Pinchi V, Yusof M, Willems G (2013) Comparing 14 country-specific populations on third molars development: consequences for age predictions of individuals with different geographic and biological origin. J Forensic Odonto-Stomatol 31(1):87–88 (**Abstract book IOFOS Conference 2013 Firenze**)
66. Cugati N, Kumaresan R, Srinivasan B, Karthikeyan P (2015) Dental age estimation of growing children by measurement of open apices: a Malaysian formula. J Forensic Dent Sci 7(3):227–231. https://doi.org/10.4103/0975-1475.172445
67. Alghali R, Kamaruddin AF, Mokhtar N (2016) Dental age estimation: comparison of reliability between Malay formula of Demirjian method and Malay formula of Cameriere method. In: AIP conference proceedings
68. Kumaresan R, Cugati N, Chandrasekaran B, Karthikeyan P (2016) Reliability and validity of five radiographic dental-age estimation methods in a population of Malaysian children. J Investig Clin Dent 7(1):102–9
69. Johan NA, Khamis MF, Abdul Jamal NS, Ahmad B, Mahanani ES (2012) The variability of lower third molar development in Northeast Malaysian population with application to age estimation. J Forensic Odontostomatol 30(1):45–54
70. Lee Yin ET, Sim KP, Mohd Yusof MYP (2020) Adaptation of Demirjian's method for age estimation via third molar development among adolescents and young adults of Malay ethnicity: a preliminary assessment. Mal J Med Health Sci 16:14–18
71. Wilson YP, Nambiar P, Yaacob H, Asif MK (2021) Age estimation from developing third molars. Med Leg J 89(4):254–259. https://doi.org/10.1177/00258172211052930
72. Willershausen B, Löffler N, Schulze R (2001) Analysis of 1202 orthopantograms to evaluate the potential of forensic age determination based on third molar developmental stages. Eur J Med Res 6(9):377–384
73. Jorde LB, Watkins WS, Bamshad MJ, Dixon ME, Ricker CE, Seielstad MT, Batzer MA (2000) The distribution of human genetic diversity: a comparison of mitochondrial, autosomal, and Y-chromosome data. Am J Hum Genet 66:979–988
74. Liversidge HM, Chaillet N, Mörnstad H, Nyström M, Rowlings K, Taylor J, Willems G (2006) Timing of Demirjian's tooth formation stages. Ann Hum Biol 33:454–470
75. Townsend G, Hughes T, Luciano M, Bockmann M, Brook A (2009) Genetic and environmental influences on human dental variation: a critical evaluation of studies involving twins. Arch Oral Biol 54:S45–S51

Authors and Affiliations

Nikolaos Angelakopoulos[1,2] · Stefano De Luca[2,3] · Inês Oliveira-Santos[4] · Isabella Lima Arrais Ribeiro[5] · Ilenia Bianchi[6] · Sudheer B. Balla[2,7] · Hatice Cansu Kis[8] · Lourdes Gómez Jiménez[9] · Galina Zolotenkova[10] · Mohd Yusmiaidil Putera Yusof[11] · Aida Hadzić Selmanagić[12] · Hemlata Pandey[13] · Palmela C. Pereira[14] · Johnys Berton Medeiros da Nóbrega[15] · Hettiarachchi Kalani[16] · Sylvia M. Mieke[17] · Akiko Kumagai[18] · Ayse Gulsahi[2,19] · Ksenija Zelić[20] · Nemanja Marinković[21] · Jeta Kelmendi[22] · Ivan Galić[2,23] · Israel Soriano Vázquez[24] · Enrico Spinas[25] · Ymelda Wendy Velezmoro-Montes[26] · Maria Moukarzel[27] · Jorge Pinares Toledo[28,29] · Amal Abd El-Salam El-Bakary[30] · Roberto Cameriere[2]

[1] Department of Orthodontics and Dentofacial Orthopaedics, University of Bern, Bern, Switzerland

[2] AgEstimation Project, Department of Medicine and Health Sciences “Vincenzo Tiberio,” University of Molise, Campobasso, Italy

[3] Panacea Cooperative Research S. Coop, Ponferrada, Spain

[4] Centre for Functional Ecology, Laboratory of Forensic Anthropology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal

[5] Postgraduate Program in Dentistry, Universidade Federal da Paraíba, Joao Pessoa, , Paraiba, Brazil

[6] Department of Law, Institute of Legal Medicine, University of Macerata, Macerata, Italy

[7] Department of Forensic Odontology, Panineeya Institute of Dental Sciences and Research Center, Hyderabad, Telangana, India

[8] Department of Orthodontics, Tokat Gaziosmanpaşa University, Tokat, Turkey

[9] Instituto Nacional de Patología “Dr. Sergio Sarita Valdez,”, Santo Domingo, Dominican Republic

[10] Department of Forensic Medicine, I.M. Sechenov First Moscow State Medical University, Moscow, Russia

[11] Centre for Oral & Maxillofacial Diagnostics and Medicine Studies, Faculty of Dentistry, Universiti Teknologi MARA, Sungai Buloh Campus, Selangor, Malaysia

[12] Department of Dental Morphology With Dental Anthropology and Forensics, Faculty of Dentistry, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

[13] Department of Forensic Medicine and Toxicology, Seth GS Medical College and KEM Hospital, Mumbai, India

[14] Facultade de Medicina Dentária, Universidade de Lisboa, Lisbon, Portugal

[15] Postgraduate Program in Dentistry, Universidade Federal da Paraíba, Joao Pessoa, Paraiba, Brazil

[16] Department of Oral Medicine and Periodontology, Faculty of Dental Sciences, University of Peradeniya, Peradeniya, Sri Lanka

[17] Department of Forensic Odontology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

[18] Division of Forensic Odontology and Disaster Oral Medicine, Department of Forensic Science, Iwate Medical University, Morioka, Iwate, Japan

[19] Dentomaxillofacial Radiology Department, Faculty of Dentistry, Baskent University, Ankara, Turkey

[20] Laboratory of Anthropology, Institute of Anatomy, School of Medicine, University of Belgrade, Belgrade, Serbia

[21] Clinic for Orthodontics, School of Dental Medicine, University of Belgrade, Belgrade, Serbia

[22] Department of Orthodontics, Faculty of Medicine Alma Mater Europaea, University of Pristina, Campus Rezonanca, Pristina, Kosovo

[23] Department of Oral Surgery, School of Medicine, University of Split, Split, Croatia

[24] Forensic Odontologist, Department of Medicine and Health Sciences “Vincenzo Tiberio,” University of Molise, Campobasso, Italy

[25] Department of Surgical Sciences, Section of Dentistry, University of Cagliari, Cagliari, Italy

[26] Forensic Dentistry, Scientific University of the South, Lima, Peru

[27] Beirut, Lebanon

[28] Departamento de Patología Y Medicina Oral, Facultad de Odontología, Universidad de Chile, Santiago, Chile

[29] Escuela de Odontología, Facultad de Medicina, Universidad Católica de Chile, Santiago, Chile

[30] Faculty of Medicine, Mansoura University, Mansoura, Egypt