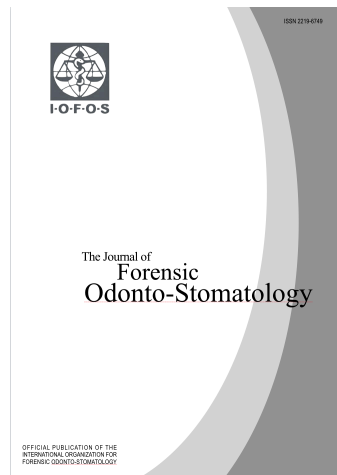




The Journal of
**Forensic
Odonto-Stomatology**

Volume 44, n. 2 - Aug 2026

JFOS
ISSN: 2219-6749



THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

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Sexual dimorphism based on metric and non-metric parameters of human teeth in a Brazilian population

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The authors declare that they
have no conflict of interest.

KEYWORDS

Forensic Anthropology,
Forensic Dentistry,
Sex Characteristics,
Sex Determination Analysis,
Tooth,
Dentition,
Permanent.

J Forensic Odontostomatol
2026. Aug; (44): 2-2:15
ISSN :2219-6749
DOI: doi.org/10.5281/zenodo.21848953

ABSTRACT

In forensic contexts, incomplete or fragmented bones are frequently encountered due to post-mortem damage and taphonomic changes, and teeth can be considered a valid and reliable source for sex estimation, as they are among the most resistant structures of the human body. Methods based on metric and non-metric dental traits are commonly used for sex estimation. The aim of this study was to evaluate the presence of sexual dimorphism in a Brazilian population through metric and non-metric analysis of teeth. A total of 73 individuals aged between 18 and 25 years were evaluated. All analyses were conducted on the upper jaw. The metric analysis consisted of obtaining buccolingual and mesiodistal measurements of the clinical crown of the teeth. In addition to measurements for each tooth, the maxillary dental arch perimeter and intercanine distance were also considered. The non-metric analysis consisted of observing and describing traits present or absent in the teeth, using the ASUDAS. Intraobserver error was assessed using the paired t-test and the Dahlberg method. Parametric or non-parametric tests were applied according to the data distribution. The mesiodistal and buccolingual dimensions proved effective for identifying sex differences, except for teeth 12/22 (mesiodistal) and 13/23 (buccolingual). The maxillary dental arch perimeter and intercanine distance were significantly larger in males. Regarding non-metric traits, the results were less significant; only a few teeth presented traits associated with sexual dimorphism: the lingual tubercle (tooth 22), the mesial marginal ridge tubercle (tooth 26), and the hypocone cusp (teeth 17 and 27). It is concluded that the findings, although relevant, should be interpreted with caution, as their consistency still needs to be confirmed. Therefore, additional studies with larger samples from different regions of Brazil are necessary to validate these parameters and consider them reliable in anthropological and forensic contexts.

INTRODUCTION

Forensic Anthropology is dedicated to the examination of human remains, with the intention of reconstructing the biological profile,¹ which encompasses a set of biological characteristics such as sex, age at death, population affinity, and stature.²⁻⁴ The purpose is to narrow down the list of missing persons and, consequently, assist in the identification process.^{1,2,5}

In anthropological analyses, sex estimation is fundamental.^{1,6,7} It is necessary to apply tests to estimate other parameters of the biological profile⁸ and, being based on sexual dimorphism, yields a dichotomous outcome (male or female).^{5,8} Among all bones, the pelvis exhibits the highest degree of sexual dimorphism, followed by the long bones and the cranium.⁸ In forensic scenarios, however, incomplete or fragmented bones are frequently encountered due to postmortem damage and taphonomic alterations.^{1,6}

Teeth can be considered a valid and reliable source for sex estimation¹ as they exhibit sexual dimorphism⁹ and are among the most resistant structures of the human body.^{5,6,9} Their lower susceptibility to physical, chemical, and biological influences compared to bones provides greater postmortem durability,^{1,6,9} which explains why, in many cases, they may be the only preserved structures.¹⁰

Methods based on both metric and non-metric dental traits are used for sex estimation.^{11,12} Among the metric approaches, analyses of the buccolingual (BL) and mesiodistal (MD) dimensions of the tooth crowns^{1,10,11,13} as well as intercanine distance (ICD),¹³ are noteworthy. In addition, the maxillary dental arch perimeter (MDAP) can also be assessed as a complementary variable for sex discrimination.^{14,15} Non-metric analyses evaluate tooth morphology and consider individual phenotypic variations,¹⁰ which can be observed in the roots and crowns of all teeth.^{11,16} The most important and widely used systematization in dental morphology is the Arizona State University Dental Anthropology System (ASUDAS), proposed by Turner *et al.* in 1991.¹⁷ It was developed to assess population affinity and is based on textual definitions of non-metric morphological traits, which can be simply observed and recorded.¹⁷ These traits undergo minimal changes due to physiological dental wear, show considerable distinction among different populations, but exhibit low sexual dimorphism.¹⁷ However, morphological discrepancies have been reported when comparing male and female teeth.^{11,12}

Therefore, considering that teeth are among the most resistant structures of the human body and also exhibit sexual dimorphism,⁹ particularly regarding their dimensions,¹⁰ it becomes essential to evaluate the applicability of metric dental methods in different populations for sex

estimation. Furthermore, the use of dental morphological traits included in the ASUDAS for sex estimation remains scarcely explored, highlighting the need to investigate their discriminative potential.

This study aimed to assess the presence of sexual dimorphism in a Brazilian population through metric and non-metric analyses (ASUDAS) of permanent teeth.

MATERIALS AND METHODS

A total of 73 individuals were assessed, including 33 males and 40 females, aged between 18 and 25 years. All participants were Brazilian, with ancestors up to the second degree also of Brazilian origin, and born in municipalities located in the South-Southwest mesoregion of the state of Minas Gerais, as established by the Brazilian Institute of Geography and Statistics (IBGE).¹⁸

All analyses were conducted on the upper jaw. Volunteers were clinically evaluated to verify whether they met the inclusion criteria for the study. To be included, all volunteers had to be fully dentate (excluding third molars) and free of anomalies, fractures, wear, or restorations on the teeth that could compromise the planned analyses. Volunteers were excluded if they were not born in, or did not have ancestors from, the regions under study; if they had missing teeth other than third molars; or if any morphological or structural alterations were clinically observed in the maxillary dental arch elements that could compromise the analyses.

Next, the sex of the volunteers was recorded, and dental impressions were taken using type II alginate to obtain plaster models. The models were then cast in type III dental stone, and both metric and non-metric analyses were performed on these models.

Metric analysis

Metric analysis consisted of obtaining the BL and MD measurements of the clinical crowns of teeth 11 (right maxillary central incisor) and 21 (left maxillary central incisor), 12 (right maxillary lateral incisor) and 22 (left maxillary lateral incisor), 13 (right maxillary canine) and 23 (left maxillary canine), 16 (right maxillary first molar) and 26 (left maxillary second molar), and 17 (right maxillary second molar) and 27 (left maxillary second molar).

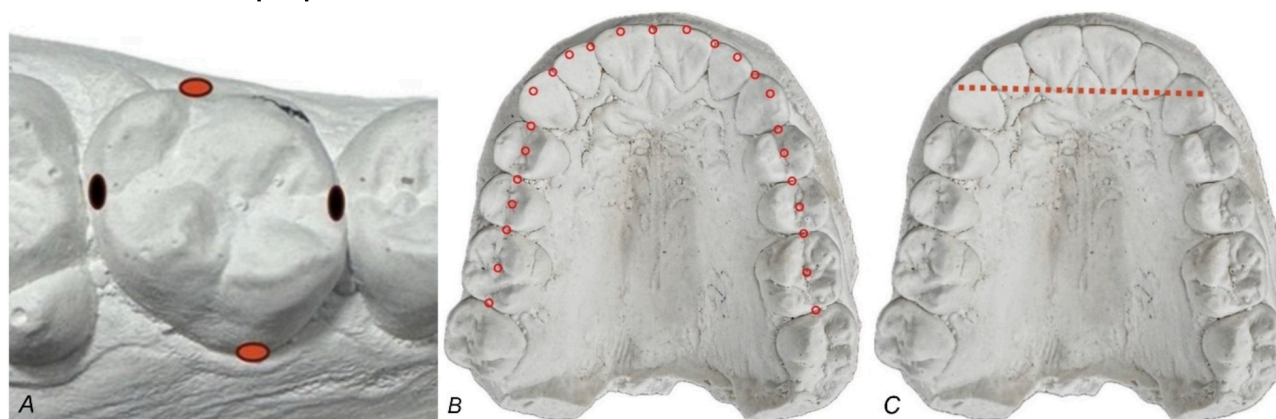
The MD measurement was obtained at the incisal third of the proximal surfaces of the incisors and canines (proximal ridges) and at the occlusal third of the proximal surfaces of the molars, at the greatest distance between the marginal ridges (proximal ridges). The BL measurement was taken at the cervical third of the tooth crown, at the region of greatest convexity of the free surfaces, that is, at the buccal and lingual ridges (Fig. 1).

In addition to the measurements of each tooth, the MDAP and ICD were also considered. The dental arch perimeter (Fig. 1) is defined as the distance from the central point of the distal marginal ridge of the right maxillary first molar to the same point on the distal marginal ridge of the

left maxillary first molar, passing through the center of the occlusal surface (central fossae) of the posterior teeth and the incisal edges of the anterior teeth.¹⁹ The ICD corresponds to the straight-line distance between the crown apex (cusp) of the right and left canines.

All measurements were performed by a single researcher on two occasions, with a fifteen-day interval, using a digital caliper with hundredth-millimeter precision (Marberg®, Marberg Ferramentas, Brazil). The MDAP was measured using a silk thread positioned along the reference points described above, and the value was subsequently obtained with the caliper. The ICD (Fig. 1) was measured directly with the caliper.

Figure 1. A – Representation of the sites where measurements of each dental element were obtained. BL – distance between the orange points; MD – distance between the black points. B – Maxillary Dental Arch Perimeter (MDAP); red points extending from the center of the distal marginal ridge of tooth 16 to the center of the distal marginal ridge of tooth 26, passing through the centers of the occlusal surfaces and incisal edges. C – Representation of the Intercanine Distance (ICD), measured between the cusp apices of teeth 13 and 23.



Non-metric analysis

Non-metric analysis consisted of observing and recording the presence or absence of traits in the teeth. For this purpose, the Arizona State University Dental Anthropology System (ASUDAS)¹⁷ was used. Although one trait analyzed is not included in this system, it corresponds to the presence or absence of the Bolk tubercle or paramolar tubercle (on the buccal surface of the mesiobuccal cusp of the maxillary second molar). All observations were performed using a five-times magnification loupe and following the ASUDAS guidelines, with adequate and sufficient lighting. Two observations were also conducted by a single observer, with a fifteen-day interval between them, to avoid any interference in decision-making.

The following traits were observed: in the maxillary central incisors: a) shoveling – presence of lingual marginal ridges; b) double shoveling – presence of buccal marginal ridges; c) cingulum – presence of one or more lingual tubercles extending from the cingulum to the lingual fossa; and d) interruption grooves – vertical grooves crossing the dental cingulum. In the maxillary lateral incisors: a) morphology – peg-shaped incisor (“reptilian” incisor); diminutive incisor – reduced crown dimensions; tri-form – a narrow projection of the cingulum extending toward the incisal edges, forming a “T” shape in incisal view; barrel-shaped – marginal ridges fold lingually and coalesce; normal-shaped – conventional morphology of a maxillary lateral incisor; b) agenesis – congenital absence; c) cingulum – presence of lingual tubercles extending from the

cingulum to the lingual fossa; and d) interruption grooves – vertical grooves crossing the dental cingulum. In the maxillary first molars: a) hypocone – presence or absence of the distolingual cusp; b) Carabelli tubercle – visible on the mesiolingual cusp; c) distal accessory tubercle (metaconule) – present on the distal marginal ridge; and d) mesial tubercles – present on the mesial marginal ridge. In the maxillary second molars: a) hypocone (distolingual cusp) – presence or absence; b) Carabelli tubercle – visible on the mesiolingual cusp; c) distal accessory tubercle (metaconule) – present on the distal marginal ridge; d) mesial tubercles – present on the mesial marginal ridge; and e) Bolk tubercle – paramolar cusp, present on the buccal surface of the mesiobuccal cusp. Each of the traits observed was classified according to the ASUDAS, considering the teeth on the right and left sides separately. As previously mentioned, the analysis of the paramolar cusp or Bolk tubercle, although not included in the ASUDAS, is reported in the literature²⁰ and was therefore considered in this study.

Statistical analysis

Statistical analysis of the data was performed using Jamovi software, version 2.3.28 (The Jamovi

Project, Australia). The normality of the metric variables was assessed with the Shapiro–Wilk test. For those with normal distribution, Student's *t*-test for independent samples was applied; in the absence of normality, the nonparametric Mann–Whitney test was used. Qualitative (non-metric) variables were described and compared using Fisher's exact test. All analyses adopted a significance level of 5% ($p < 0.05$).

Intra-observer systematic error was assessed using the paired *t*-test, while random error was estimated using the method proposed by Dahlberg, according to the formula:

$$\text{error}^2 = \frac{d^2}{2n}$$

where *d* represents the difference between two measurements and *n* the number of volunteers evaluated.

RESULTS

Intra-observer systematic error, assessed using the paired *t*-test, showed no significant differences ($p > 0.05$). Intra-observer casual error, evaluated using Dahlberg's method, presented values below 1 mm for all measurements (Table 1).

Table 1. Dahlberg test values for the two measurements performed by the same observer.

Measurement	Male	Female
BL 11	0.23	0.24
BL 21	0.33	0.44
BL 12	0.24	0.34
BL 22	0.54	0.48
BL 13	0.15	0.30
BL 23	0.17	0.29
BL 16	0.26	0.39
BL 26	0.22	0.49
BL 17	0.31	0.47
BL 27	0.35	0.68
MDAP	0.30	0.81
ICD	0.18	0.73
MD 11	0.10	0.25
MD 21	0.17	0.26
MD 12	0.17	0.55
MD 22	0.25	0.28
MD 13	0.18	0.22
MD 23	0.16	0.31
MD 16	0.28	0.34
MD 26	0.34	0.33
MD 17	0.27	0.38
MD 27	0.32	0.34

BL – buccolingual; MD – mesiodistal; MDAP – maxillary dental arch perimeter; ICD – intercanine distance. Values should be < 1.0.

Metric analysis

BL, MD, MDAP, and ICD measurements were analyzed according to the data distribution.

For the BL measurement, teeth 11, 12, 22, 13, 23, 16, and 26, which showed a normal distribution, were analyzed using the independent samples Student's *t*-test. For teeth 21, 17, and 27, which did not show normality, the nonparametric Mann-Whitney test was applied (Table 2).

The BL measurements were statistically significant between sexes (Table 2), with the exception of the right and left canines, which did not differ between the groups analyzed. However, for tooth 13, no significant difference was observed between sexes, although the *p*-value (0.053) approached the established significance level (0.05). Fig. 2 shows the means and medians of the BL distances for each tooth and sex, providing a visual illustration of the results.

Similar results were observed for the MD measurements of the analyzed teeth. Statistically significant differences between sexes were found

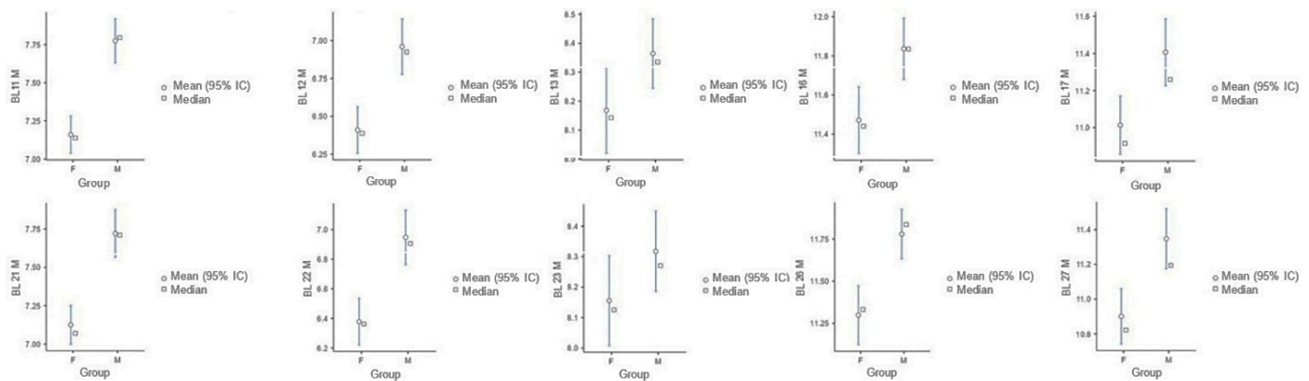
for most teeth, except for the maxillary lateral incisors, in which no sexual dimorphism was detected based on MD dimension. Table 3 presents the mean MD measurements and the statistical results. As normality was not observed for teeth 11, 13, 23, 17, and 27, the Mann-Whitney test was applied. Mean values were higher in males for all evaluated teeth. Fig. 3 shows the means and medians of the MD distances for each tooth and sex, providing a visual illustration of the results.

For the MDAP and ICD, Shapiro-Wilk test values indicated normality of the distributions of these measurements ($p > 0.05$). Analysis using Student's *t*-test revealed statistically significant differences between sexes ($p < 0.001$), with higher values in males, indicating that these linear measurements can be used for sex estimation (Table 4). Fig. 4 shows the means and medians of MDAP and ICD measurements for each sex, providing a visual illustration of the results.

Table 2. Buccolingual (BL) distance values for male and female

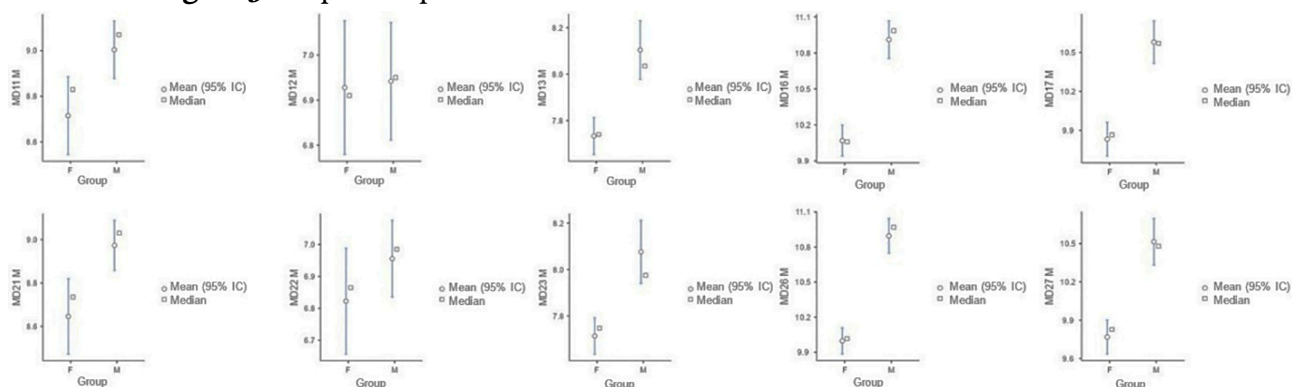
Tooth	Sex	Mean	Shapiro-Wilk(p)	Student's t-test(p)	Mann-Whitney(p)
11	Male	7.77	0.05	< 0.001 *	-
	Female	7.16			
21	Male	7.72	0.03	-	< 0.001 *
	Female	7.13			
12	Male	6.96	0.09	< 0.001 *	-
	Female	6.41			
22	Male	6.95	0.10	< 0.001 *	-
	Female	6.38			
13	Male	8.36	0.17	0.053	-
	Female	8.17			
23	Male	8.32	0.09	0.119	-
	Female	8.16			
16	Male	11.80	0.29	0.003 *	-
	Female	11.50			
26	Male	11.80	0.10	< 0.001 *	-
	Female	11.30			
17	Male	11.40	< 0.001	-	< 0.001 *
	Female	11.00			
27	Male	11.30	0.006	-	< 0.001 *
	Female	10.90			

According to the Shapiro-Wilk test, data with $p > 0.05$ are considered normally distributed; $p < 0.05$ indicates non-normality. *p*-values < 0.05 for the Student's *t*-test and Mann-Whitney test correspond to statistically significant results (*).

Figure 2. Graphical representation of the mean and median of the BL measurement**Table 3.** Mesiodistal (MD) distance values for males and females.

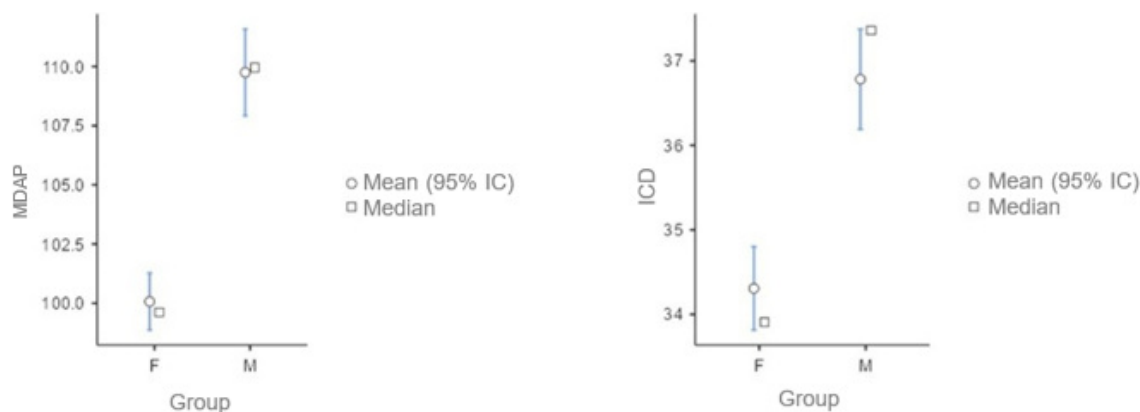
Tooth	Sex	Mean	Shapiro-Wilk(p)	Student's t-test(p)	Mann-Whitney(p)
11	Male	9.00	0.028	-	0.006 *
	Female	8.71			
21	Male	8.97	0.087	0.009 *	-
	Female	8.65			
12	Male	6.94	0.069	0.892	-
	Female	6.93			
22	Male	6.96	0.100	0.224	-
	Female	6.82			
13	Male	8.10	< 0.001	-	< 0.001 *
	Female	7.73			
23	Male	8.08	< 0.001	-	< 0.001 *
	Female	7.71			
16	Male	10.90	0.893	< 0.001 *	-
	Female	10.07			
26	Male	10.90	0.891	< 0.001 *	-
	Female	10.00			
17	Male	10.60	0.048	-	< 0.001 *
	Female	9.83			
27	Male	10.50	0.014	-	< 0.001 *
	Female	9.77			

According to the Shapiro-Wilk test, data with $p > 0.05$ are considered normally distributed; $p < 0.05$ indicates non-normality. p -values < 0.05 for the Student's t-test and Mann-Whitney test correspond to statistically significant results (*).

Figure 3. Graphical representation of the mean and median of the MD measurement**Table 4.** Values of the maxillary dental arch perimeter (MDAP) and Inter canine Distance (ICD) measurements for males and females.

Measurements	Sex	Mean	Shapiro-Wilk(p)	Student's t-test (p)
MDAP	Male	109.8	0.278	< 0.001 *
	Female	100.1		
ICD	Male	36.8	0.876	< 0.001 *
	Female	34.3		

According to the Shapiro-Wilk test, data with $p > 0.05$ are considered normally distributed; $p < 0.05$ indicates non-normality. p -values < 0.05 for the Student's t -tests correspond to statistically significant results (*).

Figure 4. Graphical representation of the mean and median of MDAP and ICD measurements

Non-metric analysis

Morphologically, using parameters established by the ASUDAS¹⁷ and Fisher's exact test, some statistically significant differences were observed for differentiating male and female sexes. Fig. 5 shows some of the morphological traits evaluated in the permanent maxillary incisors, according to the ASUDAS.¹⁷ Considering the maxillary central incisors, evaluated through non-metric traits, no significant differences were observed between sexes. For the maxillary lateral incisors, a significant difference was found in the number of lingual tubercles of tooth 22, which were more

frequent in males. Tables 5 and 6 present the descriptive values of the morphological analysis of the permanent maxillary incisors, as well as the p -values obtained from Fisher's exact test. Fig. 6 shows some of the morphological traits analyzed in the permanent maxillary molars, according to the ASUDAS.¹⁷ In the maxillary first molars, significant differences were observed in the number of mesial marginal ridge tubercles only for tooth 26 ($p = 0.001$), being more frequent in males. In the maxillary second molars, on both the right and left sides, significant differences

were found regarding the presence of the hypocone (distolingual cusp), also more frequent in males. Tables 7 and 8 present the descriptive

values of the morphological analysis of the permanent maxillary molars, as well as the p -values obtained from Fisher's exact test.

Figure 5. A – Representation of the shoveling trait on the lingual (palatal) surface of the maxillary incisors: grade 0. B – Shoveling scoring 1. C – Shoveling scoring 2. D – Black arrows indicate lingual tubercles on teeth 11 and 21, and the red arrow indicates the interruption groove on tooth 22. E – Lingual tubercles and marginal ridges outlined on teeth 12, 11, 21, and 22.

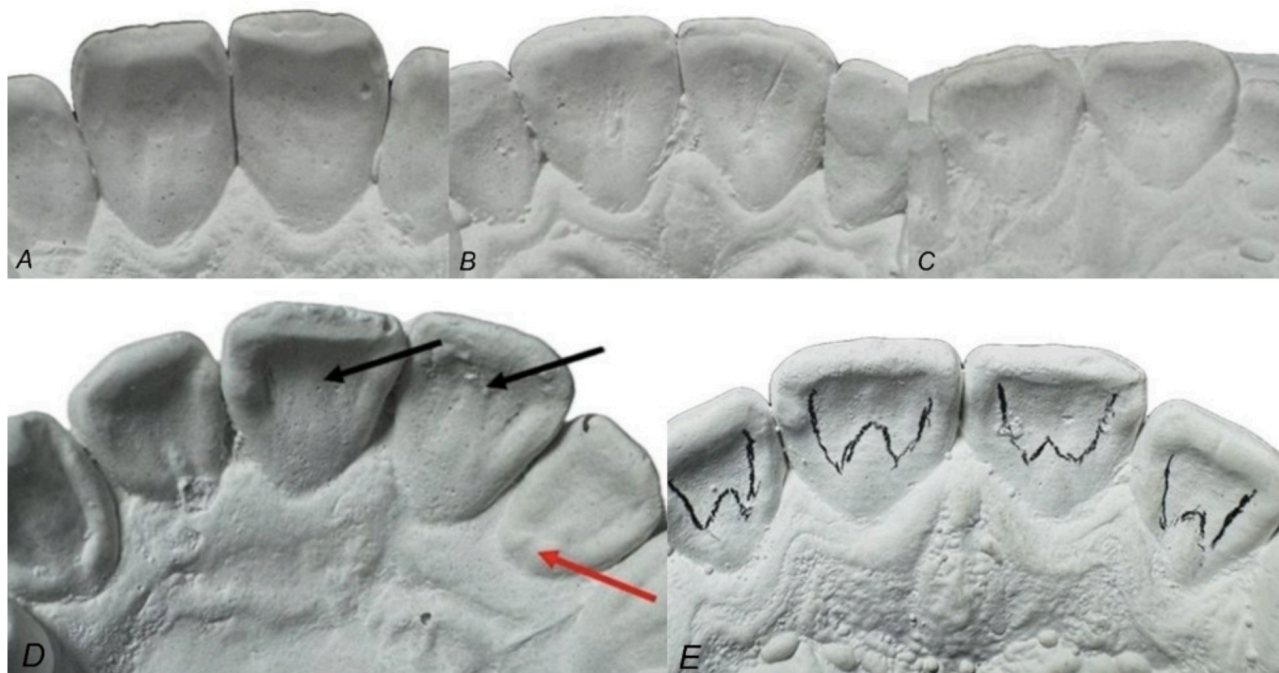


Table 5. Morphological data for the permanent maxillary central incisors

Morphological trait	11 Male	11 Female	21 Male	21 Female
Shoveling 0	0	0	0	0
Shoveling 1	25 (75.8%)	29 (72.5%)	25 (75.8%)	29 (72.5%)
Shoveling 2	8 (24.2%)	11 (27.5%)	8 (24.2%)	11 (27.5%)
Shoveling 3	0	0	0	0
p(Fisher)	0.79		0.79	
Double shoveling	0	0	0	0
p(Fisher)	-		-	
Absent tubercle	20 (60.6%)	30 (75.0%)	20 (60.6%)	32 (80.0%)
01 tubercle	5 (15.1%)	8 (20.0%)	8 (24.3%)	5 (12.5%)
02 tubercle	8 (24.3%)	2 (5.0%)	5 (15.1%)	3 (7.5%)
03 tubercle	0	0	0	0
p(Fisher)	0.07		0.20	

The double shoveling trait was not observed in any tooth; therefore, comparative statistics could not be calculated. Significant values with $p < 0.05$.

Table 6. Morphological data for the permanent maxillary lateral incisors

Morphological trait	12 Male	12 Female	22 Male	22 Female
Peg shaped	0	0	0	0
Diminute	0	0	0	1 (2.5%)
Tri-form	0	0	0	0
Barrel form	0	0	0	0
Normal shaped	33 (100%)	40 (100%)	33 (100%)	39 (97.5%)
p(Fisher)	1.00		1.00	
Agenesis	0	0	0	0
p(Fisher)	-		-	
Absent tubercle	17 (51.5%)	29 (72.5%)	17 (51.5%)	32 (80.0%)
o1 tubercle	16 (48.5%)	11 (27.5%)	16 (48.5%)	8 (20.0%)
o2 tubercle	0	0	0	0
o3 tubercle	0	0	0	0
p(Fisher)	0.08		0.01*	
Interruption groove present	13 (40.0%)	8 (20.0%)	13 (40.0%)	9 (22.5%)
Interruption groove absent	20 (60.0%)	32 (80.0%)	20 (60.0%)	31 (77.5%)
p(Fisher)	0.07		0.13	

The agenesis trait was not observed in any tooth; therefore, comparative statistics could not be calculated. Significant values with $p < 0.05$ (*).

Figure 6. A – Carabelli tubercle (arrow) present on the mesiopalatal cusp of tooth 16. B – Tooth 27 exhibiting three cusps, with absence of the hypocone. The arrow indicates a distal tubercle on the distal marginal ridge of tooth 27. C – Presence of distal tubercles on tooth 17 (arrow). D – Arrows indicate tubercles located on the mesial marginal ridges of teeth 26 and 27. E – Paramolar tubercle (Bolk's tubercle); the arrow points to the tubercle located on the buccal surface of the mesiobuccal cusp of tooth 27

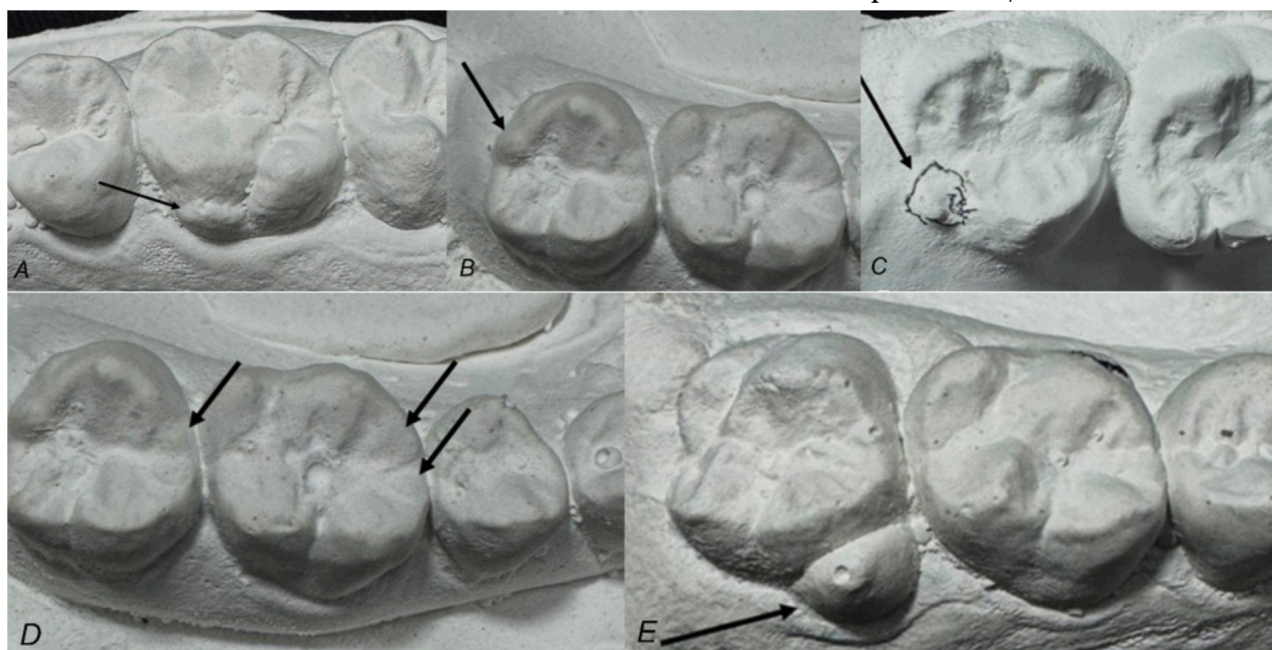


Table 7. Morphological data for the permanent maxillary first molars

Morphological trait	16 Male	16 Female	26 Male	26 Female
Hypocone present	33 (100.0%)	40 (100%)	33 (100%)	40 (100%)
Hypocone absent	0	0	0	0
p (Fisher)	1.00		1.00	
Carabelli tubercle absent	1 (3.0%)	1 (2.5%)	1 (3.0%)	2 (5.0%)
Carabelli tubercle I/II	16 (48.5%)	19 (47.5%)	15 (45.5%)	19 (47.5%)
Carabelli tubercle III/IV	8 (24.3%)	10 (25.0%)	11 (33.3%)	8 (20.0%)
Carabelli tubercle V/VI	8 (24.2%)	10 (25.0%)	6 (18.2%)	11 (27.5%)
Carabelli tubercle VII	0	0	0	0
p(Fisher)	1.00		0.58	
Distal tubercle present	4 (12.2%)	10 (25.0%)	4 (12.2%)	13 (32.5%)
Distal tubercle absent	29 (87.8%)	30 (75.0%)	29 (87.8%)	27 (67.5%)
p(Fisher)	0.23		0.05	
Mesial tubercle absent	3 (9.1%)	11 (27.5%)	5 (15.2%)	22 (55.0%)
o1 mesial tubercle	21 (63.7%)	23 (57.5%)	22 (66.7%)	18 (45.0%)
o2 mesial tubercles	6 (18.1%)	6 (15.0%)	6 (15.0%)	0
o3 mesial tubercles	3 (9.1%)	0	0	0
p(Fisher)	0.06		<0.001*	

Significant values with $p < 0.05$ (*).**Table 8.** Morphological data for permanent maxillary second molars

Morphological trait	17 Male	17 Female	27 Male	27 Female
Hypocone present	30 (90.9%)	24 (60.0%)	28 (84.8%)	24 (60.0%)
Hypocone absent	3 (9.1%)	16 (40.0%)	5 (15.2%)	16 (40.0%)
p (Fisher)	0.003*		0.022*	
Carabelli tubercle absent	25 (75.7%)	33 (82.5%)	26 (78.8%)	33 (82.5%)
Carabelli tubercle I/II	7 (21.2%)	5 (12.5%)	7 (21.2%)	5 (12.5%)
Carabelli tubercle III/IV	1 (3.1%)	2 (5.0%)	0	2 (5.0%)
Carabelli tubercle V/VI	0	0	0	0
Carabelli tubercle VII	0	0	0	0
p(Fisher)	0.57		0.45	
Distal tubercle present	13 (39.4%)	13 (32.5%)	8 (24.2%)	15 (37.5%)
Distal tubercle absent	20 (60.6%)	27 (67.5%)	25 (75.8%)	25 (62.5%)
p(Fisher)	0.62		0.31	
Mesial tubercle absent	10 (30.3%)	17 (42.5%)	18 (54.5%)	22 (55.0%)
o1 mesial tubercle	18 (54.6%)	20 (50.0%)	15 (45.5%)	13 (32.5%)
o2 mesial tubercles	5 (15.1%)	3 (7.5%)	0	5 (12.5%)

o3 mesial tubercles	o	o	o	o
p(Fisher)	0.41		0.08	
Paramolar tubercle present	o	o	1 (3,3%)	o
Paramolar tubercle absent	33 (100%)	40 (100%)	32 (96.7%)	40 (100%)
p(Fisher)	1.0		0.45	

Significant values with $p < 0.05$ (*).

DISCUSSION

The study and analysis of teeth provide essential information for establishing an individual's biological profile.⁶ Among the characteristics of this profile, sex estimation is of great importance, particularly for classifying individuals into the two main groups, male and female.^{5,8} Therefore, this study was conducted to establish data regarding the BL and MD measurements of dental elements, the MDAP, and ICD in permanent maxillary teeth in a Brazilian population, in addition to non-metric dental traits.

There is evidence that tooth size is influenced by sex chromosomes,^{5,21} with male teeth generally being larger than female teeth.¹ The results obtained in this study, shown in Tables 2 and 3, are consistent with this assertion, as most BL measurements exhibited significant differences between sexes, with the exception of the canines. Similarly, marked differences were observed in MD dimensions, except for the maxillary lateral incisors, reinforcing the influence of biological factors on dental sexual dimorphism.

Alanazi *et al.* (2022)²² analyzed morphometric parameters of permanent canines in an Arab population and observed that sexual dimorphism can be detected through MD and BL dimensions. However, in the present study, considering the BL measurement and focusing on the maxillary canines, the data differ, as no differences between sexes were found (Table 2). On the other hand, supporting the previous authors regarding MD measurement, the maxillary canines do exhibit sexual dimorphism (Table 3). Specifically, for the right maxillary canine (tooth 13), the p -value (0.053) for the BL measurement approached the significance threshold (0.05), suggesting a trend, but without a statistically significant difference. These findings also diverge from other studies,^{23,24} which indicate that all dimensions of the maxillary canines may contribute to sex estimation. One possible explanation for this discrepancy may be related to sample size or

specific characteristics of the population analyzed.

Abaid *et al.* (2021)²⁵ also reported sexual dimorphism based on MD dimensions for almost all teeth in a population of Australian adolescents, with the exception of the maxillary second premolars, which showed no differences between sexes. Other authors support these findings, showing greater MD dimensions in male teeth compared to female teeth.^{14,24} As shown in Table 3, the results of this study, although the premolar group was not analyzed, also indicate the potential for sex estimation for almost all dental elements based on MD measurements; only the maxillary lateral incisors did not show differences between males and females. However, in those teeth that did show differences, male teeth exhibited greater dimensions.

Although most studies on metric analysis of teeth primarily focus on anterior teeth (incisors and canines), with canines considered the most reliable for detecting sexual dimorphism due to their larger dimensions in males, posterior teeth, including molars, can provide highly relevant information for sex estimation.^{5,26,27} In this study, MD and BL measurements of the permanent first and second maxillary molars were evaluated, and sexual dimorphism was observed for both measurements, with males exhibiting larger dimensions than females.

When considering the ICD, that is, the distance between the apices of the cusps of these teeth, the results showed that ICD in males is significantly greater than in females (Table 4). These findings differ from those reported by Selim *et al.* (2020),²⁴ who observed a greater ICD in females compared to males. This contrast in results may be due to the different methodology used by these authors—cone-beam computed tomography—or because their study involved a population from a different region of Brazil, unlike the population analyzed in the present study.

Mazumder *et al.* (2023)¹⁴ and Ajmal *et al.* (2023)²⁸ report that the sum of MD measurements, resulting in the dental arch perimeter, is a very useful parameter for sex estimation. The present study showed that, based on isolated MD measurements, the maxillary lateral incisors do not exhibit sexual dimorphism; however, when the MDAP was analyzed considering all MD dimensions, the male maxillary dental arch was significantly larger than the female (Table 4). Thus, the findings suggest that metric analysis of the maxillary teeth, including MD, BL, MDAP, and ICD, can serve as a relevant auxiliary tool for biological sex estimation.

From the perspective of non-metric morphological aspects, there is phenotypic variation in both deciduous and permanent dentition, particularly across different population groups, and this variation can be assessed through the observation and description of various dental traits.^{17,29} The ASUDAS¹⁷ used in this study, allows a simplified recording of qualitative morphological traits that undergo minimal wear over time and are widely used to estimate an individual's population affinity, but are less frequently employed for observing sexual dimorphism, as they are considered less influenced by sex.³⁰ Nonetheless, this study showed that, despite the limited number of traits, differences between males and females can still be observed.

Moreover, some authors report that the traits analyzed in this system are extremely stable, with evolutionary changes occurring over thousands of years, and that the genetic factor influencing dental morphology is also highly stable.^{31,32} Therefore, these traits are reliable for interindividual comparisons and could serve as an additional tool in human identification processes. In the analysis of non-metric traits, it is essential to consider intra-observer error, since, despite technical rigor, a certain degree of subjectivity may exist.¹⁷ In this study, observations were performed twice by the same examiner under standardized conditions, with the paired *t*-test applied to assess systematic error and Dahlberg's formula used for casual error. In both cases, the results remained below reference limits for error consideration. In this context, the absence of significant error between observations supports the reliability of the analysis, allowing the suggestion that sexual dimorphism is expressed in some dental morphological traits.

Thus, although the literature indicates that the ASUDAS is more suitable for population affinity estimation,²⁹ the data obtained for the left maxillary lateral incisor, left maxillary first molar, and both maxillary second molars show the presence of sexual dimorphism (Tables 6, 7 and 8). The presence of lingual tubercles extending into the lingual fossa of tooth 22 was more frequent in males than in females. The same pattern was observed for the mesial marginal ridge tubercles of tooth 26 and for the presence of the hypocone cusp in the right and left maxillary second molars, being more frequent in male individuals. Tinoco *et al.* (2016)³³ also reported sex differences based on the hypocone cusp of the maxillary second molar, in agreement with the results of this study; however, they also observed sexual dimorphism for the Carabelli tubercle, which was not found in the population analyzed in the present study.

Although only three traits, out of all those analyzed, showed significant differences between sexes, they could still be used as auxiliary tools for sex estimation, as they are easily observable and recordable. This limited number of distinguishing traits between the analyzed groups may be related to the sample size used in this study, but it is always important that the evaluation of qualitative characteristics be conducted on a sample that more adequately represents the population. Therefore, the importance of further studies on non-metric traits with larger and more robust samples is emphasized, in order to accurately establish which traits may exhibit sexual dimorphism.

Additionally, the paramolar tubercle was also analyzed, although it is not part of the ASUDAS criteria and occurs at low frequency in populations. It is considered an accessory cusp located on the buccal surface of the mesiobuccal cusp of the permanent maxillary second molars (paracone).^{20,34} The data from this study are consistent with the literature reporting low frequency,^{20,34} as only a single male individual presented this tubercle. On the other hand, some researchers, such as Kustaloghi (1962),³⁵ reported a high frequency in an Indian population, although without distinction between sexes.

Therefore, the results of this study suggest that metric traits and some non-metric characteristics of human permanent maxillary teeth provide evidence that can contribute to sex estimation, especially in situations where traditionally used

structures, such as the pelvis and skull, are not available. In the absence of these elements, teeth can offer additional relevant information for sex differentiation, particularly when analyzed using quantitative data. Considering the small sample size, these findings should be interpreted with caution and regarded as indicative of the applicability of these methods in future studies.

CONCLUSION

The results of this study allow us to conclude that MD and BL measurements are effective for establishing sexual dimorphism. However, for teeth 12/22, the MD measurement did not show sexual dimorphism, and similarly, for teeth 13/23, the BL measurement did not indicate dimorphism. The MDAP and ICD were significantly greater in males than in females, suggesting that these parameters may serve as indicators for sex differentiation. Regarding non-

metric analyses, in the studied population, the results were of limited significance, with only a few teeth presenting traits associated with dimorphism: the lingual tubercle on tooth 22, the mesial marginal ridge tubercle on tooth 26, and the presence of the hypocone cusp on teeth 17 and 27. Although these findings are relevant, they should be interpreted with caution, as their consistency still needs to be confirmed. Therefore, further studies with larger samples from different regions of Brazil are recommended to validate these parameters and consider them reliable in anthropological and forensic contexts.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

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Evaluating if blanking the eyes of individuals effectively ensures anonymity: an ethical dilemma in scientific publications and lectures

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have no conflict of interest.

KEYWORDS

Anonymity,
Blanking Eyes,
Patient Consent

J Forensic Odontostomatol
2026. Aug; (44): 2 -16:22
ISSN :2219-6749
DOI: doi.org/10.5281/zenodo.21849188

ABSTRACT

Clinicians and lecturers often use clinical photographs of actual patients for teaching purposes, in congress presentations and for scientific publications. It has been customary practice to blank out the eyes on clinical photographs to ensure anonymity. Researchers have become increasingly concerned that merely covering the eyes is not an effective way to conceal identity especially if the photograph is of a person that may be known to the observer. This study blanked out the eyes of 15 photographs of staff members and well-known public figures as per routine practice in maintaining anonymity. The images were presented to dental students in the final two years of their study, and they were asked to try and identify the individuals. The students were then shown the same images in a different order with no blanked-out eyes and again asked to identify the people. Students were advised that spelling of names was not important. The results were analysed using descriptive statistics to determine the number of correct answers per illustration and in total for both the blanked out and unaltered series of photographs. The results revealed that despite the eyes being blanked out, 75% of the students were able to identify the same number of faces in both series, 21% only had 1 image they could not identify in the blanked-out images, and 4% misidentified 2 images. This study confirmed the suspicion that mere blanking out the eyes of an image is ineffectual in ensuring anonymity. It is also evident that most journals are aware of this limitation and are insisting on confirmation of consent before publishing facial photographs. However, the authors suggest that all lecturers, presenters at study groups and organisers of conferences ensure there is a declaration slide included in the presentation, in which they assure the audience they have gained consent from the respective subjects in their presentations.

INTRODUCTION

Publishing photographs of patients' faces in journal articles, or their use for teaching purposes is a challenging ethical issue.¹ Not only does it require a fully informed and comprehensive consent process, but patients, clinicians, researchers and journal editors need to be aware that if any identifiable images or data are shared, they may become widely disseminated, and available to the broader public, opening it up to potential abuse. In disciplines involving the head and neck area, it is

necessary to show patient faces to illustrate treatment outcomes.¹ It is common practice in dentistry and medicine, forensic science, legal cases, journalism, social media, or other situations where one wants to maintain the anonymity of an individual, to blank out their eyes. Despite that practice, it has been noted by many authors and journal editors that merely covering the eyes of an individual will not conceal their identity. It speaks to the idea that understanding or "seeing" someone isn't just about physical sight and explores how a person can still be "seen" by drawing on psychological, physiological, and social perspectives. We can "see" a person through many different aspects including other physical features, their actions, words, energy, situation and setting. This paper aimed to investigate how easy it is for an observer to identify a person despite the eyes being blocked out. The results may reveal if further and more refined methods of ensuring anonymity are needed when using patient photographs.

LITERATURE REVIEW

In a previous paper by the authors² the concept of human recognition, and the role of facial features, especially the eyes is discussed. It explored the more complex processes involved that extend beyond visual processing alone, and postulated that even if a person's eyes are covered, it is still possible for an observer to identify them based on a myriad of other interconnected features.² These include cognitive recognition and the brain's capacity for "multimodal integration" which allows for the blending of information from multiple senses. Tactile and kinetic cues such as a person's gait, gestures and mannerisms are also distinctive means of recognition.³ Other non-visual identifiers include voice, pitch, tone, cadence, and rhythm, as well as social environment and context.⁴

Facial photography presents a unique ethical dilemma, as faces are difficult to de-identify if one wants to use patient photographs for teaching, at conferences, in publications, as illustration for other patients, or even for advertising purposes.⁵ Blanking out of the eyes alone is no guarantee that a person's identity will be protected as there are so many other individualising features that the brain may process.⁶ There are several ethical and legal

issues to consider when patient photographs are being used. In addition to applying all possible measures to anonymise the illustrations, there is a crucial need for a more robust and fully informed consent process. The 4 key elements of consent must be met, namely voluntariness, decision capacity, disclosure and documentation of consent. Health care professionals must also take cognisance that patients come from a heterogeneous group regarding educational level and socioeconomic backgrounds.¹ Disclosure must ensure they are able to understand and comprehend all the anticipated uses for their images, the potential consequences of publication, and the possibility that the images could end up on social media platforms, especially if published in open-access media.^{1,7} Roguljic et.al, carried out a more in-depth scoping review in 2022, where they looked into the policies and practices of publishing identifiable photographs from papers published between 1914 and 2020. After screening 15 949 titles, and 98 abstracts, a final total of 30 papers were analysed. In general, there was a wide diversity of practices in publishing identifiable patient photographs despite existing standards and protocols. There was also an unsatisfactory level of knowledge on this issue amongst all stakeholders, including patients, clinicians and publishers. From an ethical aspect, it was found that generally the consent process was not conducted properly, and health professionals did not recognise the importance of obtaining written consent for taking medical photographs. They often considered verbal consent to be sufficient or to even use pictures without any consent as they considered these to be part of their own records. With regards to the use of photographs for publication purposes, both patients and doctors agreed that storage facilities need to maintain confidentiality. Patients preferred to be photographed on an institutional camera as opposed to doctors' personal cameras or smart phones. They all preferred nonidentifiable photographs for all purposes and uses.¹ Generally they all agreed that the conventional method of concealing the eyes was not sufficient to protect patient privacy, but very few suggested better alternatives. When the actual journal policies were examined, it also revealed that there was a diverse range of

requirements regarding the consent process, that consent forms were generally insufficient, and the existing ethical guidelines were not fully implemented. A more serious concern was that patient photographs from open-access journals were found on public web-based platforms. The authors concluded that there is an urgent need to improve standards and processes with regards to obtaining fully informed consent, and to apply more stringent measures to protect the privacy of patients in published articles.¹

Some journals have suggested alternative methods of de-identification such as removing all metadata, cropping of non-essential features, blurring or pixelating the entire face, using silhouette-style or high-contrast settings, shadowing or cloning and pasting over features. However, modern AI technology may make it possible to reverse or override these adjustments and render them ineffectual.³ Mere blanking out of the eyes in patient photographs is unlikely to be sufficient to ensure anonymity. This prompted the current research to explore the ability of observers to recognise and identify people from a series photograph of known acquaintances and public figures, despite the eyes having been covered.

AIM AND OBJECTIVES

The study aimed to investigate if blanking out the eyes is an effective means of ensuring anonymity when using patient photographs.

HYPOTHESIS

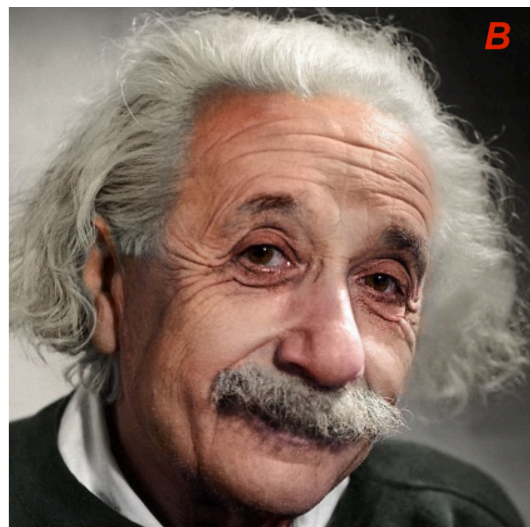
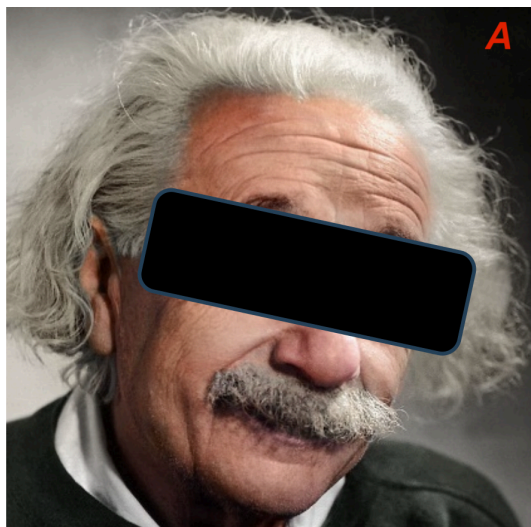
The hypothesis is that blanking out the eyes will effectively ensure anonymity of all people in the photographs for all observers.

METHODS

1. A select sample of high impact journals were reviewed to identify the most current and universally accepted guidelines for publishing images of patients faces. High impact and widely recognised journals were consulted as they generally follow shared ethical frameworks developed by international organizations. They set the standards that shape broader norms and often influence smaller journals, leading to much similarity across journals. Furthermore, due to the immense volume of medical and dental journals, those which aligned the most closely to the research topic, scope and field were chosen to provide manageable examples of ethical policies.
2. The research itself took the form of a cross-sectional descriptive statistical / analytical study at the Oral and Dental School of the University of Pretoria. Consenting staff members who have had some form of contact with final year students were approached to have their photographs taken and used in the study (Figures 1, 2 and 3). Additional photographs of popular celebrities, politicians and sports persons were taken from social media sites. All consenting students from the final two years of dentistry were asked to take part, in the study. A sample size of 120 participants was considered sufficient. They were shown a series of 15 photographs consisting of the images of staff members as well as international celebrities, where the eyes had been blanked out. Included in the sample were photographs of the same person with different hairstyles and in a shaven and unshaven state.

Figure 1. Person known to the students in real life, a. Bearded, covered eyes , b. Shaven, visible eyes



Figure 2. Famous people - Albert Einstein - a. covered eyes. b. visible eyes**Figure 3.** Famous people - Michael Jackson - a. covered eyes. b. visible eyes

They were then shown the same photographs but in a different order and with no blocking out to compare their recognition abilities and account for those persons they did not know at all. Data was analysed to compare the number of correctly identified images between the covered and uncovered examples. Descriptive statistics were used to report the results. The participants were also asked to suggest alternative ways that identity could be concealed.

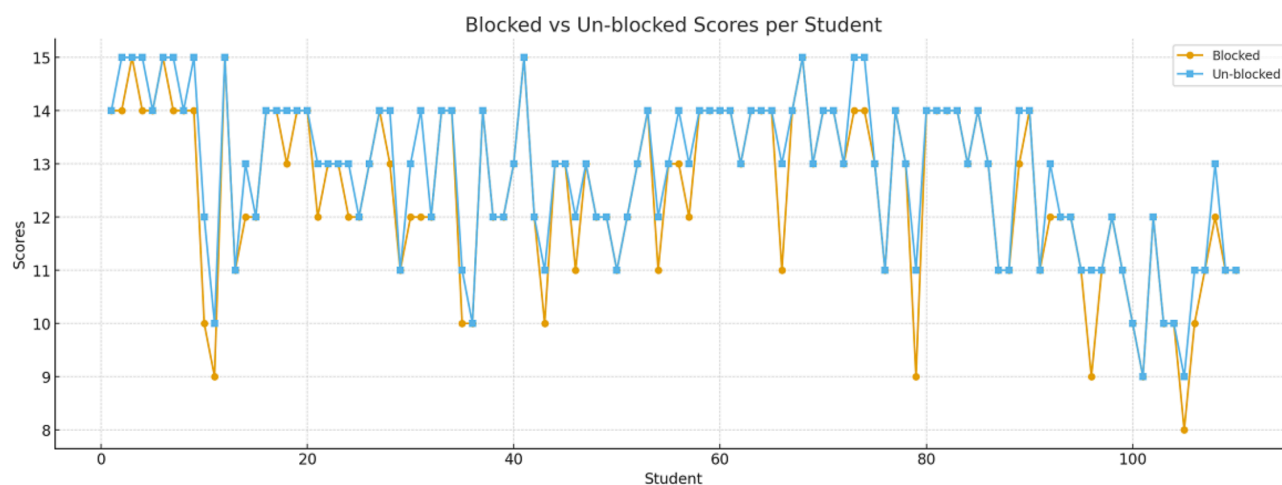
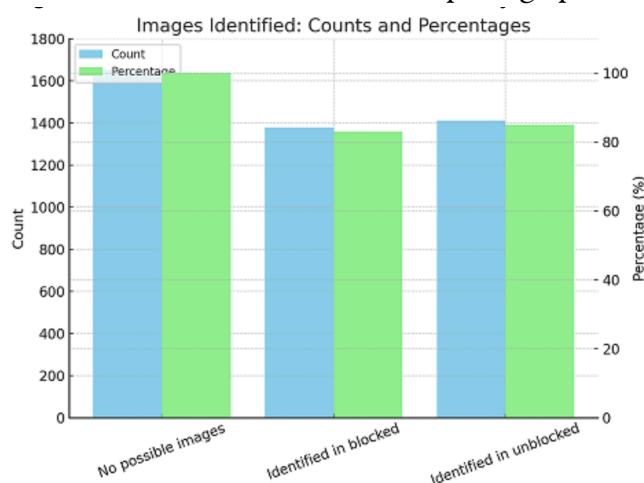
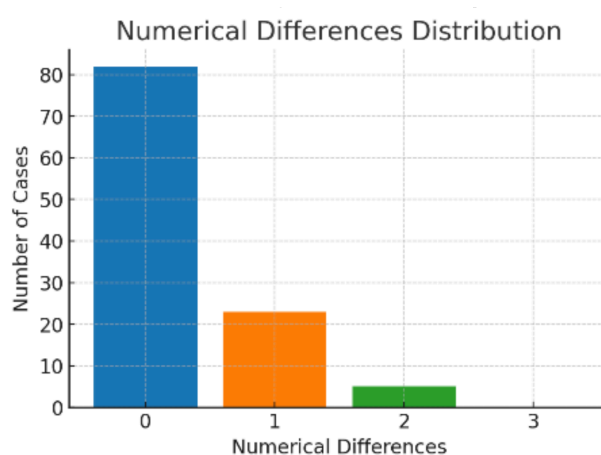
Ethical considerations

The photographs used were taken from consenting staff members in the school, as well as from freely available social media sources. The aim of the study was explained to students during academic sessions, and they were invited to participate. All consenting staff and students

were required to sign a participant informed consent form. Waiver of consent was obtained for the use of images taken from social media sites. All submissions were anonymous. Data will be stored in a secure password protected programme for 10 years as per university requirements.

RESULTS

One hundred and twelve students took part in the study. Two score sheets were incorrectly filled in and were excluded leaving a sample size of 110. Results were tabulated according to the number of correct scores for the “anonymised” images, and for the unaltered images (Figure 4). Highest and lowest number of correctly identified images were recorded in Figures 5 and 6.

Figure 4. Number of correct scores in the blocked-out images, unblocked images and difference between both**Figure 5.** Number of correctly identified images in blocked out and unblocked photographs**Figure 6.** Number of correctly identified images in blocked out and unblocked photographs

Some additional comments and suggestions to improve protection of anonymity were provided by participants. Suggestions included: placing dots over the eyes, only showing the mouth, concealing the eyes and the nose, cropping out all unnecessary features, zooming in and taking more close up photos, blurring the eyes, showing only lower half of the face, using black and white photos, using a plain background and having all patients wear a white bib, editing out hair, editing the skin colour and blurring most of the face with mosaics or pixelating.

DISCUSSION

The results of this study clearly revealed that blanking of the eyes in facial photographs is no guarantee of anonymity, yet this has been the most widely used practice in journal publications

and congress presentations. The researchers consulted the author guidelines of a selection of high impact journals to establish their current requirements. The relevant sections of these are presented as follows:

The Journal of Prosthodontics⁸ - “*Photographs of People. The Journal of Prosthodontics follows current HIPAA guidelines for the protection of patient/subject privacy. If an individual pictured in a digital image or photograph can be identified, his or her permission is required to publish the image. Otherwise, the image/photo must be altered such that the individual cannot be identified (black bars over eyes, tattoos, scars, etc.). The Journal of Prosthodontics will not publish patient photographs that will in any way allow the patient to be identified unless the patient has given their express consent*”.

The Journal of Forensic Odonto-Stomatology (JFOS)⁹ - Tables, photographs, and illustrations: *"All photographs need to be of high resolution: image files at least 6cm wide and 300 dpi, JPEG. Authors should have WRITTEN PERMISSION to use illustrations that may identify human subjects or to disclose sensitive personal information about identifiable persons".*⁹

The International Journal of Forensic Odontology¹⁰ - *"If photographs of individuals are used, their pictures must be accompanied by written permission to use the photograph".*¹⁰

Journal of the Colleges of Medicine South Africa¹¹ - Ethical considerations: *"Papers based on a case study that involves the treatment of humans must adhere to the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects. Case series must have the consent of the patient(s) or waiver of consent approved by an ethics committee. Where individual human subjects or case studies are discussed, e.g. as in medicine, psychology, criminology, books/journals should protect confidentiality and should not permit publication of items that might upset or harm participants/subjects, or breach confidentiality of, for example, the doctor-patient relationship. AOSIS will not publish individual information and identifiable images from patients/human subjects. We will also require explicit consent from any patients described in case studies or shown in photographs".*¹¹

Nature¹² - Publishing images from human research participants. *"When publishing identifiable images from human research participants in Nature Portfolio journals, authors include a statement in the published paper affirming that they have obtained informed consent for publication of the images. All reasonable measures must be taken to protect patient anonymity. Black bars over the eyes are not acceptable means of anonymization. Images without appropriate consent will be removed from publication".*¹²

Forensic Science International¹³ - Informed consent and patient details. *"Authors must document in the manuscript that ethics committee approval and informed consent have been obtained for studies involving patients or volunteers (including organ/tissue donors). Key guidelines: Appropriate consents, permissions and releases must be obtained if case details, personal information and images of patients or any other individuals are included in a publication, even if anonymized. Patient and research subjects' names, initials, hospital or social security numbers, dates of birth or any other personal or identifying information*

*should never be used, even where consent has been provided".*¹³

Although not formally documented, currently it is a strict requirement in scholarly journals that eyes and facial features be obscured to protect individual identity. This practice aligns with established ethical protocols. However, covering eyes or obscuring facial features may appease clinicians and publishers that the authors have tried to ensure anonymity, but in reality, it is ineffectual.

Medical law and ethics aim to protect patient privacy, confidentiality and dignity. Facial photographs can contain highly identifiable information especially if it includes a full face, or has distinctive features such as scars, tattoos, facial asymmetry, characteristic nose or lip anatomy, distinguishing skin tones, or any other unique traits that could reveal the person's identity. Even partial images can allow for identification if combined with other information such as background clues or settings. Therefore, any use of photographs requires strict safeguards and adherence to core ethical principles. Autonomy dictates that patients have the right to control how their personal information and images will be used; confidentiality must protect all information obtained during treatment; the right to dignity and privacy should protect them from exposure to embarrassment or harm; and beneficence and non-maleficence must ensure that the scientific benefits outweigh potential harm.

This research highlights the ethical and legal need to ensure a more robust and comprehensive informed consent process whenever clinicians need to take photographs of patients. Not only do they need to know the exact intended uses of their images (lecture, conference, teaching, publication, advertising), and where they will be used, but they must also be made fully aware that despite blocking out or masking certain features, the chances of them still being recognised are very high. They must also be informed that once published, control over the images is lost and there is a risk that they may become widely and freely available on worldwide internet sites. It would also be prudent to allow the patient to view their anonymised images prior to them being used. Clinicians must realise that blanking out of eyes is not a sufficient guarantee of anonymity. One may argue that one needs to look for a more robust way to anonymise photographs,

but at the same time most journals have strict policies with regards to not accepting any form of “doctoring” as this opens up the opportunity for fraud and deception. In addition, just as certain AI tools can be used to manipulate an image in order to conceal certain features, there are other programmes that can re-insert morphed out facets to re-create a recognisable face. Journals should be required to adhere to the guidelines stipulated by the Committee on Publication Ethics (COPE) that require written patient consent for use of all images; removal of all names and personal data; images only used if scientifically necessary; consent forms to be retained by the authors and institutions; and ethical committee approval for use.¹⁴

International legal frameworks have data protection laws. Patient photographs are considered as personal health data under many of these laws and have to be protected and treated as sensitive personal information that requires explicit consent for use outside of clinical diagnosis and treatment. Unauthorised use of this protected health information can result in legal penalties. In South Africa, healthcare practitioners have to adhere to the Health Professions Council of South Africa (HPCSA) ethical and legal regulations. These state that “express consent must be obtained before publishing case histories

or patient photographs in journals or other scientific media. This is required even if the practitioner believes the patient cannot be identified”. Failure to obtain consent may lead to professional misconduct charges, disciplinary action or civil legal liability.¹⁵

CONCLUSION

This study highlights the need for all clinicians to ensure that they gain fully informed consent from patients before they take clinical photographs. This includes making them aware of all the intended uses for the images, of that fact that a wider than anticipated audience may get to see the photographs and that despite all attempts to conceal their identity, there is a high chance that they may still be recognisable to people who know them. In the absence of a foolproof method of anonymizing images, obtaining informed consent becomes the gold standard, not only in publications but also in presentations. To this end, the authors suggest that all lecturers, presenters at study groups and organisers of conferences ensure there is a declaration slide included in the presentation in which they assure the audience they have gained consent from the respective subjects in their presentations.

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The use of canine dimensions as markers for sex estimation in biological profiling

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for Forensic Odonto-Stomatology - IOFOS

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The authors declare that they have no conflict of interest.

KEYWORDS

Dental Records,
Dental Impressions,
Mandibular Canine Index,
Maxillary Canine Index,
Mesiodistal Width,
Inter canine Distance

J Forensic Odontostomatol
2026. Aug; (44): 2-23:34
ISSN :2219-6749
DOI: doi.org/10.5281/zenodo.21849228

ABSTRACT

Forensic dentistry focuses on identifying individuals through distinctive characteristics of their dental structures. There is a lack of documentation of dental records in developing countries, particularly in Ghana. Therefore, the study aimed to generate detailed baseline data on the sexually dimorphic features of the canine tooth. A convenience sampling technique was employed to recruit a total of 280 participants (140 males and 140 females) aged 18-42 years. Ethical approval was obtained from the Committee on Human Research, Publications and Ethics, Kwame Nkrumah University of Science and Technology. Informed consent was sought from the participants. Dental impressions were made using alginate powder, and study models were prepared using dental stone. Measurements of right and left mesiodistal width of the mandibular and maxillary canines, and Inter canine distances were taken using Digital Vernier Calipers. The means of the Right and Left Mandibular, Maxillary Mesiodistal Widths, Inter canine Distances, and the Right Mandibular Canine index were sexually dimorphic. Binary logistic regression analyses showed that models employing the Right and Left Mandibular Mesiodistal Width, Mandibular Inter canine Distance, Maxillary Inter canine Distance, Right and Left Maxillary Mesiodistal Width, and Right Mandibular Canine Index were good and statistically significant in predicting sex. The best-performing model was the Left Maxillary Mesiodistal Width, which correctly predicted 65% of males and 67.9% of females, with an overall prediction accuracy of 66.40%. Microdont was the predominant tooth type for left and right maxillary canines, followed by mesodont and microdont among both sexes. The results of this study indicate sexual dimorphism, providing a baseline for further research. Canine dimensions, combined with other dental and skeletal indicators, can enhance the accuracy of sex estimation.

INTRODUCTION

The teeth and their dimensions appear to be the most accurate approach in sex estimation, since they are the most resilient and long-lasting component of the skeleton. The fact that the majority of teeth reach their full development before skeletal maturation makes them a valuable indicator of sex, particularly in young persons.¹ The alveolar processes of the maxilla and mandible contain the sockets that house the teeth.

Odontometric features are useful indicators in the estimation of sex.^{2,3} Estimating sex is an important step in the identification of fragmented human remains in forensic anthropology.⁴ The most stable teeth, the canines, exhibit the greatest degree of sexual dimorphism. The canine index and tooth measurements have thus been used exclusively in odontometric sex assessment.⁵ The Mandibular and Maxillary Canine Indices are the ratio between the mesiodistal measurement of the mandibular and maxillary canine teeth and the lower intercanine distance, expressed as a percentage.^{6,7} The size of the tooth is influenced by ethnic, cultural, environmental, and genetic factors. The largest dimension disparities are found in the mandibular canines, where males have larger teeth than females. This could be attributed to longer amelogenesis for both the temporal and permanent dentition, which results in bigger tooth crowns in males than in females.^{8,9} Maxillary odontometric indices can be used to model crime scenes or other situations when the only available skull has a maxilla, establishing dimorphism¹⁰. According to a study by Gupta *et al*¹¹ in North India, males had significantly wider maxillary canines at the midline than females. The maxillary canine exhibited a higher degree of sexual dimorphism compared to the mandibular canine in a Japanese population and a Swedish population.⁸ Dental records include clinical images, radiographs, dental casts, and the medical history of patients. Dental casts allow for highly accurate analysis of tooth size.¹ Dental practitioners in developed countries are required by law to keep and create sufficient patient records.¹³ However, there are poor dental records practices and a dearth of data on the canine index as an identification tool in Ghana. There are differences in odontometric features in different populations. There is therefore a need for population-specific data. This study sought to generate detailed baseline data on the use of the canine index for identification among young adults in the Ghanaian population.

MATERIALS AND METHODS

Study Design and Area

The study design was quantitative and cross-sectional. This study was conducted from July

2023 to December 2024. A convenience sampling technique was employed to sample a total of 280 participants (140 males, 140 females) aged 18-42 years. This age range was selected because canine eruption is completed by early adolescence. However, because minors cannot provide informed consent, the minimum age for participation in the study was set at 18 years. The study adhered to the Helsinki Declaration, and ethical approval was obtained from the Committee on Human Research, Publications Ethics, Kwame Nkrumah University of Science and Technology (KNUST), with reference number CHRPE/AP/131/23. Informed consent was sought from the students of KNUST, School of Medical Sciences.

Sample Size

A calculated sample size of 280 was used. The sample size was calculated via Yamane's formula (1967).

$$n = \frac{N}{1 + N(e)^2}$$

Where n = sample size

N = study population

α = margin of error = 0.05; confidence level of 95% (Adam,¹⁴)

Inclusion and Exclusion Criteria

Participants without anterior teeth spacing, a complete set of fully erupted teeth, noncarious teeth, nonattrited, intact teeth, satisfactorily aligned mandibular and maxillary teeth were included in the study. Participants with dental caries, teeth attrition, impacted canines, retained deciduous canines, morphologically altered teeth, developmental abnormalities of the jaws, crowded or excessive spacing in the anterior teeth, orthodontic treatment, and trauma to the canine teeth were all excluded from the study.

Data collection

- *Materials for data collection*
- Chromatic Hygedent (ISO 21563 EN 21563, ANSI ADA Standard Number 128, 454g, China)
- Dental mixing bowl (U-life, UYKWN0101, China)
- Perforated Aluminium dentulous dental impression (ITRLDPU4) trays, (GDC fine crafted Dental Pvt. Ltd, India)

- Dental stone, super hard Dental Plaster Gypsum powder (HTBR, HT-2308, HT-2309), 25Kg, China)
- Shahe 5110-150 precision stainless steel 150mm IP54 Digital Vernier Caliper, China (Resolution of 0.01mm, accuracy ± 0.02 mm).
- Hospital Disposable Vinyl latex gloves, Dongguan Godetia Medical Supplies Technology Co., Ltd. (Dongguan City, Guangdong Province, China)

Data collection procedures

The participants were seated on a dental chair and a clinical examination was performed using a mouth mirror and a probe. Alginate impressions of the mandibular and maxillary teeth were obtained using perforated metal trays, an alginate bowl, a kidney tray, and gloves. The alginate impression material (Chromatic Hygedent) was mixed with water according to the manufacturer's instructions. The mixed material was loaded onto perforated aluminium dentulous dental impression trays of appropriate sizes with large and extra-large mostly used. Each tray was then placed in the participant's mouth over the mandibular and maxillary dental arch, one at a time, to obtain the impressions. Once set, the mandibular and maxillary impressions were removed from the participants' mouths and disinfected with a hypodisinfectant. Dental stone powder was then mixed with water according to the manufacturer's recommended water-to-powder ratio and carefully poured into the mandibular and maxillary alginate impressions. The mixture was also poured onto a model base to create a flat surface for the final study models, facilitating accurate measurement of the canine teeth. The impressions, now embedded in the dental stone, were allowed to set and dry for at least four hours. Finally, the mandibular and maxillary dental casts were separated from the model base for subsequent study. Measurements of the canines were done using a Shahe Digital Vernier Caliper with a resolution of 0.01mm. The following measurements were taken from the study model of each participant: right and left mesiodistal width of the mandibular canine, right and left mesiodistal width of the maxillary canine, and Intercanine distance. Measurements obtained from dental stone models may be subject to minor dimensional

changes, such as shrinkage, expansion, or air entrapment. However, alginate impressions and stone casts are well-established and widely used in odontometric and forensic studies, and standardized protocols such as impressions were quickly poured using dental stone and vibration technique to minimize air bubbles and potential errors. Advanced digital imaging techniques such as intraoral scanners or Cone Beam Computed Tomography (CBCT) can further reduce measurement variability. However, such technologies have cost and accessibility implications in the study setting. To assess the reproducibility and precision of the measurements, fifty randomly chosen dental casts were measured. All measurements were performed by a single trained observer; therefore, interobserver reliability was not assessed. Intraobserver reliability was assessed using the intraclass correlation coefficient (ICC) based on a two-way mixed-effects model with absolute agreement. Excellent reliability was observed across all parameters, with ICC values ranging from 0.929 to 0.981: Right Mandibular Mesiodistal width (RMMW) 0.964 (95% CI: 0.937–0.979), Mandibular Intercanine Distance (MICD) 0.958 (95% CI: 0.928–0.976), Left Mandibular Mesiodistal Width (LMMW) 0.957 (95% CI: 0.926–0.976), Left Maxillary Mesiodistal Width (LMAMW) 0.959 (95% CI: 0.929–0.977), Maxillary Intercanine Distance (MAICD) 0.981 (95% CI: 0.967–0.989), and Right Maxillary Mesiodistal Width (RMAMW) 0.929 (95% CI: 0.878–0.959).

Mesiodistal width of the canine

The mesial and distal surfaces of the teeth were identified, and the distance between the crest of curvature on the mesial surface and the distal surface was measured with a Digital Vernier calliper (Shanghai, China), accurate to 0.01 mm. The mean mesiodistal widths of the left, right mandibular and maxillary canines in males and females were measured on the dental stone models (Figure 1).

Intercanine Distance

It was measured from the cusp tip of one upper canine to the cusp tip of the opposite canine for both mandibular and maxillary canines. The Vernier calipers were attached to the tips of the mandibular and maxillary canines (Figure 2).

Figure 1. A photograph showing the measurement of the maxillary mesiodistal width of a canine on a study model ($\times 0.5$)



Figure 2. A photograph showing the measurement of mandibular intercanine distance of canines on a study model ($\times 0.5$)



Observed Mandibular/Maxillary Canine Index (MCI)

The mandibular and maxillary canine indices were calculated by dividing the mesiodistal width of the canine by the Intercanine distance expressed as a percentage.

Mandibular/Maxillary Canine index

$$= \frac{\text{Mesiodistal width of canine}}{\text{Intercanine distance}} \times 100$$

Canine Index Classification

A novel method for classifying the tooth size was devised according to the indices obtained from the ratio of the canine dimensions.

Microdont (small-sized teeth): Canine index ≤ 25.0

Mesodont (medium size tooth) Canine index $25.1-37.5$

Megadont (large size tooth) Canine index ≥ 37.6

RESULTS

The age of the participants ranged from 18 to 42 years, with a mean age of 22.04 ± 3.41 years for males and 22.58 ± 3.72 years for females. The mandibular and maxillary canine dimensions are shown in Table 1. The mean values of the mandibular and maxillary mesiodistal widths were significantly higher in males than in females. All the canine dimensions exhibited sexual dimorphism ($p < 0.05$). The maxillary dimensions were numerically higher than the mandibular dimensions in both sexes. The mean values for the right mandibular mesiodistal width of the canine were 7.06 ± 0.53 mm for males, whereas females had 6.67 ± 0.53 mm. The left mandibular mesiodistal width of the canine tooth was 7.05 ± 0.56 mm for males and 6.67 ± 0.47 mm for females. The mean values for the right maxillary mesiodistal widths were 7.76 ± 0.58 mm and 7.32 ± 0.54 mm for males and females, respectively. The maxillary intercanine distance was numerically higher than the mandibular intercanine distance. The mean maxillary mesiodistal width was numerically higher than that of the pooled mandibular mesiodistal width (Table 1).

Table 1. Independent samples T-test comparing mandibular and maxillary canine dimensions between males and females

Parameter	Sex	N	Mean $\pm$ SD (mm)	t	p-Value
Right Mandibular Mesiodistal Width	M	140	7.06 ± 0.53	6.37	<0.001
	F	140	6.67 ± 0.50		
Left Mandibular Mesiodistal Width	M	140	7.05 ± 0.56	6.22	<0.001
	F	140	6.67 ± 0.47		
Mean Mandibular Mesiodistal Width	M	140	7.06 ± 0.56	7.07	<0.001
	F	140	6.67 ± 0.42		

Right Maxillary Mesiodistal Width	M	140	7.76 ± 0.58	6.34	<0.001
	F	140	7.32 ± 0.54		
Left Maxillary Mesiodistal Width	M	140	7.75 ± 0.62	6.24	<0.001
Mean Maxillary Mesiodistal Width	F	140	7.33 ± 0.5	6.85	<0.001
	M	140	7.76 ± 0.56		
	F	140	7.32 ± 0.50		
Mandibular Inter canine Distance	M	140	27.84 ± 2.23	3.02	<0.001
	F	140	26.80 ± 2.06		
Maxillary Inter canine Distance	M	140	37.01 ± 2.50	4.45	<0.001
	F	140	35.35 ± 2.22		

mm= millimetres, SD= Standard Deviation, t= t-statistic; p= probability, statistically significant difference ($p < 0.05$), M = Male, F = Female

Paired Sample t-test was conducted to assess the significant difference between the means of the left and right mandibular, maxillary, and canine indices in both sexes. There was no significant difference between all the paired canine dimensions in both sexes. Paired dimensions were bilaterally symmetrical (Table 2).

The right and left mandibular canine indices were numerically higher than the right and left maxillary canine indices. The right mandibular

canine index for males was 25.48 ± 2.33 mm and 24.83 ± 1.92 mm for females whereas the right maxillary canine indices recorded 20.97 ± 1.42 mm and 20.65 ± 1.62 mm for males and females respectively (Table 2). The right mandibular canine index and the mean mandibular canine index were the only parameters that exhibited sexual dimorphism ($p < 0.05$). All the other canine indices were bilaterally symmetrical and sexually non-dimorphic (Table 3).

Table 2. Paired Sample T-Test of Right and Left Mandibular and Maxillary Canine Dimensions

Sex	Parameter	Mean CD ± SD (mm)	t	P-value	95% C.I Lower	Upper
Male	RMMW	7.05 ± 0.53	0.04	0.96	-0.07	0.07
	LMMW	7.05 ± 0.56				
	RMAMW	7.74 ± 0.56	-0.41	0.69	-0.09	0.05
	LMAMW	7.76 ± 0.58				
	RMCI	25.46 ± 2.34	0.08	0.93	-0.26	0.29
	LMCI	25.46 ± 2.42				
	RMACI	20.95 ± 1.41	-0.56	0.57	-0.26	0.14
	LMACI	21.01 ± 1.51				
	RMMW	6.67 ± 0.48	0.44	0.65	-0.05	0.07
	LMMW	6.66 ± 0.48				

Female	RMAMW	7.33 ± 0.55	-0.58	0.56	-0.09	-0.04
	LMAMW	7.34 ± 0.58				
	RMCI	24.80 ± 1.99	0.38	0.71	-0.19	0.28
	LMCI	20.72 ± 1.59				
	RMACI	20.67 ± 1.62	-0.50	0.61	-0.24	0.14
	LMACI	20.72 ± 1.59				

mm: millimetres, CD: Canine Dimensions; CI: Confidence Interval SD: Standard Deviation, t: t-statistic; p: probability, statistically significant difference ($p < 0.05$). RMMW: Right Mandibular Mesiodistal Width; MICD: Mandibular Inter canine Distance; LMMW: Left Mandibular Mesiodistal Width; RMAMW: Right Maxillary Mesiodistal Width; LMACI: Left Maxillary Canine Index; LMAMW: Left Maxillary Mesiodistal Width; RMCI: Right Mandibular Canine Index; LMCI: Left Mandibular Canine Index; RMACI: Right Maxillary Canine Index; LMACI: Left Maxillary Canine Index

Table 3. Independent Samples T-Test Comparing Canine Indices Between Males and Females

Parameter	Sex	N	Mean ± SD (mm)	t	p-Value
Right Mandibular Canine Index	M	140	25.48 ± 2.33	2.17	0.03
	F	140	24.83 ± 1.92		
Left Mandibular Canine Index	M	140	25.47 ± 2.42	1.80	0.07
Mean Mandibular Canine Index	F	140	24.83 ± 2.04	2.10	0.03
	M	140	25.47 ± 2.23		
	F	140	24.96 ± 1.81		
Right Maxillary Canine Index	M	140	20.97 ± 1.42	0.97	0.33
	F	140	20.65 ± 1.62		
Left Maxillary Canine Index	M	140	21.03 ± 1.52	1.82	0.07
Mean Maxillary Canine Index	F	140	20.70 ± 1.57	1.50	0.13
	M	140	21.00 ± 1.35		
	F	140	20.74 ± 1.45		

N= Number; mm= millimetres; SD= Standard Deviation, t= t-statistic; p= probability, statistically significant difference ($p < 0.05$), M = Male, F = Female

Binary logistic regression analyses were performed to assess the association between mandibular and maxillary dimensions, indices, and sex (Table 4). All predictor variables were statistically significant ($p < 0.05$) except for the right mandibular canine index, right, and left maxillary canine indices. Regression coefficients (B) ranged from 0.07 to 1.68, with standard errors between 0.05 and 0.28. Corresponding odds ratios (Exp(B)) ranged from 1.08 to 5.42. The strongest association was observed for Left Maxillary Mesiodistal Width (LMaMW) (B = 1.49, Exp(B) = 4.46). The binary logistic regression model was statistically significant,

Omnibus Test of Model Coefficients ($p < 0.001$). The model demonstrated good fit to the data, evidenced by a non-significant Hosmer-Lemeshow test ($p = 0.435$), and explained approximately 19.6% of the variance in sex. LMaMW emerged as a strong and significant predictor, increasing the likelihood of male classification by more than fourfold. Although the overall classification accuracy was moderate (66.4%), the findings support the utility of LMaMW as a reliable odontometric parameter for sex estimation. Incorporation of additional dental variables may further enhance predictive performance (Table 5).

Table 4. Binary Logistic Regression Equations for sex prediction using Mandibular and Maxillary Dimensions

Logistic regression equations	B	S.E	Wald	P-value	Exp(B)
$\log_e (p/1-p) = -11.58 + 1.68 (\text{RMMW})$	1.68	0.287	34.63	< 0.01	5.42
$\log_e (p/1-p) = -5.87 + 0.22(\text{MICD})$	0.22	0.058	13.76	< 0.01	1.24
$\log_e (p/1-p) = 10.10 + 1.47 (\text{LMMW})$	1.47	0.272	29.36	< 0.01	4.36
$\log_e (p/1-p) = -10.83 + 0.29 (\text{MaICD})$	0.29	0.057	27.66	< 0.01	1.35
$\log_e (p/1-p) = -9.17 + 1.21(\text{RMaMW})$	1.21	0.23	27.68	< 0.01	3.35
$\log_e (p/1-p) = -11.26 + 1.49 (\text{LMaMW})$	1.49	0.251	35.38	< 0.01	4.46
$\log_e (p/1-p) = -3.16 + 0.13 (\text{RMCI})$	0.13	0.059	4.53	0.03	1.13
$\log_e (p/1-p) = -3.79 + 0.10 (\text{LMCI})$	0.10	0.056	3.15	0.07	1.10
$\log_e (p/1-p) = -1.60 + 0.07(\text{RMaCI})$	0.07	0.07	0.93	0.33	1.08
$\log_e (p/1-p) = -3.03 + 0.14 (\text{LMaCI})$	0.14	0.08	3.25	0.07	1.15

RMMW: Right Mandibular Mesiodistal Width ; MICD: Mandibular Inter canine Distance; LMMW: Left Mandibular Mesiodistal Width; RMaMW: Right Maxillary Mesiodistal Width; LMaCI: Left Maxillary Canine Index; LMaMW: Left Maxillary Mesiodistal Width; RMCI: Right Mandibular Canine Index; LMCI: Left Mandibular Canine Index; RMaCI: Right Maxillary Canine Index ; LMaCI: Left Maxillary Canine Index ; S.E: Standard Error; p = probability, statistically significant difference ($p < 0.05$); B: regression coefficient; Exp(B) : Exponent of Beta (Odds ratio)

Table 5. Classification Accuracy of Binary Logistic Regression Models for Mandibular and Maxillary Dimensions

Predictor	Correctly classified Males (%)	Correctly classified females (%)	Overall Correctly classified (%)
RMMW	62.1	70.0	66.1
MICD	55.7	61.4	58.6
LMMW	61.4	63.6	62.5
RMaMW	62.9	62.9	62.9
MaICD	65.7	65.7	65.7
LMaMW	65.0	67.9	66.4
RMCI	56.4	56.4	56.4
LMCI	51.4	57.1	54.3
RMaCI	53.6	96.7	58.6
LMaCI	56.4	53.6	55.0

%: Percentage; RMMW: Right Mandibular Mesiodistal Width; MICD: Mandibular Inter canine Distance; LMMW: Left Mandibular Mesiodistal Width; RMaMW: Right Maxillary Mesiodistal Width; LMaCI: Left Maxillary Canine Index; LMaMW: Left Maxillary Mesiodistal Width; RMCI: Right Mandibular Canine Index; LMCI: Left Mandibular Canine Index; RMaCI: Right Maxillary Canine Index ; LMaCI: Left Maxillary Canine Index

Microdont was the most dominant tooth type for right maxillary indices for both sexes. Males and females recorded 49.46% and 49.82% respectively. A few participants, 0.36% recorded mesodont for both sexes. None of the participants recorded the megadont tooth type (Figure 3). Left Maxillary canine index was predominantly microdont; females recorded 50% whereas males recorded 49.29%. Only a few of the males (0.71%) had the mesodont tooth type (Figure 4). For the right mandibular canine index, mesodont was the most predominant tooth type, males and females recorded 30.4% and 23.08% respectively. Microdont was the commonest tooth type in females (26.74%) whereas males had 19.78%. Megadont tooth type was not recorded. (Figure 5). For the Left Mandibular Canine index, mesodont was the commonest tooth type in males (27.27%) whereas microdont was the most predominant tooth type in females (26.91%). Only a few (0.36%) of males were megadont (Figure 6).

Figure 3. A bar graph showing the right maxillary indices of both sexes

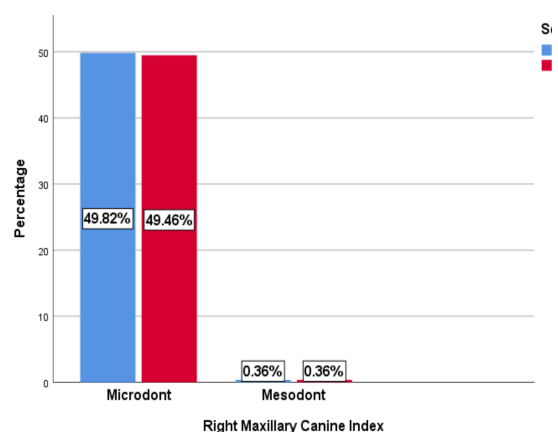


Figure 4. A bar graph showing the left maxillary canine indices of both sexes

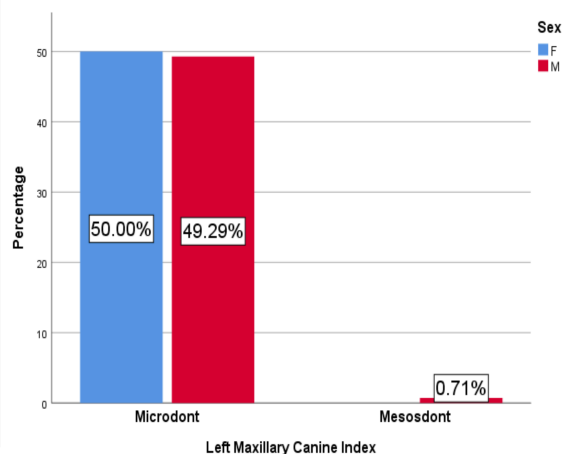


Figure 5. A bar graph showing the right mandibular canine indices of both sexes

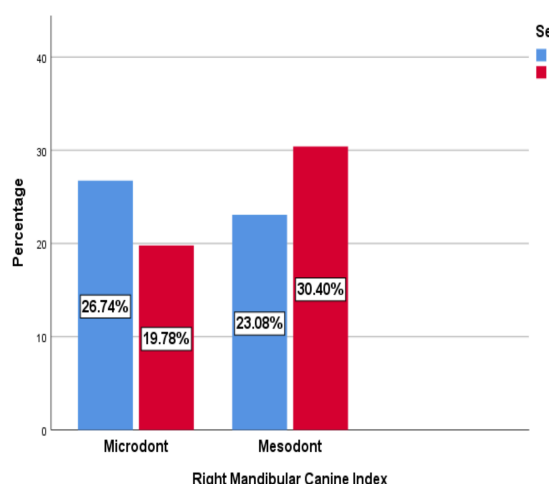
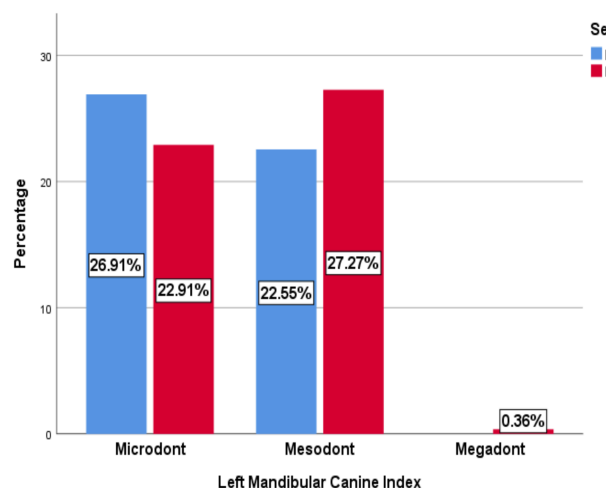


Figure 6. A bar graph showing the left mandibular canine indices of both sexes



DISCUSSION

The young age range in this study was chosen to reduce age-related tooth wear and changes in tooth shape. Sex estimation through dental morphometrics, especially mesiodistal (MD) tooth widths, is a well-known method in forensic anthropology and human anatomy due to the durability of dental tissues and their genetically controlled growth patterns. Odontometric sexual dimorphism refers to the noticeable difference in tooth size between males and females, with males usually having larger teeth. This trend is linked to sex-related growth hormones and hormonal effects during tooth development (Banerjee *et al.*¹⁵). In this study, the average mandibular and maxillary mesiodistal widths were significantly greater in males than in females ($p < 0.05$). Several past studies have shown similar patterns

of sexual dimorphism across different populations in South Asia, the Middle East, and Africa. This aligns with findings in studies from Egypt, Nepal, India, and Nigeria (Awwad ¹⁶; Bajracharya *et al.*¹⁷; Gandhi *et al.*¹⁸; Jain and Kuriakose ¹⁹; Nuhu *et al.*²⁰; Otuaga *et al.*²¹; Rani ²²). It is also supported by reports from Karaman ²³ in Turkey, Kapila *et al.* ² in South India, Rani ²² among the Nalgondian population in India, as well as Zirahei ⁷ among Kogi Polytechnic students in Nigeria, and Edibamode *et al.* ²⁴ among the Ijaw ethnic group in Nigeria, where a significant difference between males and females was observed in the canine teeth. This may be due to males having more developed chewing systems than females, as confirmed by Ates *et al.* ²⁵, who noted that males tend to consume more meat than females.

Similar studies by Rajarathnam *et al.* ¹² and Vishwakarma and Guha ²⁶ asserted that the sexual dimorphism of the mandibular and maxillary mesiodistal widths could be due to genetic variables and amelogenesis which make the X chromosome of males hold 90% of the genetic code for ameloglobin, and the Y chromosome holds the remaining 10% and that leading to longer enamel creation, which leads to larger canine size in males than that of females. Moreover, during the early bell stage, enamel knots form on the cusp of tooth development, controlled by activators and inhibitors prior to calcification. The gene that regulates the expression of activators and inhibitors affects the rate and amount of enamel deposition. Some teeth can be dimorphic across both sexes due to genetic influences (Mazumder *et al.*²⁷). On the contrary, the mean mesiodistal width of mandibular and maxillary canines was not sexually dimorphic for the Saudi Arabian population ²⁸. The variation in mesiodistal widths of both sexes could be a result of the narrow age range of 15-18 years, Moire's topography, and Fourier's analysis in the previous study, as opposed to 18 - 42 years and the use of vernier calipers in the present study.

The statistical thresholds used in different studies all affect the results. Some conflicting results can be explained by the fact that smaller or heterogeneous samples do not detect considerable dimorphism, but larger, well-standardized samples typically do.

In contrast, other similar studies by Boaz and Gupta *et al.* ²⁹ in South India, Alanazi *et al.*³⁰ in

Saudi Arabia, Acharya and Mainali ³¹ among the Nepalese population, reported the reverse dimorphism of mandibular and maxillary mesiodistal widths. These findings could be related to evolution leading to a reduction in sexual dimorphism, generating an overlap of tooth dimensions in males and females.

The maxillary dimensions were numerically higher in both sexes than the mandibular dimensions in this study. Maxillary growth is influenced by complex interactions, including nasal cavity development, paranasal sinus expansion, and midfacial skeletal growth, which results in a broader and longer maxillary dental arch than the mandible (Jacobson ³²). Transverse maxillary dimensions are further influenced by functional variables, such as masticatory muscle activity (He *et al.* ³³). From an evolutionary perspective, the maxillary arch has adapted to accommodate a broader occlusal table, improving masticatory efficiency in humans (Lieberman ³⁴). This confirms the work of Zirahei ⁷, who indicated that maxillary canines were the most sexually dimorphic due to their high rate of survival.

The mean values of the mandibular and maxillary intercanine distance of the present study were sexually dimorphic. This is similar to what was reported by Parekh *et al.* ³⁵ in the Gujarat population and Gupta *et al.*¹¹, where there was a statistical significance between males and females. Multiple factors, including genetic determinants, hormonal impacts, skeletal growth patterns, masticatory function, and evolutionary adaptation, contribute to sexual dimorphism in mandibular and maxillary intercanine distance. Due to increased transverse jaw growth and skeletal robusticity caused by testosterone and hereditary variables linked to the Y chromosome, males usually exhibit noticeably larger intercanine lengths (Ajmal *et al.* ³⁶ ; Herrera-Escudero *et al.*³⁷).

The right mandibular canine index and the mean mandibular canine index were the only canine indices in this study that exhibited significant differences between both sexes. This aligns with earlier findings of Parekh *et al.* ³⁵ among the Gujarat population, where sexual dimorphism was observed in the mandibular canine index. In contrast, Gupta *et al.*¹¹ in the North Indian population observed no sexual dimorphism for right and left maxillary canine indices.

Binary logistic regression analyses showed that the best model for sex prediction was the left mandibular mesiodistal width. The left mandibular mesiodistal width could correctly predict 65.0% males and 67.9% females, with an overall prediction of 66.4%. This moderate accuracy indicates that canine dimensions alone may not be sufficiently reliable as a sex estimation tool and should ideally be used in conjunction with other skeletal or dental indicators. Meanwhile, the findings of Kumawat *et al.*³⁸ in Central India, where the mandibular canine index showed sex prediction accuracy of 81.33 % males and 78 % females, with an overall prediction accuracy of 79.66%. The observed differences in prediction accuracies could be attributed to race, differences in sample size, and age group.

The Right Mandibular Canine Index of the present study could predict 56.4% of males, 56.4% of females, with an overall prediction accuracy of 56.4%. A related study by Mohsenpour *et al.*¹ predicted sex using right mandibular canine index recorded accuracies; 44% for males and 62% for females, with an overall accuracy of 53%. However, the Left mandibular canine index sex prediction accuracy in this study was 54% for males and 64% for females, with an overall accuracy of 59%. Mohsenpour *et al.*¹ also reported prediction accuracies of 54% for males, 64% for females, with an overall prediction accuracy of 59% for the left mandibular canine index.

Microdont was the commonest tooth type for right and left maxillary canine indices for both sexes. For the right mandibular canine indices, mesodont was the most predominant, whereas microdont and mesodont were the most frequent tooth types for Left Mandibular Canine Indices. Tooth size can be influenced by nutritional status during development. Undernutrition and environmental stresses during tooth growth can decrease overall tooth dimensions.

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CONCLUSION

Mandibular and Maxillary mesiodistal widths of the canine tooth exhibited sexual dimorphism. The mandibular canine indices were numerically higher than the maxillary canine indices. However, only the right mandibular canine index exhibited sexual dimorphism. The Left mandibular mesiodistal width was the best predictor of sex. Microdont was the predominant tooth type for both sexes. Canine dimensions alone may not be sufficiently reliable as a sex estimation tool and should ideally be used in conjunction with other skeletal or dental indicators.

LIMITATIONS

- A University-based population may limit full generalizability to the wider Ghanaian population.
- Participants' age range of 18–42 years, with a mean age in the early twenties, may limit generalizability of the study.
- Although measurements obtained from dental stone models may be affected by minor dimensional changes such as shrinkage, expansion, or air entrapment, standardised procedures during impression making, model preparation, and measurement were employed to minimise these potential errors.

ACKNOWLEDGEMENTS

I am highly indebted to Dr. Joseph Abu Sakyi, Dr. Emmanuel Kwadwo Owusu and all the dental staff of Dental clinic, KNUST Hospital for their support and assistance during my data collection. I would also like to appreciate the students of the School of Medical Sciences as well as of allied health sciences and all participants who were recruited in the data collection. I would also appreciate all the Lecturers and senior research assistants of the anatomy department, KNUST for their support.

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Assessing the comparative efficacy of forensic torch excitation wavelengths for dental restoration differentiation: a pilot study

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The authors declare that they
have no conflict of interest.

KEYWORDS

Forensic Odontology,
Dental Fluorescence,
Restorative materials,
Canonical Discriminant Analysis,
Post-mortem identification

J Forensic Odontostomatol
2026. Aug; (44): 2-35:42
ISSN :2219-6749
DOI:doi.org/10.5281/zenodo.21849045

ABSTRACT

Background: The increasing sophistication of restorative materials in replicating natural tooth fluorescence poses a challenge for both antemortem and postmortem dental charting. This study evaluated the efficacy of various discrete excitation wavelengths, including those from common forensic torches, in differentiating modern restorative materials from tooth. **Methods:** Conducted within the Institute for Photonics and Advanced Sensing (IPAS) laboratory framework, six extracted human molars and five contemporary A3-shade restorative materials were examined via spectrophotometry across five excitation wavelengths: 266, 300, 365, 395 and 426nm. Canonical Discriminant Analysis (CDA) was applied to Peak Intensity and Peak Emission Wavelength data to quantify the multivariate statistical separation between groups. **Results:** UV-A wavelengths of 365nm and 395nm produced the most robust group separation. While 365nm excitation produced the highest absolute emission intensities for visual detection, 395nm excitation elicited unique spectral red-shifts in composite resins, providing the highest mathematical discrimination. Visible violet excitation at 426nm was the least reliable differentiator. **Conclusion:** For forensic practitioners, UV-A torches (365–395 nm) remain effective for detecting the tooth-coloured restorations tested here. While 365nm is optimal for rapid visual screening 395nm provides a more distinctive spectral fingerprints for formal characterisation. Despite improved fluorescence matching under visible light, the modern restorative materials tested here retain unique spectral signatures under UV-A illumination, though variable fluorescence intensity influenced detectability and may limit generalisation to other restorations or teeth.

INTRODUCTION

Forensic odontology has become a well-established and respected dental specialisation, providing a highly reliable means of identification.¹ This efficient method relies on the systematic comparison of clinical records with post-mortem examinations, identifying unique markers such as patterns of missing teeth and discernible restorations.^{1,2} In modern dentistry tooth-coloured restorations have become the norm and their increasing aesthetic quality makes them, in some instances, remarkably difficult to distinguish from natural tooth. This evolution, while aesthetically pleasing, poses

significant challenges for both clinical practitioners and forensic odontologists. The limited detectability of tooth-coloured restorations complicates accurate dental charting and may result in incomplete or erroneous records. Detection of composite fillings during forensic examination has been shown to be possible using ultraviolet (UV) torches of various types.³⁻⁶ However, how well this works on modern materials, which are designed to mimic natural tooth fluorescence has not been thoroughly investigated.

The physical phenomenon of UV-induced fluorescence from refracted sunlight was first described in 1845 by Frederick Herschel, but its application in dental science emerged significantly later. While early investigations in the 1920s reported the red fluorescence of dental tissues, the documented history of dental fluorescence as a rigorous scientific subject truly began in the mid-twentieth century. Research from the 1950s onwards into enamel and dentine fluorescence established its relevance for clinical diagnostics, and it has since been extensively studied for diagnostic and aesthetic purposes, such as caries detection, restorative material design and polymerisation control.⁷⁻¹⁰

Historically, research has relied on UV excitation (300-400nm), which is favoured for its high-energy photons that efficiently induce fluorescence in both natural teeth and restorative materials.^{11, 12} Under UV illumination, human teeth typically exhibit peak fluorescence around 440nm, and dental materials are often engineered to mimic this wavelength for aesthetic compatibility^[13-16]. However, a major limitation in existing published research is the variable nature of excitation sources, which complicates comparisons between studies. Most laboratory investigations employ wide-band spectra that do not replicate the discrete wavelengths used in common forensic UV torches.¹⁷⁻¹⁹ While such devices are favoured in forensic settings because they are portable and battery-operated, their usefulness in their current form may decline as modern materials increasingly mimic tooth fluorescence.

High-energy visible violet light (around 405-430nm) is gaining interest as a safer and potentially more discriminative light source.^{20, 21} Recent findings indicate that 426nm excitation induces maximum fluorescence emissions in human teeth¹¹, suggesting that visible violet

wavelengths may elicit distinctive responses not yet fully considered by manufacturers of tooth-coloured restorative materials. Natural tooth fluorescence originates predominantly from dentine because of its organic content.²²⁻²⁴ Restorative materials are comparatively homogeneous and largely derive fluorescence from added phosphors.²⁵⁻²⁷ Although manufacturers aim to produce materials to reproduce the blue fluorescence of teeth under UV, their behaviour of these materials under visible violet light remains relatively unexplored.

Given the sensitivity of fluorescence spectra to excitation methods, this pilot study compares the emission spectra of natural teeth and a selection of contemporary restorative materials under discrete UV wavelengths corresponding to forensic torch LEDs (266, 300, 365 and 395nm) and a visible violet wavelength (426nm).^{11, 18, 19} The primary aim is to assess the efficacy of these different wavelengths in differentiating natural teeth from a selection of contemporary tooth-coloured restorative materials and to contribute to best practices for material identification in forensic odontology.

MATERIALS AND METHODS

This study was conducted in accordance with the *National Statement on Ethical Conduct in Human Research 2007 (Updated 2018)*. The research protocol, encompassing the evaluation of forensic torch wavelengths (Project ID: 35804), was authorised as exempt from formal ethical review by the University of Adelaide Human Research Ethics Committee. This exemption was granted on the basis that the research involved only negligible risk using non-identifiable, archived dental specimens.

Sample Selection and Preparation: To facilitate integration into the Institute for Photonics and Advanced Sensing (IPAS) laboratory workflows and ensure high-throughput spectral consistency, a standardised sample set was used. Six intact, disease-free human adult molars were sourced from the University of Adelaide Dental School archives. Molars were selected primarily for their large, relatively flat surface areas, which permitted stable orientation during spectrophotometric measurement and provided a consistent volume of dentine to induce fluorescence.

Similarly, all restorative materials were prepared exclusively in A3 shade. As a common mid-range

clinical shade, A3 served as a representative baseline to establish the fundamental discriminative capacity of the selected wavelengths across different brands and compositions.

While it is acknowledged that tooth and material type and shade variations may influence fluorescence intensity and peak emission, this narrowed scope was essential for the primary objective of this pilot investigation. This evaluation establishes a protocol that can be expanded in future research to include a broader range of shades, materials and different tooth morphologies.

The materials included:

- capsule-delivered Filtek Z250 composite (3M)
- syringe-delivered Universal Flo composite (Gaenial)
- syringe-delivered Universal Injectable composite (Gaenial)
- syringe-delivered Supreme XTE Flowable composite (3M)
- capsule-delivered Fuji2 LC glass ionomer cement (GC Corporation)

Each material was dispensed and shaped into an approximate molar crown and light-cured according to the manufacturer's instructions. Post-curing, samples were not altered with polishing discs. Materials and teeth were stored in deionised water at room temperature for a minimum of 24 hours and air dried prior to spectral analysis.

Optical Excitation Setup

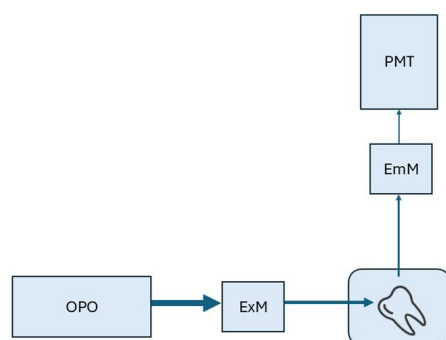
Fluorescence excitation was generated using a tuneable Optical Parametric Oscillator (OPO) (Opotek OPO), pumped by a Q-switched Nd:YAG laser integrated into an Edinburgh Instruments FLS980 Spectrometer.

As illustrated in the schematic Figure 1, the raw laser output was directed through an excitation monochromator (ExM) to isolate discrete wavelengths at 266, 300, 365, 395 and 426 nm. Emissions were processed through an emission monochromator (EmM) and detected via an air cooled R928 photomultiplier tube (PMT) (Hamamatsu Company, USA) to ensure high signal-to-noise ratio for lower-intensity UV-C and UV-B excitations. This dual-monochromator configuration ensured that the data used for subsequent Canonical Discriminant Analysis (CDA) represented true material fluorescence, free from scattered excitation light.

Statistical Analysis

The multivariate spectral differences (Peak Intensity and Peak Emission Wavelength) were analysed using Canonical Discriminant Analysis (CDA) to determine the potential for separation between the Molar and Material groups. CDA was selected for its efficacy in pilot studies with small sample sizes to provide Wilks' Lambda, classification accuracy and canonical function score plots. Analysis was performed using IBM SPSS Statistics v28

Figure 1. Schematic representation of the experimental optical geometry using the Edinburgh Instruments FLS980 Spectrometer. The setup illustrates the discrete excitation path (incident at 45° to the sample surface) and subsequent fluorescence emission collection at a 90° angle to minimise specular reflection while maximising signal-to-noise ratios.



RESULTS

This spectrophotometric analysis revealed distinct fluorescence profiles for natural tooth and restorative materials across five excitation wavelengths. Wide variability in intensities was noted within groups.

UV-C and UV-B Excitation (266nm and 300nm)

At 266nm and 300nm, both natural teeth and restorative materials exhibited relatively low-intensity fluorescence. While the groups remained statistically distinct, overlapping spectral ranges resulted in lower

canonical scores that indicate less robust differentiation compared to longer wavelengths.

UV-A Excitation (365nm and 395nm)

The 365nm wavelength achieved correct classification for all samples in the exploratory transformed spectral space (Figure 2, Panel A). 365nm produced the highest absolute emission intensities for restorative materials, with peaks reaching up to 50,000 counts. However, the emission peaks demonstrated considerable variability, appearing staggered across a range from 439nm to 490nm (Figure 2, Panel B). In contrast, 395nm excitation demonstrated the greatest statistical separation as evidenced by the largest distance between cluster centroids on the first canonical function (Figure 2, Panel C), yet it resulted in significantly lower photon counts (Figure 2, Panel D). This 395nm separation was driven by a consistent, uniform red-shift in the peak emission of the composite resins, which created a more predictable spectral signature than the high-variance peaks observed at 365 nm.

Visible Violet Light Excitation (426nm)

The 426nm visible light excitation proved to be the least reliable differentiator among the wavelengths tested. While two specific composite resins exhibited significantly higher intensities and distinct spectral morphologies, the remaining four restorative materials, including the Fuji 2 LC glass ionomer cement (GIC), demonstrated a high degree of spectral overlap with the natural molar cluster (Figure 3).

The high degree of separability observed between natural tooth structure and restorative materials was driven by distinct spectral signatures, particularly under UV-A excitation. As shown in Table 2, natural molars exhibited a consistent fluorescence peak in the 440–460nm region. In contrast, several composite resins demonstrated a characteristic red-shift in their peak emission wavelength, moving towards the 480–510nm spectrum. This wavelength-dependent shift was most pronounced at 395nm excitation, which provided the greatest mathematical distance between cluster centroids.

Figure 2. Canonical Discriminant Analysis (CDA) scatter plots and corresponding fluorescence emission spectra for natural molars and restorative materials under 365nm and 395nm excitation. A. CDA for 365 nm, illustrating clear group clustering and high classification accuracy. B. CDA for 395nm demonstrating the greatest statistical separation, evidenced by the increased distance between the Molar and Material centroids. C. Emission spectra for 365nm, highlighting high absolute photon counts and the variable, staggered peak positions among the different restorative resins. D. Emission spectra for 395nm, illustrating the lower signal magnitude and the consistent, diagnostic red-shift observed in restorative materials. Note the significant difference in y-axis scales between B and D.

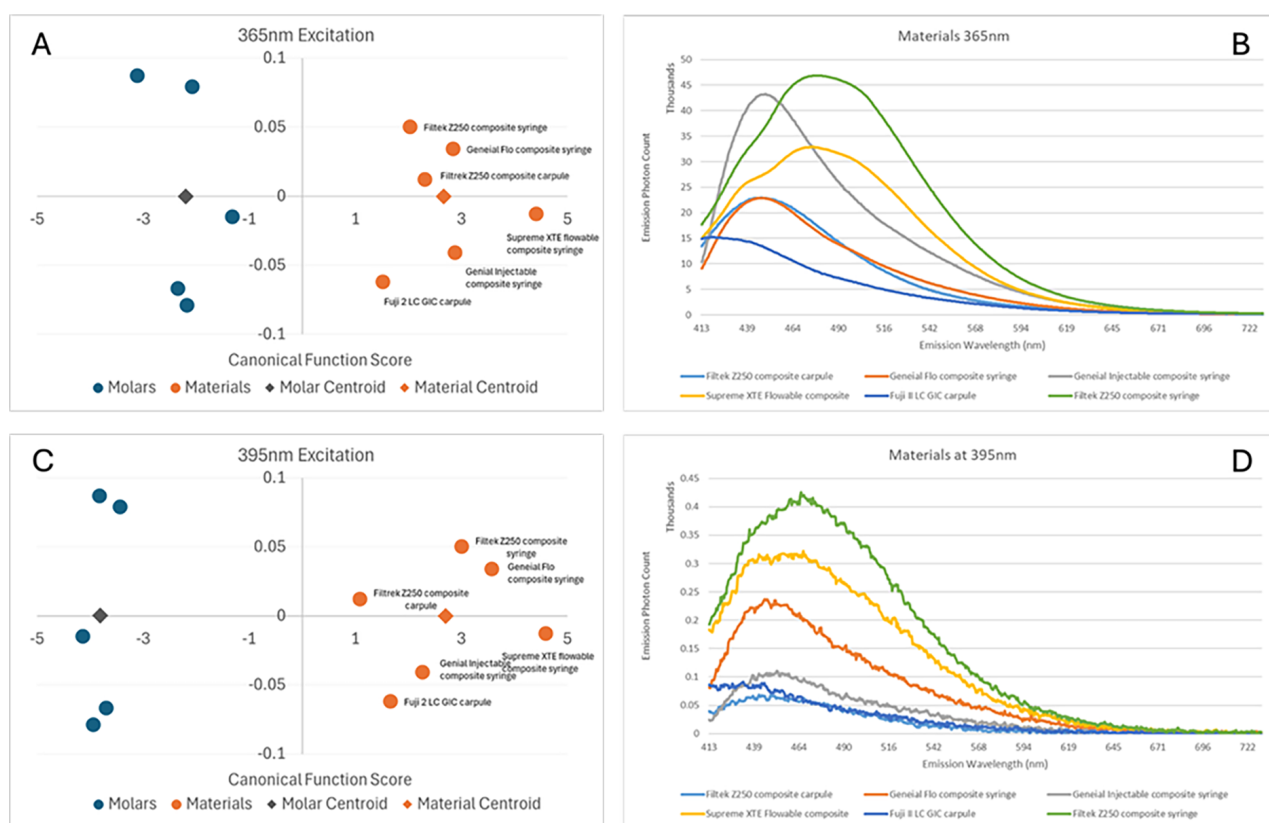


Figure 3. Canonical Discriminant Analysis (CDA) scatter plot and corresponding fluorescence emission spectra for 426nm excitation. A illustrates that 426nm is a less effective wavelength for fluorescence differentiation, evidenced by the misclassification of the 'Fuji II LC GIC carpule' within the natural molar cluster. B displays the emission spectra, where although Genial Flo and Genial Injectable showed higher relative photon counts, the overall signal magnitude and overlapping spectral characteristics limit the accuracy of material differentiation.

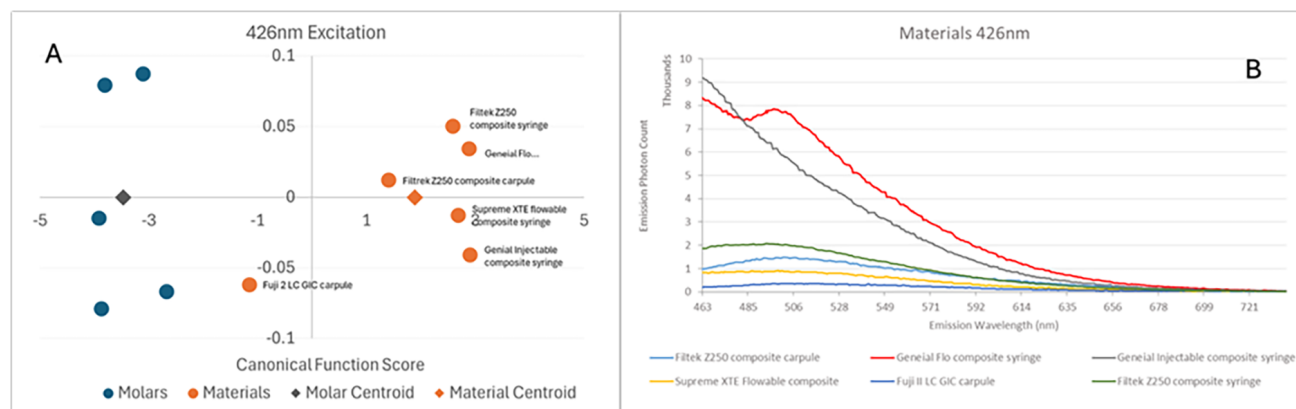


Table 1. Canonical Discriminant Analysis results showing statistical significance and classification accuracy for Molar vs. Restorative Material differentiation across five excitation wavelengths

Excitation Wavelength (nm)	Wilks' Lambda (Λ)	p-value	% Correctly Classified	Classification Result
266 (UV-C)	0.052	0.003	100%	Perfect differentiation
300 (UV-B)	0.057	0.004	90.9%	One molar misclassified
365 (UV-A)	0.057	0.004	100%	Perfect differentiation
395 (UV-A)	0.016	<0.001	100%	Most statistically significant
426 (Visible violet)	0.089	0.012	90.9%	One material misclassified

Table 2. Intensity (Photon Count) and corresponding Emission Wavelength (nm) at the peak of the spectral curve for all five excitation wavelengths

Sample ID	266nm		300nm		365nm		395nm		426nm	
	Intensity	λ_{Em}	Intensity	λ_{Em}	Intensity	λ_{Em}	Intensity	λ_{Em}	Intensity	λ_{Em}
Molar 1	143	439.9	300	444.2	5,414	451.5	194	442.0	1,589	487.1
Molar 2	343	463.6	122	473.9	3,038	466.3	168	452.3	1,088	487.5
Molar 3	101	455.9	99	515.2	1,680	477.4	50	456.9	2,865	502.7
Molar 4	138	448.9	201	447.7	4,172	461.9	94	446.4	959	501.8
Molar 5	265	461.9	905	439.9	12,524	451.9	8,023	451.1	2,401	500.3
Material 1	1,183	436.0	1,440	439.9	23,002	446.8	69	441.6	1,477	500.9
Material 2	3,493	477.7	15,185	434.7	22,094	446.4	237	444.7	9,338	463.0
Material 3	984	443.3	4,393	446.4	4,230	443.8	111	451.9	9,173	445.5
Material 4	6,040	487.3	17,037	477.1	32,074	476.3	322	457.9	933	496.0
Material 5	290	441.6	3,724	412.6	15,230	436.3	91	432.4	364	515.6
Material 6	3,519	479.1	20,294	477.4	46,095	477.8	426	485.8	3,085	499.3

DISCUSSION

The present study advances our understanding of material identification in forensic odontology by elucidating the discriminative power of different excitation wavelengths in detecting restorative materials. The findings underscore the critical role of wavelength selection in fluorescence analysis. While the findings of this pilot study indicate distinct fluorescence profiles between the teeth and materials tested, the variance observed in peak intensities and spectral morphology suggests that a larger sample size, encompassing different tooth morphologies and a broader range of restorative shades, would likely reveal points of spectral overlap. This variability is important, since the discriminative boundaries apparent in a controlled laboratory setting are likely to become less distinct in clinical or forensic scenarios.

Although modern restorative materials are engineered to mimic the fluorescence of natural teeth, the spectral signatures of the materials tested here remained distinct under specific UV-A excitation wavelengths. A key finding is the superior performance of 365nm and 395nm excitation wavelengths, which correspond to standard emissions from commonly used forensic torches. The high photon counts at 365nm facilitate rapid, preliminary screening, enabling practitioners to swiftly identify restorative materials in a clinical or forensic setting. This wavelength's high-intensity output ensures that restorations remain clearly visible even when viewed through filtered lenses required for UV torch use. Conversely, the subtle red-shift, a phenomenon likely linked to the specific phosphors and photo-initiators, such as Lucirin TPO or Ivocerin, used in modern universal shades^[15, 25], induced by 395nm excitation offers a unique molecular fingerprint for synthetic materials, providing a robust signature for confirmatory, computer-aided analysis. Ultimately, the high inter-sample variability at 365nm ensures that most materials will exhibit some form of intense fluorescence, whereas the subtle but reliable red-shift at 395nm ensures that these materials are fundamentally distinguishable from natural tooth structure on a molecular level. Because natural dentine fluorescence is primarily driven by collagen and amino acid cross-links that do not exhibit this specific shift, 395nm provides a unique optical fingerprint for synthetic materials.^{8, 23} The Canonical Discriminant

Analysis demonstrated that separation between the Molar and Material clusters is not only based on intensity variations, but on fundamental differences in peak emission wavelength.

While these results are likely affected by the pilot nature of the study and its limited sample size and type, the significant canonical function scores for both 365nm and 395nm confirm that these wavelengths elicit distinctive optical fingerprints that are statistically superior to those elicited by 266nm or 426nm excitation.

The trade-off between visual accessibility and mathematical distinctiveness is crucial for practitioners to understand, as it guides the appropriate use of these wavelengths in different stages of material identification. While 365nm offers high visual contrast for initial identification, 395nm provides the most mathematically distinct signature for formal, high-certainty material characterisation where mathematics outweighs any need for apparent visual contrast.

The results of this investigation suggests that using a single wavelength alone is not sufficient for tooth coloured differentiation. Therefore, a forensic torch with at least 2 selectable wavelengths of 365nm and 395nm is suggested for differentiation.

Our findings also underscore the limitations of visible violet or blue light torches for restoration detection. The high inter-sample variance and spectral blending with natural molars observed at 426nm render this wavelength unsuitable for consistent forensic differentiation. Methodologically, while our study's exclusive use of molars provided a consistent experimental baseline, it also introduced constraints. Future research should expand sample sizes to include anterior teeth with varying dentine-to-enamel ratios, as these may exhibit greater fluorescence intensity and affect contrast ratios observed in this study.¹² Furthermore, while the decision to use only A3 shade materials was necessary for high-throughput analysis, this limits the generalisability regarding full VITA shade options. Darker shades or highly translucent incisal resins may contain different concentrations of fluorescing agents that alter classification accuracy. Additionally, the consideration of stratified layering techniques, where multiple resins are used in a single restoration, can help determine the extent to

which laminated materials improve or reduce detection efficacy under UV forensic torch use.^{28, 29}

CONCLUSION

This pilot study confirms that despite the increasing aesthetic sophistication of modern dental materials, the ones tested here remain statistically and visually distinct from natural tooth structure under specific ultraviolet excitation. However, larger studies are required to determine whether overlaps between tooth and materials fluorescence become apparent when a larger population is sampled. Practitioners must recognise that the use of a UV forensic torch does not guarantee all composites are apparent, given the range of shades and brands available on the market. Nevertheless, the 365nm and 395nm

wavelengths, common to many forensic torches, demonstrated the highest efficacy for differentiation, with 395nm providing the most robust mathematical separation through unique spectral shifts in restorative resins. Conversely, visible violet light at 426nm proved less reliable, particularly for identifying glass ionomer cements, suggesting it should not be the primary tool for forensic dental identification. We recommend that forensic practitioners prioritise the use of 365–395nm UV-A torches during post-mortem examinations to ensure the accurate detection of clinically valuable restorative identifiers. Future research should expand sample sizes and consider stratified layering techniques to enhance understanding of material differentiation under UV torch use.

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SUPPLEMENTARY MATERIAL

The supplementary figures provide additional visualisation of the fluorescence responses observed across the excitation wavelengths investigated in this study. This data supports the primary findings presented in the main manuscript by illustrating the Canonical Discriminant Analysis outcomes at 266 nm and 300 nm excitation (Figure S4, S5), alongside comparative fluorescence emission profiles for both natural molars and restorative materials across the investigated wavelength range (Figure S6, S7, S8, S9).

Figure 4s. Canonical function at 266nm excitation.

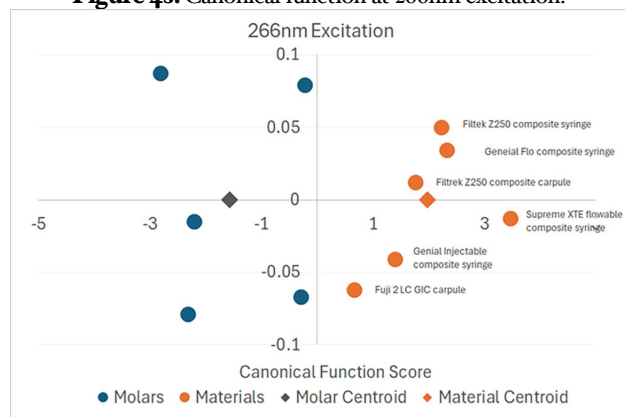


Figure 5s. Canonical function at 300nm excitation

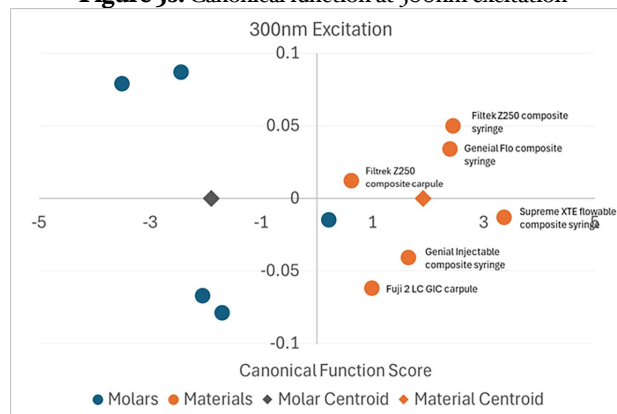


Figure 6s. Fluorescence emission for molar samples at each of the UV excitation wavelengths

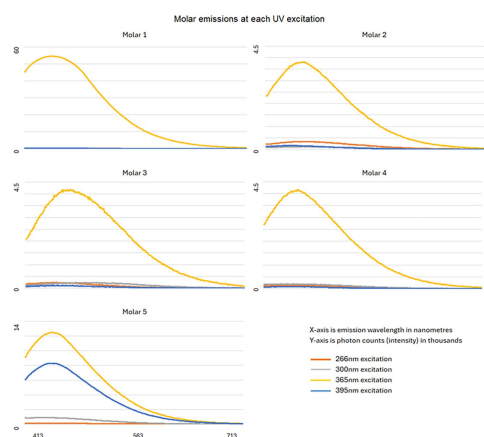


Figure 7s. Fluorescence emission for material samples at each of the UV excitation wavelengths

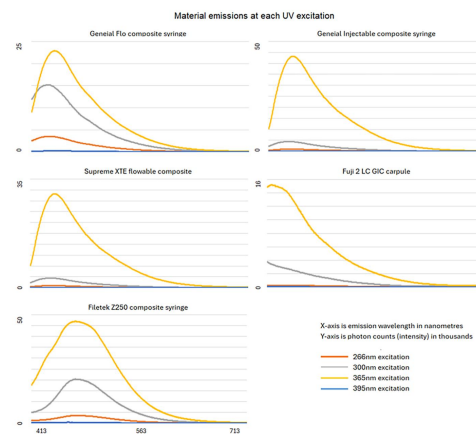


Figure 8s. Comparative emission spectra for materials under each excitation wavelength

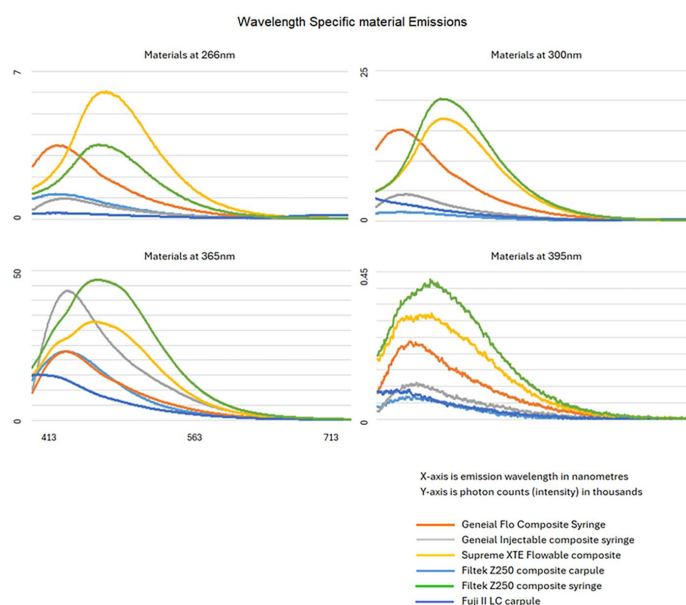
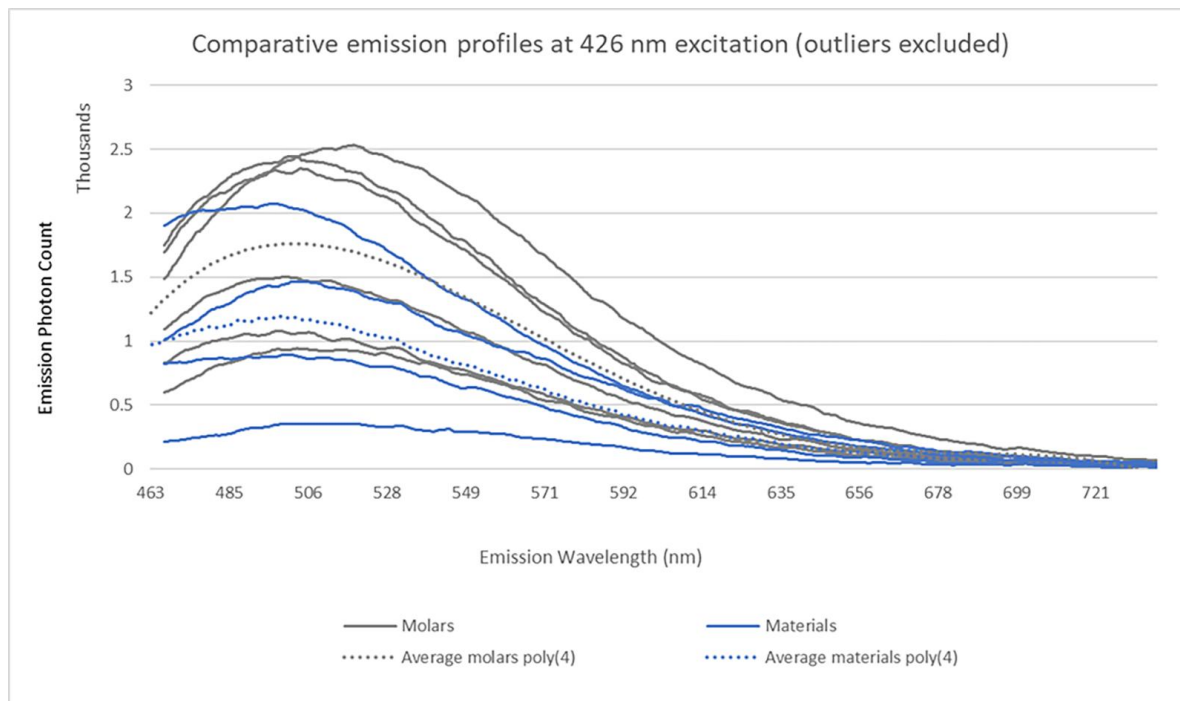


Figure 9s. Comparative fluorescence emission profiles under 426nm excitation. Individual spectral curves for natural molar specimens (grey) and restorative materials (blue) illustrate the significant overlap in emission morphology and intensity at this wavelength. To highlight the lack of diagnostic separability, two high-intensity material outliers were excluded. The dotted trendlines represent the polynomial-smoothed averages for the molar and material groups, respectively, confirming that the mean spectral signatures are nearly indistinguishable. This overlap demonstrates why 426nm was excluded from formal Canonical Discriminant Analysis and is considered unsuitable for consistent forensic differentiation.



Modified radiographic age estimation criteria based on third molar eruption and mineralization status

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The authors declare that they have no conflict of interest.

KEYWORDS

Chronological,
Eruption,
Maturation,
Radiographic Indicators,
Regression Analysis,
Third Molar

J Forensic Odontostomatol

2026. Aug; (44): 2-43:56

ISSN :2219-6749

DOI:doi.org/10.5281/zenodo.21849077

ABSTRACT

Background: Radiographic assessment of the eruption and maturation status of third molars is widely used for age estimation.

Aim: This study compared the effectiveness of radiographic age estimation based on the eruption and mineralization status of third molars and proposed a new regression equation that combined both factors.

Methodology: Eruption and mineralization status of 995 third molars from 269 individuals (North-Indian Subpopulation) were assessed using panoramic radiographs (OPGs) of individuals aged 12-25 years. The Olze et al. (2007) criterion was applied to evaluate eruption levels, whereas a new simplified criterion was proposed to assess mineralization status. 71 additional OPGs were used to validate the proposed regression equations.

Results: A significant correlation was observed between chronological age and the stages of both methods; however, this correlation was slightly stronger for the mineralization method than for the eruption method. Regression Equation 1 (status of all four third molars was determined) consistently showed lower mean absolute difference (MAD) (females: 1.89 years; males: 1.22 years) and root mean square error (RMSE) (females: 2.22 years; males: 1.57 years), indicating smaller prediction errors and greater precision. Equation 2 (applied in cases where any third molar was impacted) exhibited higher MAD (females: 2.35 years; males: 1.90 years) and RMSE (females: 3.13 years; males: 3.09 years), reflecting comparatively larger deviations between estimated dental and chronological ages.

Conclusion: The mineralization method provides a more balanced and diagnostically reliable approach for age estimation, with a higher area under the curve, sensitivity, better overall accuracy, and superior discriminative ability for the estimation of 18 years. In contrast, Olze's eruption method demonstrated higher specificity, making it more useful when minimizing false-positive classification is a priority. Although Regression Equation 1 could estimate dental age more accurately, in cases where third molars are impacted, Regression Equation 2 could be useful.

INTRODUCTION

The assessment of dental age (DA) during the mid-teens to early twenties is based mainly on the development and

eruption status of the third molars, as they are the only teeth that develop and erupt during this period.^{1,3} Numerous radiographic studies have been conducted in the last few decades in various ethnic groups to corroborate the role of third molars in age estimation. However, the majority of them evaluated mineralization status, while a few studies considered the eruption stages.^{4,5} Although tooth eruption appears to follow a predictable pattern and timing, it is governed by a complex interaction of biological (gender, ethnicity, physical and sexual development), genetic, environmental (socioeconomic status), local (infection, premature loss of primary teeth, lack of space, impaction, crowding of permanent teeth, decayed teeth), craniofacial morphology and systemic (hormonal, nutritional factors and systemic diseases) factors, each contributing to the normal or pathological progression of tooth emergence.⁶⁻⁹ On the contrary, tooth mineralization is a continuous process largely driven by genetic factors with less influence from other factors and can be assessed radiographically over a long period, using different stages of tooth formation as a criterion.¹⁰⁻¹³

Numerous classification systems have been proposed to categorize the mineralization status of the tooth based on crown and root development status, featuring a variable number of stages.^{10,14} The most commonly employed criteria were Demirjian et al. 1973 and Nolla 1960, which have 8 and 10 developmental stages, respectively.¹⁵⁻¹⁶ However, evaluating the large number of stages in available systems limits their reliability for accurate stage determination.^{5,17} The relative morphological comparison (crown height/root length) is also inevitable in most staging techniques.¹⁴ Therefore, researchers proposed modifications and adaptations of these criteria, including fewer stages.¹⁸⁻²⁰ Sant Anna et al. 2010 modified Nolla's criteria and proposed five stages to assess dental maturity.²¹ Olze et al. in 2005 examined the validity of five different stage-based systems to assess the mineralization of third molars and found that the Harris and Nortje criteria (five stages) yielded results comparable to those of Demirjian et al. criteria. However, the Gustafson and Koch criteria (which involved four stages, commencement of mineralization; completion of crown; eruption

when cusp(s) penetrate gingiva; completion of the root) have slightly lower accuracy than the Demirjian et al. criteria.²² Moreover, determining apex closure, one of the major criteria, is particularly difficult, especially in maxillary molars, due to the superimposition of surrounding structures and compromised radiographic quality. In addition, literature reports the delayed closure of the apices due to poor socioeconomic status.^{4,14,23} After considering the modifications and limitations of established criteria and the fact that Nolla's criteria has been developed to be applied to third molars, this study adapted and modified his criteria and proposed a newer classification system to determine the mineralization status of a tooth with four stages, without considering the comparison of crown height: root length and assessment of apex closure.

Moreover, most studies available in the literature present population-specific data independently based on either the eruption or mineralization status of the third molars. Thus, the present study was designed to compare the effectiveness of radiographic criteria for age estimation based on the eruption and mineralization status of third molars and to amalgamate these two independent elements by formulating a regression equation to improve the accuracy and reliability of DA estimation among individuals reporting to our institute, catering primarily to the North-Indian subpopulation.

METHODOLOGY

Study design and settings:

This retrospective institution-based observational study was conducted among individuals who reported to the Department of Oral and Maxillofacial Radiology, Post Graduate Institute of Dental Sciences, Rohtak, Haryana, catering primarily to the North-Indian subpopulation for routine radiographic examinations. Ethical approval was obtained from the institutional Biomedical Health and Research Committee (PGIDS/BHRC/23/35 dated 27.06.2023).

Sample size calculation:

After deciding on a timeframe of six months, the Raosoft sample size calculator was used to determine the sample size with 600 as the

number of eligible OPGs at a 95% confidence level, 5% margin of error and 50% response distribution rate. The minimum sample size was calculated as 235. The following formula was used to calculate the sample size:

$$x = Z(c/100)^2 r(100-r)$$

$$n = Nx / ((N-1)E^2 + x)$$

$$E = \text{Sqrt}[(N - n)x / n(N-1)]$$

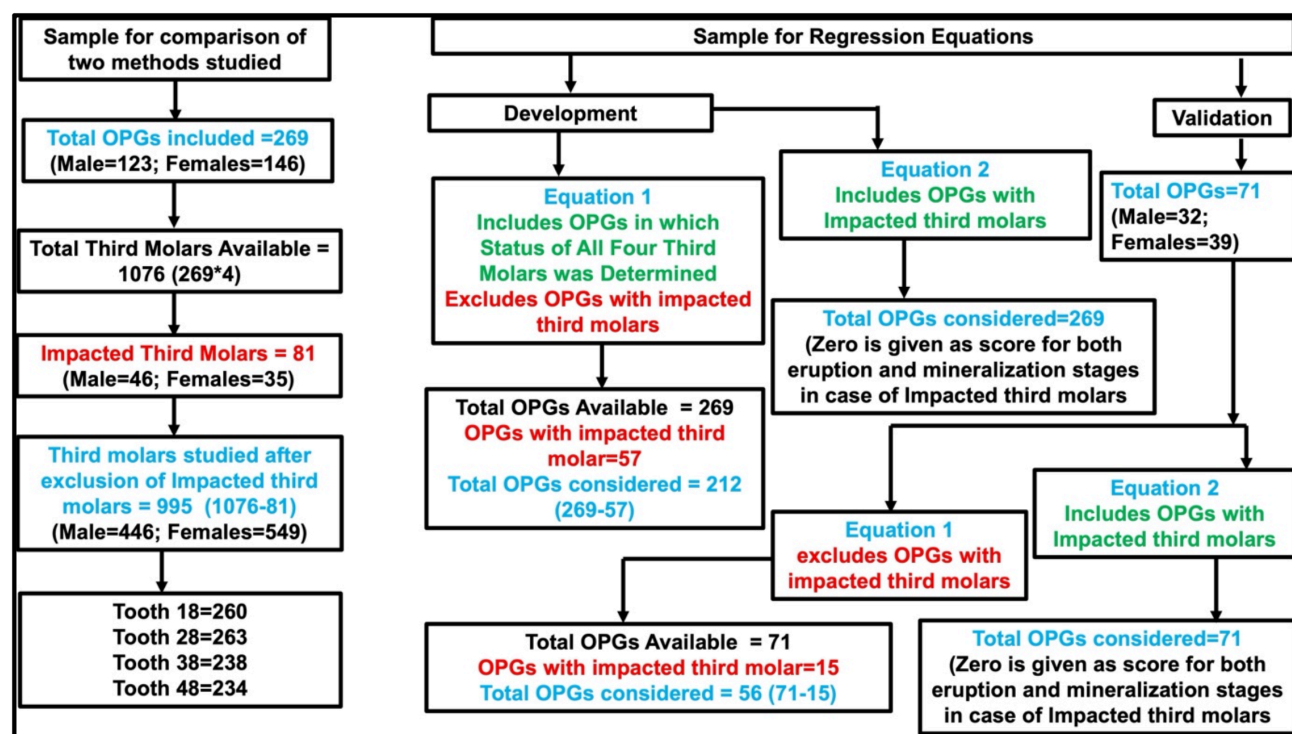
where N is the population size, r is the fraction of responses, Z(c/100) is the critical value for the confidence level c, n is the sample size, and E is the margin of error.²⁴

Participants:

Panoramic radiographs (OPGs) from 350 individuals aged between 12 and 25 years who reported in the past six months were retrieved from the archives of the department.

Radiographs were obtained using an orthopantomography machine (Papaya Plus, M/s Genoray Co. Limited, South Korea), and all scans were evaluated on a desktop monitor with an 8-bit depth and a resolution of 1920×1080. A total of 269 OPGs from the retrieved sample that showed all four third molars (developing or developed) were included in the study. OPGs showing developmental anomalies, trauma, orthodontic treatment or distortion were excluded from the study. Individual third molars that were impacted or had indiscernible root structures were also excluded. Thus, this study evaluated a total of 995 third molars, of which the maxillary right (18), maxillary left (28), mandibular left (38) and mandibular right (48) were 260, 263, 238 and 234, respectively. The FDI tooth-numbering system was used to represent third molars. Presents a detailed outline of the sample distribution for comparison of the two methods, formulation, and validation of regression equations.

Figure 1. Detailed Outline of Sample Distribution



Data Collection:

One investigator retrieved and assigned a unique identification number to each OPG. The date of birth and the date on which the radiograph was taken were recorded, and the chronological age (CA) was calculated by subtracting the date of birth from the date of the radiograph. Two examiners blinded to the CA reviewed the radiographs and recorded the eruption and mineralization status of the tooth. In the case of disagreement, the least developed stage was chosen.^{15,25}

Olze et al.'s (2007)²⁶ criterion was used to appraise the eruption status, which is as follows: Stage A: The occlusal plane is covered with alveolar bone; Stage B: Alveolar emergence; complete resorption of alveolar bone over the occlusal plane; Stage C: Gingival emergence; penetration of gingiva by at least one dental cusp; and Stage D: Complete emergence in the occlusal plane.

The mineralization status of third molars was assessed using the newly proposed mineralization criteria, as follows:

Stage A- Crown formation completed
 Stage B- 1/3rd of root formation completed
 Stage C- 2/3rd of root formation completed
 Stage D- Complete root formation without considering apex closure

Statistical Analysis:

The data collected were subjected to statistical analysis using SPSS Software version 23.0 (IBM Corp., Armonk, NY) and Jamovi Software version 2.6.0. Parametric data are expressed as mean and standard deviation, and categorical data are presented as frequencies and percentages. To assess inter- and intra-observer reliability, Cohen's kappa and intraclass correlation coefficients were applied. Pearson's chi-square test was used to compare categorical data. Pearson's correlation test was used to correlate the two variables. Paired t-tests and one-way ANOVA were used to compare the means between groups. The receiver operator characteristic (ROC) curve was plotted to compare the efficacy of the two methods in estimating 18 years of age. DeLong's test was employed to compare the area under the curve (AUC) of the two methods. In addition, diagnostic test parameters (sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), positive

predictive value (PPV), negative predictive value (NPV), accuracy and Youden's index) were used to compare the efficacy of the stages for two methods. Regression analysis was performed to formulate an equation to predict DA using the combined eruption and mineralization status of the third molars. The predictive accuracy of the formulas for estimating DA was established based on the mean absolute difference (MAD) and root mean square error (RMSE) values. $p < 0.05$ was considered significant.

RESULTS*Inter- and Intra-observer reliability:*

Cohen's kappa was computed from 995 third molars to assess the inter-observer reliability, which was 0.799 and 0.973 for Olze's eruption and new mineralization criteria, respectively, indicating good and very good inter-observer agreement, respectively.²⁷ The intraclass correlation coefficient was applied to randomly selected 100 third molars to assess intra-observer reliability, which was 0.987 and 0.998 for Olze's eruption and the new mineralization method, representing excellent reliability.²⁸

Demographic Details:

Of 269 subjects, 146 were female, and 123 were male. The mean CA of the 269 included participants was 17.57 ± 3.32 . The mean CA for females and males was 17.97 ± 3.33 and 17.10 ± 3.25 years, respectively. Of the 995 third molars assessed, 446 were from males, and 549 from females. The difference in the mean CA between males (16.878 ± 3.202) and females (17.839 ± 3.322) was found to be statistically significant ($p < 0.001$). (Table S1) A statistically significant difference was observed in the mean CA between different stages of Olze's eruption (Stage A: 15.204 ± 2.080 ; Stage B: 17.466 ± 2.613 ; Stage C: 19.636 ± 2.872 ; Stage D: 21.171 ± 2.315 ; $p < 0.001$) and the new mineralization (Stage A: 14.729 ± 1.799 ; Stage B: 16.438 ± 1.973 ; Stage C: 17.494 ± 2.415 ; Stage D: 20.787 ± 2.382 ; $p < 0.001$) method. (Table S2) However, no significant difference was observed in the mean CA for individual third molars ($p = 0.871$) and arch-

wise ($p=0.449$) comparisons. (Table S3 and S4).

Comparison of Olze's eruption and the newer mineralization method:

A statistically significant correlation was observed between CA and stages of both methods for all four third molars combined, individual third molars, sex and arch. However, the correlation coefficient was higher for the mineralization method than for Olze's eruption method for all the evaluated parameters. (Table 1) When all four third molars were considered together, the difference between the mean CA of the two methods was statistically significant for stages A, B and C; however, this difference was not significant for stage D. (Table 2) The stage-wise comparison of the mean CA of the two methods regarding individual third molars, sex and arches are presented in Table S5-S7.

A statistically significant difference was observed in the number of third molars in

different stages of the two methods for all four third molars combined, individual third molars, sex and arches. (Table 3, S8-10)

When all four third molars were considered together, the ROC curve revealed that the AUC was statistically significantly higher for the mineralization method than for the eruption method for the estimation of 18 years of age. (Figure 2) Considering stage D as the cut-off stage, the new mineralization method had higher sensitivity, NPV, accuracy and Youden's index compared to Olze's eruption method. However, a higher LR+ of 15.50 for stage D of Olze's eruption method suggests that there is a greater likelihood of an individual being ≥ 18 years for stage D of Olze's eruption. (Table 4)

Table 5 shows the stage-wise percentage probability of an individual being ≥ 18 years for all four third molars together, individual third molars, arches and sex for both methods.

Table 1. Correlation of chronological age and stages of both methods

Parameters		CA-Olze's Eruption Method			CA-New Mineralization Method			Olze's Eruption- New Mineralization Method		
		n	r	p-value	n	r	p-value	n	r	p-value
Combined all four third molars		995	0.695	<0.001	995	0.758	<0.001	995	0.847	<0.001
Individual Tooth-wise	Tooth 18	260	0.655	<0.001	260	0.704	<0.001	260	0.850	<0.001
	Tooth 28	263	0.677	<0.001	263	0.726	<0.001	263	0.855	<0.001
	Tooth 38	238	0.732	<0.001	238	0.803	<0.001	238	0.840	<0.001
	Tooth 48	234	0.727	<0.001	234	0.815	<0.001	234	0.845	<0.001
Sex-wise	Male	446	0.738	<0.001	446	0.817	<0.001	446	0.854	<0.001
	Female	549	0.672	<0.001	549	0.718	<0.001	549	0.842	<0.001
Arch-wise	Maxillary	523	0.666	<0.001	523	0.715	<0.001	523	0.852	<0.001
	Mandibular	472	0.729	<0.001	472	0.809	<0.001	472	0.842	<0.001

Pearson's Correlation Test

CA-Chronological age; n-Number of third molars; r- Pearson Correlation Coefficient

For the maxillary arch, Tooth 18 and 28 were considered together

For the mandibular arch, Tooth 38 and 48 were considered together

Table 2. Stage-wise comparison of chronological age between the two methods concerning all four third molars together

Stage	Method	n	Mean	St. Deviation	Minimum	Maximum	p-value
A	Olze's Eruption	434	15.204	2.080	12.08	23.89	0.001
	New Mineralization	342	14.729	1.799	12.08	23.89	
B	Olze's Eruption	263	17.466	2.613	12.58	24.32	<0.001
	New Mineralization	208	16.438	1.973	12.90	23.22	
C	Olze's Eruption	117	19.636	2.872	12.96	24.62	<0.001
	New Mineralization	117	17.494	2.415	14.00	24.32	
D	Olze's Eruption	181	21.171	2.315	12.96	24.62	0.079
	New Mineralization	328	20.787	2.382	12.96	24.62	

One-way ANOVA

n-Number of third molars

Table 3. Distribution of third molars in different stages of two methods concerning all four third molars together

Olze's Eruption Method Stages	New Mineralization Method Stages					p-value
	A	B	C	D	Total	
A	326	88	16	4	434	<0.001
B	16	118	70	59	263	
C	0	2	28	87	117	
D	0	0	3	178	181	
Total	342	208	117	328	995	

Pearson's Chi-Square Test

Table 4. Diagnostic test parameters to predict the efficacy of stages to determine ≥18 years

Method	Cut-off Stages	n	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	Accuracy (%) (95% CI)	LR+ (95% CI)	LR- (95% CI)	Youden's Index (95% CI)
Olze's Eruption	C	995	64.85% (59.72%, 69.73%)	90.45% (87.87%, 92.63%)	79.87% (75.51%, 83.62%)	81.49% (79.27%, 83.53%)	81.01% (78.43%, 83.40%)	6.79 (5.27, 8.73)	0.39 (0.34, 0.45)	0.55 (0.48, 0.62)
	D	995	44.41% (39.26%, 49.66%)	97.13% (95.51%, 98.29%)	90.06% (84.99%, 93.54%)	74.94% (73.16%, 76.63%)	77.69% (74.97%, 80.24%)	15.50 (9.69, 24.78)	0.57 (0.52, 0.63)	0.42 (0.35, 0.48)
New Mineralization	C	995	85.29% (81.24%, 88.75%)	78.98% (75.58%, 82.10%)	70.34% (66.95%, 73.51%)	90.18% (87.74%, 92.18%)	81.31% (78.74%, 83.68%)	4.06 (3.47, 4.75)	0.19 (0.15, 0.24)	0.64 (0.57, 0.71)
	D	995	75.2% (70.46%, 79.54%)	91.72% (89.28%, 93.75%)	84.15% (80.25%, 87.39%)	86.36% (84.10%, 88.34%)	85.63% (83.29, 87.75%)	9.08 (6.96, 11.86)	0.27 (0.23, 0.32)	0.67 (0.60, 0.73)

CI: Confidence Interval; LR+: Positive Likelihood Ratio; LR-: Negative Likelihood Ratio; NPV: Negative Predictive Value; PPV: Positive Predictive Value; n: Number of third molars evaluated; Bracketed values represent Lower Bound, Upper Bound for 95% Confidence Interval

Figure 2. ROC curve depicting the Area Under the Curve (AUC) comparing the accuracy of Olze's Eruption and New Mineralization methods for estimation of 18 years when all third molars were considered together. The difference between AUCs was calculated by subtracting the AUC of the New Mineralization method from the AUC of Olze's Eruption method; Bracketed values represent Lower Bound, Upper Bound for 95% Confidence Interval

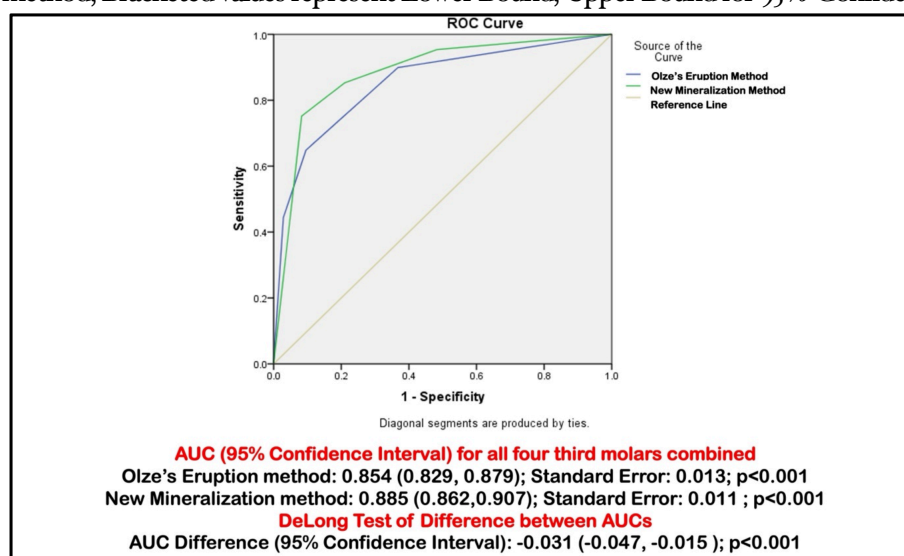


Table 5. Percentage probability of an individual being ≥ 18 years

Parameters		Age (years)	n	Olze's Eruption Method				New Mineralization Method			
				A	B	C	D	A	B	C	D
Combined all four third molar		<18	628	91.5%	65.0%	35.9%	9.9%	95.0%	82.2%	68.4%	15.9%
		≥ 18	367	8.5%	35.0%	64.1%	90.1%	5.0%	17.8%	31.6%	84.1%
Tooth-wise	Tooth 18	<18	162	87.7%	60.0%	55.0%	9.4%	91.3%	74.1%	71.4%	18.3%
		≥ 18	98	12.3%	40.0%	45.0%	90.6%	8.7%	25.9%	28.6%	81.7%
	Tooth 28	<18	163	87.9%	61.8%	60.0%	7.4%	92.6%	75.5%	68.8%	16.7%
		≥ 18	100	12.1%	38.2%	40.0%	92.6%	7.4%	24.5%	31.3%	83.3%
	Tooth 38	<18	152	96.0%	67.7%	23.1%	11.4%	98.7%	88.2%	72.4%	13.4%
		≥ 18	86	4.0%	32.3%	76.9%	88.6%	1.3%	11.8%	27.6%	86.6%
	Tooth 48	<18	151	95.9%	70.8%	21.2%	12.8%	98.8%	93.5%	60.7%	15.0%
		≥ 18	83	4.1%	29.2%	78.8%	87.2%	1.3%	6.5%	39.3%	85.0%
Arch-wise	Maxillary	<18	325	87.8%	60.9%	57.8%	8.4%	91.9%	74.8%	70.0%	17.5%
		≥ 18	198	12.2%	39.1%	42.2%	91.6%	8.1%	25.2%	30.0%	82.5%
	Mandibular	<18	303	95.9%	69.2%	22.2%	12.2%	98.7%	90.7%	66.7%	14.2%
		≥ 18	169	4.1%	30.8%	77.8%	87.8%	1.3%	9.3%	33.3%	85.8%
Sex-wise	Male	<18	304	94.9%	73.7%	44.0%	9.9%	98.8%	89.1%	82.8%	8.5%
		≥ 18	142	5.1%	26.3%	56.0%	90.1%	1.3%	10.9%	17.2%	91.5%
	Female	<18	324	88.6%	57.9%	29.9%	10.0%	91.8%	76.7%	50.9%	20.7%
		≥ 18	225	11.4%	42.1%	70.1%	90.0%	8.2%	23.3%	49.1%	79.3%

n - Number of third molars

For the maxillary arch, Tooth 18 and 28 were considered together

For the mandibular arch, Tooth 38 and 48 were considered together

Regression analysis

Development of the regression equation: The current study proposed two regression equations using the combined eruption and mineralization statuses of the third molars. To formulate a regression equation, numerical values were

assigned to the stages of both methods: stage A=1, stage B=2, stage C=3 and stage D=4.

Regression Equation 1:

This was developed from 212 subjects, including only OPGs, where the status of all four third molars was determined (i.e. excluding OPGs with any of the impacted third molars). (Figure 1)

	Regression Equation -1	R square
Female	$12.445 + 0.674 (T18\ O) + 0.055 (T18\ M) - 0.810 (T28\ O) - 0.043 (T28\ M) + 1.328 (T38\ O) + 0.169 (T38\ M) - 0.522 (T48\ O) + 1.337 (T48\ M)$	0.660
Male	$11.513 + 1.015 (T18\ O) + 1.227 (T18\ M) - 0.586 (T28\ O) - 1.204 (T28\ M) - 0.331 (T38\ O) + 0.967 (T38\ M) + 0.532 (T48\ O) + 0.573 (T48\ M)$	0.746

Regression Equation 2:

It was derived from OPGs of all 269 included participants, with a score of zero for both the eruption and mineralization stages for the impacted third molar. (Figure 1)

In the regression equations, O represents Olze's eruption stage, and M represents the mineralization stage of the tooth. T18, T28, T38 and T48 represent the maxillary right, maxillary left, mandibular left and mandibular right third molars, respectively.

	Regression Equation -2	R square
Female	$13.328 + 0.758 (T18\ O) + 0.031 (T18\ M) + 0.048 (T28\ O) + 0.361 (T28\ M) + 1.164 (T38\ O) - 0.142 (T38\ M) - 1.385 (T48\ O) + 1.225 (T48\ M)$	0.507
Male	$12.169 - 0.499 (T18\ O) + 0.134 (T18\ M) + 0.950 (T28\ O) + 1.369 (T28\ M) - 1.199 (T38\ O) + 0.804 (T38\ M) + 0.460 (T48\ O) - 0.020 (T48\ M)$	0.676

Tooth-wise predictors for regression equations:

The analyses of individual third molar eruption and mineralization stages revealed variable contributions to age estimation, with noticeable sex-specific differences between the equations. In Regression Equation 1, females showed significant positive associations for T38 eruption ($B = 1.328$, $p = 0.014$) and T48 mineralization ($B = 1.337$, $p = 0.015$), with T48 mineralization being the most influential ($\beta = 0.531$). In males, only T18 mineralization was statistically significant ($B = 1.227$, $p = 0.048$). Most other parameters in Equation 1 were not significant, indicating limited predictive contribution. In contrast, Regression Equation 2 demonstrated a greater number of significant predictors. Among females, T38 eruption ($B = 1.164$, $p = 0.022$), T48 eruption ($B = -1.385$, $p = 0.009$), and T48 mineralization ($B = 1.225$, $p = 0.005$) significantly contributed to age estimation, with T48 mineralization again showing the strongest positive influence ($\beta = 0.529$). Among males,

significant predictors included T28 eruption ($B = 0.950$, $p = 0.036$), T28 mineralization ($B = 1.369$, $p = 0.003$), T38 eruption ($B = -1.199$, $p = 0.008$), and T38 mineralization ($B = 0.804$, $p = 0.027$). (Table S11 and S12)

Validation of proposed regression equations:

To validate the proposed regression equations, an additional 71 OPGs from individuals aged 12-25 years were retrieved from the departmental archives of our institute. Regression Equation 1 was validated using 56 OPGs, whereas all 71 OPGs were used to validate Regression Equation 2. (Figure 1) For both sexes, statistically significant correlations were observed between CA and estimated DA using Regression Equations 1 and 2. In addition, no statistically significant difference was observed between the estimated DA and CA for either regression equation. Among females, the MAD and RMSE for Equation 1 were 1.89 ± 1.20 years and 2.22 years, and for Equation 2, $2.35 \pm$

2.09 years and 3.13 years, respectively. Among males, Regression Equation 1 yielded the lowest MAD (1.22 ± 1.01 years) and RMSE (1.57 years), indicating the highest predictive accuracy among all evaluated equations. In contrast, Regression Equation 2 showed a higher MAD (1.90 ± 2.48 years) and RMSE (3.09 years). The MAD values, ranging from 1.22 to 2.35 years, suggest that the estimated DA was reasonably

close to the CA in both males and females, with relatively small average prediction errors. The RMSE values, ranging from 1.57 to 3.13 years, indicate the presence of occasional larger prediction errors. (Table 6) Thus, the proposed regression equations provide reasonably reliable estimates, albeit with some variability across cases, demonstrating that individual-level prediction errors remain clinically relevant.

Table 6. Relationship between chronological age and estimated dental age using regression equations

Sex	Regression Formula	Paired Samples Statistics			Paired Differences (Chronological Age- Estimated Dental Age)		Absolute Difference		RMSE	p-value
		n	Chronological Age	Estimated Dental Age						
			Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	95% Confidence Interval of the Difference (Lower, Upper)	Mean $\pm$ SD	Range		
Female	Regression equation 1	29	18.49 $\pm$ 3.78	18.56 $\pm$ 3.03	-0.07 $\pm$ 2.26	-0.93, 0.79	1.89 $\pm$ 1.20	0.05-4.25	2.22	0.868
	Regression equation 2	39	19.45 $\pm$ 3.79	18.52 $\pm$ 2.59	0.93 $\pm$ 3.03	-0.06, 1.91	2.35 $\pm$ 2.09	0.05-9.03	3.13	0.064
Male	Regression equation 1	27	16.49 $\pm$ 3.07	16.59 $\pm$ 2.69	-0.10 $\pm$ 1.60	-0.73, 0.53	1.22 $\pm$ 1.01	0.02-3.76	1.57	0.747
	Regression equation 2	32	17.17 $\pm$ 3.33	16.78 $\pm$ 2.86	0.39 $\pm$ 3.12	-0.73, 1.52	1.90 $\pm$ 2.48	0.04-11.36	3.09	0.475
Paired Samples Correlations										
Sex	Regression Formula	n	Correlation				p-value			
Female	Regression equation 1	29	0.801				<0.001			
	Regression equation 2	39	0.607				<0.001			
Male	Regression equation 1	27	0.854				<0.001			
	Regression equation 2	32	0.501				0.003			

Paired T-test

n-Number of OPGs evaluated; SD- Standard Deviation; RMSE – Root Mean Square Error

Regression Equation 1 was applied in cases where the status of all four third molars was determined (i.e. excluding OPGs with any of the impacted third molars)

Regression Equation 2 included OPGs with impacted third molars.

Comparison of two regression equations:

A comparative evaluation of the two regression equations revealed that Regression Equation 1 had a higher predictive ability ($R^2 = 0.660$ and 0.746 for females and males, respectively) than Regression Equation 2 ($R^2 = 0.507$ and 0.676 for females and males, respectively). Regression

Equation 1 demonstrated greater consistency, with fewer but more stable predictors. However, Regression Equation 2 identified a greater number of statistically significant predictors in both sexes, suggesting that it captures a broader range of developmental indicators contributing to age estimation. (Table S11 and S12) In addition,

Equation 1 showed minimal mean differences between CA and estimated DA, lower MADs and RMSE values, indicating greater accuracy and precision. Moreover, Equation 1 exhibited stronger correlations with CA in both females ($r = 0.801$) and males ($r = 0.854$) than Equation 2, which showed comparatively weaker correlations ($r = 0.607$ in females and $r = 0.501$ in males). Neither equation showed statistically significant differences between CA and estimated DA. Thus, the findings indicate that while Regression Equation 2 may offer broader insights into multiple developmental variables, Regression Equation 1 demonstrates better overall predictive performance and reliability.

DISCUSSION

Third molar eruption and maturation status play vital roles in age estimation. Various qualitative and categorical tooth staging techniques that utilize dental radiographs have been employed for this purpose. However, the precision of these techniques is influenced by certain factors, such as biological variability, sample quality, and correct radiographic interpretation.¹⁴

One of the most commonly used radiographic criteria for determining the eruption status of third molars for age estimation was proposed by Olze et al. in 2007. It has the advantage of being able to identify both missing and impacted teeth, thus providing a better age estimate than clinical eruption techniques.²⁹ In addition, this method shows a good assessment of DA in young adults and is suggested to be clinically acceptable in terms of accuracy and ease of assessment.¹ The tooth emergence process is directly affected by several factors; hence, eruption methods are prone to errors and inaccuracies.³⁰ A few shortcomings of Olze's criteria that were comprehended during this study include discrepancies in the determination of stages following positional changes due to missing first and second molars, which may hasten the eruption of third molars, the presence of bony hindrance in the path of eruption and buccolingual eruption of third molars, which can radiographically appear below the occlusal plane. Moreover, delayed calcification could affect the accuracy of age estimation.

Radiographic interpretation of tooth development is complex, subjective and less reliable as it is affected by rater characteristics such as knowledge, experience, and expertise.¹⁴

The inter-observer variability noted during the determination of eruption status in this study could be attributed to the inherent difficulty in distinguishing developmental stages B and C. Moreover, radiographic interpretation of gingival eruption was difficult or often impossible because of the gingiva being partially radiolucent, which could also introduce error.²⁹⁻³¹ Considering this fact, Olze et al. (2012)³² modified their criteria to determine stage C based on the eruption of the third molar according to the length of the crown of the adjacent second molar. However, the varied directions of eruption, impaction of third molars and absence or positional variability of second molars could further complicate the stage assessment. This signifies a higher chance of imprecision if radiographs alone are used to determine eruption status.

A comparison of the findings of the present study with those of other studies regarding the eruption and mineralization patterns and timings of third molars is presented in the tables $SI3^{1-3,5,26,29,33-42}$ and $SI4^{5,10,17,20,36,39-40,43-50}$. The diagnostic accuracy findings of this study were not associated with those reported by Shi et al.⁴⁰, except for specificity, LR+, and LR- for stage D of Olze's eruption method in determining 18 years of age. The likelihood of an individual being ≥ 18 years of age in this study was consistent with stage D of Widek et al.'s study but not with stage C.³⁹ Our findings for stage D of the newly proposed mineralization methods corresponded to the diagnostic accuracy findings (specificity, PPV, NPV, accuracy, LR+, and LR-) of stage G of Demirjian et al.'s method in determining 18 years as reported by Costa et al.²⁵ Regarding the likelihood of an individual being ≥ 18 years, the findings of this study (stage D of the new mineralization method) were in agreement with those of Widek et al.'s study (stage G of the Demirjian et al.'s method).³⁹ Our findings for LR- were in line with those of Liversidge and Masden (2010) for root stages combined $R_{3/4}$ and Rc in determining 18 years; however, sensitivity, specificity and LR+ were in contrast.⁵¹ As this study did not consider apex closure as a separate stage, the findings of stage H of Demirjian et al.'s method are not applicable for comparison, as most studies used stage H as a cut-off to determine 18 years. The differences between the findings of this study and those of other studies could be due to variations in ethnicity, geographical regions, age groups, and the number

of participants. The classification systems referenced, types of third molars evaluated (mandibular, maxillary, or both), and subjectivity in assessing the developmental stage could also contribute to the varied findings.

In the present study, inter-observer variability was lower with the mineralization method than with the eruption technique. A higher correlation coefficient was observed between CA and the stages of the mineralization method than with the eruption stages. In addition, the AUC was higher for the mineralization method than for the eruption method when estimating 18 years. Considering stage D as the cut-off stage, the new mineralization method had considerably higher sensitivity (75.2%) than Olze's eruption method (44.41%), while maintaining high specificity (91.72%). In contrast, Olze's eruption method exhibited extremely high specificity (97.13%) but poor sensitivity, indicating accurate exclusion of non-target individuals while failing to detect a substantial proportion of true positive individuals. The new mineralization method also achieved higher accuracy (85.63% vs. 77.69%) and a superior Youden's index (0.67 vs. 0.42), indicating stronger overall discriminatory performance. Furthermore, the lower LR- value of the new mineralization method (0.27) compared with Olze's eruption method (0.57) suggests better exclusion capability. However, higher LR+ and the probability of an individual being ≥ 18 years old for stage D of Olze's eruption method suggest that there is a greater likelihood of an individual being ≥ 18 years old for stage D of Olze's eruption. Literature suggests that all available third molars should be considered in DA estimation as mineralization and eruption differ between quadrants within the same individual.³⁹ Therefore, this study proposes two regression equations: one considering the status of all four third molars and the other applicable in cases where third molars are impacted. The rationale for including impacted third molars in Regression Equation 2 is that third molars are the most commonly impacted teeth.⁵² Although Regression Equation 2 demonstrated lower predictive performance and greater estimation variability than Regression Equation 1, it could still provide clinically useful age estimates. However, a major drawback of Regression Equation 2 is that it interprets age as 12.169 and 13.328 for males and females, respectively, if all third molars are impacted, which could also be one of the reasons

for the higher RMSE. Although the mineralization stage could be evaluated in the case of impacted third molars, when formulating and validating Regression Equation 2, both the eruption and mineralization stages were scored zero because existing studies reported that impacted third molars exhibit slower mineralization and that impaction is a consequence of late maturation.⁵²⁻⁵³

Although the third molar is the only tooth that develops and erupts during the mid-teens to early twenties and is thus widely used as a dental indicator to differentiate juveniles from adults, it shows a large variation in the time of emergence and development.⁵⁴ This study also reported a wide variation in CA for stage D of eruption (12.96-24.62 years) and mineralization (12.96-24.62 years) methods, similar to previous studies.^{17,29} The present study observed a statistically significant correlation between the stages of eruption and mineralization method, consistent with literature reporting a strong correlation between eruption time and dental maturity. Furthermore, literature documented a weak correlation between CA and eruption time, suggesting that the increasing maturity of the teeth and eruption proceed independently.⁵² In the present study, third molars attained all mineralization stages earlier than the corresponding eruption stages. In addition, when all four third molars were considered together, stage D (root completion) was observed in 328 third molars. However, among these, stage D of Olze's eruption (complete emergence) was observed in only 178 third molars, followed by third molars in stage C (87), B (59), and A (04). The mandibular arch has been reported to have a higher tendency for impacted third molars.⁵³ The difference between the number of third molars which achieved stage D of mineralization and eruption was higher for the mandibular arch (74) than for the maxillary arch (59). These findings highlight the importance of clinical correlation with radiological evaluation to determine eruption status, as relying only on radiographic criteria makes the interpretation of impaction difficult. Thus, the third molar alone may not be an ideal developmental marker. Nonetheless, in the absence of other biological age indicators in late teens and early adulthood, third molar maturation and eruption status should be used cautiously alongside other skeletal factors to estimate age.³⁷

One of the main limitations of this study was its retrospective design, as medical records were unavailable for assessment. Another limitation involved determining the mineralization stage when the distal root exhibited a lower developmental level than the mesial root. However, as suggested by the literature, whenever such a situation was encountered, a lower stage was assigned. Furthermore, assessment of the eruption status of the tooth radiographically alone has inherent reliability limitations. Another limitation is the lack of validation of the newly proposed mineralization stages, requiring comparison of the accuracy of this criterion with that of previously established methods across different population groups. The regression equations proposed in this study are population-specific and therefore cannot be generalized universally. Consequently, prospective

studies in different populations with combined clinico-radiological evaluations of third molars are warranted.

CONCLUSION

The mineralization approach offers a more balanced and diagnostically robust method for age estimation, demonstrating higher accuracy and better discriminative power than the eruption method. A statistically significant correlation was observed between the stages of eruption and the mineralization method. This study introduced regression equations that integrate the eruption and mineralization stages of third molars. Both equations effectively estimated age in the study population; however, Equation 1 showed improved predictions based on the MAD and RMSE metrics.

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SUPPLEMENTARY MATERIAL**Table S1.** Relationship of chronological age concerning sex

Sex	n	Mean	Standard Deviation	Minimum	Maximum	p-value
Female	549	17.839	3.322	12.29	24.59	<0.001
Male	446	16.878	3.202	12.08	24.62	
Total	995	17.408	3.302	12.08	24.62	

One-way ANOVA

n-Number of third molars

Table S2. Relationship of chronological age concerning stages of Olze's eruption method and new mineralization method

Method	Stage	n	Mean	Standard Deviation	Minimum	Maximum	p-value
Olze's Eruption Method	A	434	15.204	2.080	12.08	23.89	<0.001
	B	263	17.466	2.613	12.58	24.32	
	C	117	19.636	2.872	12.96	24.62	
	D	181	21.171	2.315	12.96	24.62	
	Total	995	17.408	3.302	12.08	24.62	
New Mineralization Method	A	342	14.729	1.799	12.08	23.89	<0.001
	B	208	16.438	1.973	12.90	23.22	
	C	117	17.494	2.415	14.00	24.32	
	D	328	20.787	2.382	12.96	24.62	
	Total	995	17.408	3.302	12.08	24.62	

One-way ANOVA

n-Number of third molars

Table S3. Relationship of chronological age concerning individual third molars

Tooth	n	Mean	Standard Deviation	Minimum	Maximum	p-value
18	260	17.443	3.245	12.08	24.62	0.871
28	263	17.524	3.322	12.08	24.62	
38	238	17.361	3.338	12.08	24.59	
48	234	17.288	3.321	12.08	24.62	
Total	995	17.408	3.302	12.08	24.62	

One-way ANOVA

n-Number of third molars

Table S4. Relationship of chronological age concerning arches

Arch	n	Mean	Standard Deviation	Minimum	Maximum	p-value
Maxillary	523	17.484	3.281	12.08	24.62	0.449
Mandibular	472	17.325	3.326	12.08	24.62	
Total	995	17.408	3.302	12.08	24.62	

One-way ANOVA

n-Number of third molars

For the maxillary arch, Tooth 18 and 28 were considered together

For the mandibular arch, Tooth 38 and 48 were considered together

Table S5. Stage-wise comparison of chronological age between the two methods concerning individual third molars

Stage	Tooth	Method	n	Mean	Standard Deviation	Minimum	Maximum	p-value
A	18	Olze's Eruption	122	15.498	2.254	12.08	23.89	0.110
		New Mineralization	92	15.015	2.080	12.08	23.89	
	28	Olze's Eruption	116	15.457	2.361	12.08	23.89	0.084
		New Mineralization	94	14.925	1.996	12.08	23.89	
	38	Olze's Eruption	99	14.873	1.747	12.08	20.97	0.087
		New Mineralization	76	14.447	1.443	12.08	18.83	
	48	Olze's Eruption	97	14.868	1.702	12.08	19.43	0.072
		New Mineralization	80	14.436	1.419	12.08	18.83	
B	18	Olze's Eruption	65	17.805	2.811	12.80	24.32	0.029
		New Mineralization	58	16.781	2.242	13.14	22.09	
	28	Olze's Eruption	68	17.558	2.617	13.14	24.32	0.120
		New Mineralization	53	16.864	2.141	13.14	23.22	
	38	Olze's Eruption	65	17.320	2.398	12.58	23.30	0.002
		New Mineralization	51	16.037	1.706	12.90	19.86	
	48	Olze's Eruption	65	17.178	2.627	12.58	24.32	0.005
		New Mineralization	46	15.961	1.492	12.90	19.86	
C	18	Olze's Eruption	20	18.475	2.402	14.71	23.30	0.201
		New Mineralization	28	17.526	2.570	14.16	24.32	
	28	Olze's Eruption	25	18.640	2.461	14.71	23.30	0.164
		New Mineralization	32	17.652	2.748	14.16	24.32	
	38	Olze's Eruption	39	20.388	3.033	12.96	24.59	<0.001
		New Mineralization	29	17.366	2.262	14.00	23.30	
	48	Olze's Eruption	33	20.204	2.894	12.96	24.62	<0.001
		New Mineralization	28	17.415	2.103	14.16	23.30	
D	18	Olze's Eruption	53	21.085	2.416	12.96	24.62	0.265
		New Mineralization	82	20.606	2.437	12.96	24.62	
	28	Olze's Eruption	54	21.407	2.401	12.96	24.62	0.153
		New Mineralization	84	20.802	2.423	12.96	24.62	
	38	Olze's Eruption	35	21.105	2.191	16.17	24.18	0.635
		New Mineralization	82	20.884	2.341	12.96	24.59	
	48	Olze's Eruption	39	21.023	2.225	15.90	24.18	0.716
		New Mineralization	80	20.858	2.358	12.96	24.62	

One-way ANOVA

n-Number of third molars

Table S6. Stage-wise comparison of chronological age between the two methods concerning arches

Stage	Arch	Method	n	Mean	Standard Deviation	Minimum	Maximum	p-value
A	Maxillary	Olze's Eruption	238	15.478	2.302	12.08	23.89	0.018
		New Mineralization	186	14.969	2.033	12.08	23.89	
	Mandibular	Olze's Eruption	196	14.870	1.721	12.08	20.97	0.013
		New Mineralization	156	14.442	1.427	12.08	18.83	
B	Maxillary	Olze's Eruption	133	17.679	2.706	12.80	24.32	0.008
		New Mineralization	111	16.821	2.185	13.14	23.22	
	Mandibular	Olze's Eruption	130	17.249	2.507	12.58	24.32	<0.001
		New Mineralization	97	16.001	1.600	12.90	19.86	
C	Maxillary	Olze's Eruption	45	18.567	2.409	14.71	23.30	0.055
		New Mineralization	60	17.593	2.645	14.16	24.32	
	Mandibular	Olze's Eruption	72	20.304	2.951	12.96	24.62	<0.001
		New Mineralization	57	17.390	2.166	14.00	23.30	
D	Maxillary	Olze's Eruption	107	21.247	2.402	12.96	24.62	0.071
		New Mineralization	166	20.705	2.425	12.96	24.62	
	Mandibular	Olze's Eruption	74	21.062	2.194	15.90	24.18	0.555
		New Mineralization	162	20.871	2.342	12.96	24.62	

One-way ANOVA

n-Number of third molars

For the maxillary arch, Tooth 18 and 28 were considered together

For the mandibular arch, Tooth 38 and 48 were considered together

Table S7. Stage-wise comparison of chronological age between the two methods concerning the sex of an individual

Stage	Sex	Method	n	Mean	Standard Deviation	Minimum	Maximum	p-value
A	Female	Olze's Eruption	237	15.565	2.117	12.29	23.89	0.060
		New Mineralization	182	15.179	2.033	12.29	23.89	
	Male	Olze's Eruption	197	14.769	1.953	12.08	23.22	0.002
		New Mineralization	160	14.217	1.320	12.08	18.71	
B	Female	Olze's Eruption	145	18.118	2.838	12.58	24.32	<0.001
		New Mineralization	116	16.752	2.024	13.14	22.09	
	Male	Olze's Eruption	118	16.666	2.051	12.80	23.22	0.023
		New Mineralization	92	16.043	1.841	12.90	23.22	
C	Female	Olze's Eruption	67	20.098	2.924	12.96	24.59	0.003
		New Mineralization	53	18.444	3.012	14.00	24.32	
	Male	Olze's Eruption	50	19.015	2.708	14.88	24.62	<0.001
		New Mineralization	64	16.708	1.365	14.51	21.19	
D	Female	Olze's Eruption	100	21.311	2.210	12.96	24.59	0.062
		New Mineralization	198	20.759	2.493	12.96	24.59	
	Male	Olze's Eruption	81	20.999	2.442	15.62	24.62	0.602
		New Mineralization	130	20.829	2.210	16.00	24.62	

One-way ANOVA

n-Number of third molars

Table S8. Tooth-wise distribution of third molars in different stages of two methods

Tooth	Olze's Eruption Method Stages	New Mineralization Method Stages					p-value
		A	B	C	D	Total	
Tooth 18	A	89	28	4	1	122	<0.001
	B	3	29	16	17	65	
	C	0	1	8	11	20	
	D	0	0	0	53	53	
	Total	92	58	28	82	260	
Tooth 28	A	89	21	4	2	116	<0.001
	B	5	32	19	12	68	
	C	0	0	8	17	25	
	D	0	0	1	53	54	
	Total	94	53	32	84	263	
Tooth 38	A	73	20	5	1	99	<0.001
	B	3	31	17	14	65	
	C	0	0	6	33	39	
	D	0	0	1	34	35	
	Total	76	51	29	82	238	
Tooth 48	A	75	19	3	0	97	<0.001
	B	5	26	18	16	65	
	C	0	1	6	26	33	
	D	0	0	1	38	39	
	Total	80	46	28	80	234	

Pearson's Chi-Square Test

Table S9. Arch-wise distribution of third molars in different stages of two methods

Arch	Olze's Eruption Method Stages	New Mineralization Method Stages					p-value
		A	B	C	D	Total	
Maxillary	A	178	49	8	3	238	<0.001
	B	8	61	35	29	133	
	C	0	1	16	28	45	
	D	0	0	1	106	107	
	Total	186	111	60	166	523	
Mandibular	A	148	39	8	1	196	<0.001
	B	8	57	35	30	130	
	C	0	1	12	59	72	
	D	0	0	2	72	74	
	Total	156	97	57	162	472	

Pearson's Chi-Square Test

For the maxillary arch, Tooth 18 and 28 were considered together

For the mandibular arch, Tooth 38 and 48 were considered together

Table S10. Sex-Wise distribution of third molars in different stages of two methods

Sex	Olze's Eruption Method Stages	New Mineralization Method Stages					p-value
		A	B	C	D	Total	
Female	A	172	54	11	0	237	<0.001
	B	10	61	32	42	145	
	C	0	1	10	56	67	
	D	0	0	0	100	100	
	Total	182	116	53	198	549	
Male	A	154	34	5	4	197	<0.001
	B	6	57	38	17	118	
	C	0	1	18	31	50	
	D	0	0	3	78	81	
	Total	160	92	64	130	446	

Pearson's Chi-Square Test

Table S11. Tooth-wise Parameters for Formulating Regression Equation 1

Sex		Unstandardized Coefficients		Standardized Coefficients	p-value	95.0% Confidence Interval for B	
		B	Standard Error	Beta		Lower Bound	Upper Bound
Females	(Constant)	12.445	0.400		<0.001	11.652	13.237
	T18 (Olze's eruption)	0.674	0.438	0.239	0.127	-0.194	1.542
	T18 (New Mineralization)	0.055	0.561	0.022	0.922	-1.056	1.166
	T28 (Olze's eruption)	-0.810	0.465	-0.282	0.084	-1.732	0.112
	T28 (New Mineralization)	-0.043	0.611	-0.017	0.945	-1.253	1.168
	T38 (Olze's eruption)	1.328	0.535	0.451	0.014	0.269	2.388
	T38 (New Mineralization)	0.169	0.570	0.066	0.767	-0.960	1.298
	T48 (Olze's eruption)	-0.522	0.540	-0.175	0.336	-1.593	0.548
	T48 (New Mineralization)	1.337	0.544	0.531	0.015	0.259	2.414
Males	(Constant)	11.513	0.347		<0.001	10.823	12.204
	T18 (Olze's eruption)	1.015	0.561	0.395	0.074	-0.102	2.132
	T18 (New Mineralization)	1.227	0.610	0.514	0.048	0.014	2.440
	T28 (Olze's eruption)	-0.586	0.627	-0.232	0.353	-1.834	0.663
	T28 (New Mineralization)	-1.204	0.673	-0.500	0.077	-2.544	0.136
	T38 (Olze's eruption)	-0.331	0.516	-0.118	0.523	-1.358	0.695
	T38 (New Mineralization)	0.967	0.705	0.394	0.174	-0.436	2.369
	T48 (Olze's eruption)	0.532	0.455	0.196	0.246	-0.373	1.437
	T48 (New Mineralization)	0.573	0.646	0.236	0.377	-0.712	1.859

Table S12. Tooth-wise parameters for formulating regression equation 2

Sex		Unstandardized Coefficients		Standardized Coefficients	p-value	95.0% Confidence Interval for B	
		B	Standard Error	Beta		Lower Bound	Upper Bound
	(Constant)	13.328	0.456		<0.001	12.425	14.231
	T18 (Olze's eruption)	0.758	0.444	0.278	0.090	-0.121	1.637
	T18 (New Mineralization)	0.031	0.436	0.012	0.944	-0.832	0.894

Females	T28 (Olze's eruption)	0.048	0.476	0.017	0.920	-0.894	0.990
	T28 (New Mineralization)	0.361	0.442	0.145	0.415	-0.512	1.234
	T38 (Olze's eruption)	1.164	0.503	0.420	0.022	0.170	2.158
	T38 (New Mineralization)	-0.142	0.430	-0.060	0.741	-0.992	0.708
	T48 (Olze's eruption)	-1.385	0.519	-0.499	0.009	-2.412	-0.358
	T48 (New Mineralization)	1.225	0.426	0.529	0.005	0.384	2.067
Males	(Constant)	12.169	0.398		<0.001	11.381	12.957
	T18 (Olze's eruption)	-0.499	0.429	-0.183	0.248	-1.349	0.352
	T18 (New Mineralization)	0.134	0.434	0.053	0.759	-0.726	0.994
	T28 (Olze's eruption)	0.950	0.448	0.350	0.036	0.063	1.837
	T28 (New Mineralization)	1.369	0.451	0.531	0.003	0.476	2.261
	T38 (Olze's eruption)	-1.199	0.445	-0.446	0.008	-2.080	-0.318
	T38 (New Mineralization)	0.804	0.359	0.354	0.027	0.093	1.514
	T48 (Olze's eruption)	0.460	0.449	0.183	0.308	-0.430	1.350
	T48 (New Mineralization)	-0.020	0.378	-0.009	0.958	-0.769	0.729

Table S13. Comparison of findings of our study with other studies concerning the eruption status of third molars

Study	Population	Number of OPGs included	Age (years)	Method	Inference
Olze et al., 2007 ²⁶	German, Japanese, Black South African	2,482 German: 666(144:522) Japanese: 1300 (549:751) Black South African: 519 (410:106)	12-26	Olze et al., 2007	Tooth 18 Mean Age (years) M:F German Stage A: 18.9:16.6; Stage B: 20.8:18.9; Stage C: 22.1:20.3; Stage D: 23.4:24.0 Japanese Stage A: 19.9:19.9; Stage B: 20.9: 20.6; Stage C: 21.5: 21.2; Stage D: 22.4: 22.3; Black South African Stage A: 15.7: 14.8; Stage B: 17.8: 18.0; Stage C: 20.7: 18.7; Stage D: 22.7: 22.0
Olze et al., 2008 ³³	Japanese	1300 (549:751)	14-26	Olze et al., 2007	Range (years) M:F Stage A: 14.6-25.4:14.2-25.8; Stage B: 15.1-26.8:15.2-26.2; Stage C: 15.9-25.9: 16.1-26.3; Stage D: 17.5-26.6: 17.6-26.2 Mean Age (years) M:F Tooth 18: Stage A-19.9:19.9; Stage B- 20.9:20.6; Stage C: 21.5:21.2; Stage D: 22.4:22.3 Tooth 28: Stage A-19.2:20.0; Stage B- 21.0:20.5; Stage C: 21.3:21.2; Stage D: 22.5:22.3 Tooth 38: Stage A-19.2:19.5; Stage B- 21.7:20.9; Stage C: 21.6:21.6; Stage D: 22.5:22.2 Tooth 48: Stage A-18.9:18.4; Stage B- 22.1:21.2; Stage C: 21.3:21.2; Stage D: 22.5:22.2 No statistically significant difference was observed in the study population concerning side or jaw With regard to sex of an individual, statistically significant differences were only observed for tooth 48 in stage A

Olze et al., 2008 ³⁴	German	666 (144:522)	12-26	Olze et al., 2007	Range (years) M:F Stage A: 14.7-26.1:12.0-25.4; Stage B: 14.7-26.6:12.0-25.4; Stage C: 18.7-26.6:13.7-25.4; Stage D: 19.0-26.9:17.4-25.8 Mean Age (years) M:F Tooth 18: Stage A-18.9:16.6; Stage B- 20.8:18.9; Stage C: 22.1:20.3; Stage D: 23.4:24.0 Tooth 28: Stage A-18.8:16.6; Stage B- 21.2:18.6; Stage C: 21.4:20.6; Stage D: 23.7: 22.0 Tooth 38: Stage A-18.6:15.5; Stage B- 22.4:18.9; Stage C: 22.8:20.2; Stage D: 23.6:21.9 Tooth 48: Stage A-19.9:15.5; Stage B- 21.3:18.7; Stage C: 22.7:20.3; Stage D: 23.7:22.0 Depending on the tooth, the males were between 1.7 and 4.4 years older than the females at the different eruption stages Concerning sex, statistically significant differences were observed for tooth 18 at eruption stages A and B, for tooth 28 at stages A, B and D, and for teeth 38 and 48 at all eruption stages.
Olze et al., 2010 ³⁵	First Nations people of Canada	605 (258:347)	11-29	Olze et al., 2007	Range (years) M:F Stage A: 11.7-18.9:11.0-25.5; Stage B: 13.0-26.7:12.4-27.5; Stage C: 13.6-29.8:13.7-27.4; Stage D: 14.5-29.8: 15.9-29.4 Mean Age (years) M:F Tooth 18: Stage A-14.9:15.1; Stage B- 17.8:18.2; Stage C: 21.6:20.2; Stage D: 22.2:22.3 Tooth 28: Stage A-14.6:14.8; Stage B- 18.1:18.4; Stage C: 20.6:20.0; Stage D: 22.8:22.2 Tooth 38: Stage A-12.8:13.3; Stage B- 15.9:17.3; Stage C: 18.1:17.5; Stage D: 21.6:20.6 Tooth 48: Stage A-13.6:13.7; Stage B- 16.2:16.7; Stage C: 17.2:18.5; Stage D: 20.5:21.3 A good correlation was observed between the stages and the chronological ages of the subjects for both sexes.
Scheurer et al., 2011 ³⁶	Central European individuals living in Switzerland	307 (139:168)	17.5 and 18.5 years	Olze et al., 2007	All men and 75% of the women were overestimated by up to 7 years. Number of Third Molars in each stage (M:F) Tooth 18: Stage A- 18:41; Stage B-78:84; Stage C- 14:16; Stage D- 19:13 Tooth 28: Stage A- 14:37; Stage B-83:91; Stage C- 12:15; Stage D- 21:12 Tooth 38: Stage A- 17:27; Stage B-48:70; Stage C- 22:22; Stage D- 11:12 Tooth 48: Stage A- 12:36; Stage B-46:59; Stage C- 22:17; Stage D- 12:14
Karadai et al., 2012 ⁵	Turkish Caucasian origin and Turkish nationality	768 (374:394)	8-22	Olze et al., 2007	Range (years) M:F Stage A: 7.67-19.94:7.54-20.96; Stage B: 12.42-22.49:12.67-22.22; Stage C: 14.17-22.45:13.64-22.02; Stage D: 15.47-22.43:15.89-22.48 Mean Age (years) M:F Tooth 28: Stage A-11.37:11.93; Stage B- 17.27:18.22; Stage C: 17.75: 18.44; Stage D: 20.14:20.02 Tooth 38: Stage A-11.37:11.83; Stage B- 16.79:17.64; Stage C: 17.92 :18.33; Stage D: 20.04:20.27 No statistical difference with respect to eruption was found among the jaws Concerning sex of an individual, Statistically significant were only observed for tooth 28 at eruption stage B

Tuteja et al., 2012 ³⁷	Indian	150 (77:73)	12-26	Olze et al., 2007	Range (years) M:F Stage A: 11.50-15.91; 12.16-23.25; Stage B: 15.16-25.33; 14.66-23.66 years; Stage C: 19.58-25.16; 14.66-25.50; Stage D: 16.58-26.25; 14.66-26.58 Mean Age (years) M:F Tooth 18: Stage A-13.65; 14.55; Stage B- 21.75; 16.93; Stage C: 21.58; 21.56; Stage D: 22.60; 22.60 Tooth 28: Stage A-13.65; 14.55; Stage B- 21.54; 17.58; Stage C: 24.21; 20.41; Stage D: 22.50; 22.67 Tooth 38: Stage A-13.39; 13.14; Stage B- 19.07; 18.12; Stage C: 25.16; 21.98; Stage D: 22.52; 22.41 Tooth 48: Stage A-13.39; 13.29; Stage B- 19.61; 19.06; Stage C: 25.17; 21.61; Stage D: 22.51; 22.58 Although, the females were approximately 1.3-3.6 years ahead of the males at the different eruption stages of the third molar, however this difference was non-significant suggesting that this method of age estimation using third molar cannot be used to differentiate gender.
Raj et al., 2014 ³⁸	Kerala, South India Moderate to High Socioeconomic Status	25 (10:15)	12-26	Olze et al., 2007	Mean estimated age (years) Whole Sample:M:F 16.05; 15.76; 16.25 (no statistically significant difference between mean estimated age and mean CA) Mean Age (years) M:F Tooth 18: Stage A-14.9; 15.1; Stage B- 17.8; 18.2; Stage C: 21.6; 20.2; Stage D: 22.2; 22.3 Tooth 28: Stage A-14.6; 14.8; Stage B- 18.1; 18.4; Stage C: 20.6; 20.0; Stage D: 21.6; 22.2 Tooth 38: Stage A-12.8; 13.3; Stage B- 15.9; 17.3; Stage C: 18.1; 17.5; Stage D: 21.6; 20.6 Tooth 48: Stage A-13.6; 13.7; Stage B- 16.2; 16.7; Stage C: 17.2; 18.5; Stage D: 20.5; 21.3
Akki et al., 2016 ¹	Indian	349 (180:169)	12-25	Olze et al., 2007	Range (years) M:F Stage A: 12-17; 12-18; Stage B: 12-19; 13-23; Stage C: 17-24; 14-24; Stage D: 18-25; 16-25 Mean Age (years) M:F Tooth 18: Stage A-12.8; 14.4; Stage B-16.8; 17.5; Stage C: 19.8; 19.3; Stage D-22.3; 22.6 Tooth 28: Stage A-12.5; 13.4; Stage B-16.7; 16.9; Stage C: 19.4; 21.8; Stage D-22.3; 22.7 Tooth 38: Stage A-12.3; 12.6; Stage B-15.1; 15.9; Stage C: 19.7; 19.5; Stage D-22.2; 22.4 Tooth 48: Stage A-12.1; 12.6; Stage B-14.9; 15.7; Stage C: 19.5; 18.9; Stage D- 22; 22.2
Kutesa et al., 2019 ²⁹	Ugandan	1012	10-20	Olze et al., 2007	Mean Age (years) M:F Tooth 18: Stage A-13.1; 12.6; Stage B- 15.8; 15.2; Stage C: 16.8; 16.6; Stage D: 18.6; 18.0 Tooth 28: Stage A-13.1; 12.6; Stage B- 15.7; 15.3; Stage C: 17.2; 16.5; Stage D: 18.6; 18.0 Tooth 38: Stage A-12.3; 12.0; Stage B- 15.7; 14.8; Stage C: 16.5; 15.8; Stage D: 18.2; 17.5 Tooth 48: Stage A-12.3; 12.0; Stage B- 15.4; 14.9; Stage C: 16.8; 15.2; Stage D: 18.2; 17.6 Range (years) M:F: Stage A: 10-19; 10-19; Stage B: 11-19; 10-19; Stage C: 12-20; 10-19; Stage D: 13-20; 13-20 Significantly higher mean age in males as compared to females with regard to stage D. No significant difference in the mean age between the homologous pairs of third molar teeth.

Widek et al. 2019 ³⁹	Austrian	316; all males	13-25	Olze et al., 2007	Mean Age (years) Tooth 18: Stage A-16.65; Stage B-20.01; Stage C: 20.94; Stage D: 21.67 Tooth 28: Stage A-16.92; Stage B- 19.82; Stage C: 20.28; Stage D: 21.87 Tooth 38: Stage A- 15.49; Stage B-18.94; Stage C: 20.56; Stage D: 21.54 Tooth 48: Stage A-15.50; Stage B- 18.61; Stage C: 19.82; Stage D:21.65 Range (years): Stage A:13.01-24.77; Stage B:13.84-24.98; Stage C:14.92-24.63; Stage D: 16.59-24.96
Shi et al., 2024 ⁴⁰	Croatian	583 (283;300)	10-21.99	Olze et al. 2007	Tooth 38 Range (years) M:F Stage A: 10.20-20.06:10.02-19.50; Stage B: 12.60-20.90:12.48-20.92; Stage C: 15.35-20.08:14.35-20.27; Stage D: 16.48-20.70:13.92-20.97 Mean Age (years) M:F Stage A: 13.64:13.70; Stage B:16.27:16.43; Stage C:17.78:17.39; Stage D: 18.63:18.30
Timme et al., 2024 ⁴¹	German	605 (303;302)	15-26	Olze et al. (2012) (Modification: Stage C: At least half the crown length of the second molar has been reached, the occlusal plane has not been reached.)	Range (years) M:F Stage A: 15.07-25.64:15.04-25.92-; Stage B: 15.27-25.72:15.09-25.93; Stage C: 17.03-25.99:15.43-25.92; Stage D: 17.10-25.95: 16.11-25.98 Mean Age M:F Tooth 38: Stage A: 16.94:17.59; Stage B: 18.91:19.66; Stage C: 20.35:20.97; Stage D: 22.29: 22.60 Tooth 48: Stage A: 16.78:17.49; Stage B: 19.77:19.43; Stage C: 20.21: 21.34; Stage D: 22.08: 22.27
Angela kopoulos et al., 2025 ²	black South African	877 (357;520)	14-24	Gambier et al., 2019	Range (years) M:F Stage 1: 14.01-23.39:14-23.27; Stage 2: 14.07-23.73:14.13-23.99; Stage 3: 14.30-23.85:14.28-23.99 Mean Age (years) M:F Tooth 18: Stage 1-16.19:16.38; Stage 2- 17.97:17.82; Stage 3: 19.59:19.84 Tooth 28: Stage 1-16.17:16.35; Stage 2- 17.88:17.92; Stage 3: 19.57:19.71 Tooth 38: Stage 1-15.74:16.12; Stage 2- 17.66:17.73; Stage 3: 19.29:19.66 Tooth 48: Stage 1-15.72:16.19; Stage 2- 17.88:17.71; Stage 3: 19.15:19.71
De Luca et al., 2025 ⁴²	Lebanese	537 (298:239)	15-24	Gambier et al., 2019	Range (years) M:F Stage 1: 15.02-23.38:15.01-23.29; Stage 2: 15.05-23.57:15.02-23.45; Stage 3: 15.14-23.57:15.34-23.61 Mean Age (years) M:F Tooth 18: Stage 1:17.37:17.51; Stage 2- 19.16:19.54; Stage 3: 21.01:21.11 Tooth 28: Stage 1:17.35:17.57; Stage 2: 19.46:19.21; Stage 3: 20.92:21.14 Tooth 38: Stage 1-16.94:17.41; Stage 2: 19.18:19.15; Stage 3: 20.60:20.48 Tooth 48: Stage 1:17.06:17.26; Stage 2: 19.13:19.34; Stage 3: 20.50:20.58

Vadivel et al., 2025 ³	Srikakulam, Andhra Pradesh, India	400 (200:200) 800 Mandibular Third molars	18-25	Modified Olze's et al. criteria (Modifications: Level A: Retromolar space is much less than the width of the 2nd molar. Level B: Retromolar space is almost equal to that of the 2nd molar.)	Number of Third Molars in each Level M:F Level A: 16:22; Level B: 92:139; Level C: 188:184; Level 4:104:55
Our Study	North-Indian	269 OPGs (123:146) 995 Third molars	12-25	Olze's et al. 2007	Range (years) M:F Stage A: 12.08-23.22; 12.29-23.89; Stage B: 12.80-23.22:12.58-24.32; Stage C: 14.88-24.62:12.96-24.59; Stage D: 15.62-24.62: 12.96-24.59 Mean Age (years) (all four third molars combined) Stage A: 15.204; Stage B: 17.466; Stage C: 19.636; Stage D: 21.171 Tooth-Wise Mean Age (years) Tooth 18: Stage A: 15.498; Stage B: 17.805; Stage C: 18.475; Stage D: 21.085 Tooth 28: Stage A: 15.457; Stage B: 17.558; Stage C: 18.640; Stage D: 21.407 Tooth 38: Stage A: 14.873; Stage B: 17.320; Stage C: 20.388; Stage D: 21.105 Tooth 48: Stage A: 14.868; Stage B: 17.178; Stage C: 20.204; Stage D: 21.023

Table S14: Comparison of Findings of Our Study with Other Studies Concerning the Mineralization Status of Third Molars

Study	Population	Number of OPGs included (M:F)	Age (years)	Method	Inference
Sarnat et al., 2003 ²⁰	Israel	674 Third molars evaluated =2696	7-16	Modified Gat et. al criteria with addition of Stage 0	Age wise number of third molars in each stage 7 years: Stage 3: 0; Stage 4: 0; Stage 5: 0; Stage 6: 0 8 years: Stage 3: 10; Stage 4: 4; Stage 5: 0; Stage 6: 0 9 years: Stage 3: 27; Stage 4: 0; Stage 5: 0; Stage 6: 0 10 years: Stage 3: 112; Stage 4: 4; Stage 5: 1; Stage 6: 0 11 years: Stage 3: 167; Stage 4: 19; Stage 5: 0; Stage 6: 0 12 years: Stage 3: 168; Stage 4: 43; Stage 5: 0; Stage 6: 0 13 years: Stage 3: 134; Stage 4: 95; Stage 5: 6; Stage 6: 0 14 years: Stage 3: 79; Stage 4: 111; Stage 5: 8; Stage 6: 0 15 years: Stage 3: 24; Stage 4: 99; Stage 5: 24; Stage 6: 2
Sisman et al., 2007 ¹⁷	Turkish	900 (380:520)	8-25	Demirjian et al.	Tooth 48 (in case 48 not present than 38 was considered) Mean Age(M:F) Stage D: 12.90:13.60; Stage E: 14.42:15.42; Stage F: 16.90: 16.84; Stage G: 18.08:19.29; Stage H: 22.10:22.66 Range (years): M:F Stage D: 10.00-17.00:8.00-22.00; Stage E: 10.00-18.00:10.00-18.00; Stage F: 12.00-22.00; 14.00-24.00; Stage G: 10.00-22.00: 14.00-24.00; Stage H: 10.00-25.00: 15.00-25.00
Scheurer et al., 2011 ³⁶	Central European individuals living in Switzerland	307 (139:168)	17.5 and 18.5 years	Demirjian and Mincer et al.	Number of Third Molars in each stage (M:F) Tooth 18: Stage D- 4:7; Stage E-9:8; Stage F- 23:30; Stage G- 39:52; Stage H: 29:21 Tooth 28: Stage D- 2:8; Stage E-9:8; Stage F- 19:28; Stage G- 40:43; Stage H: 37:19 Tooth 38: Stage D- 3:14; Stage E-14:13; Stage F- 27:51; Stage G- 61:62; Stage H: 11:8 Tooth 48: Stage D- 4:8; Stage E-12:25; Stage F- 28:44; Stage G- 61:54; Stage H: 10:9 Overestimations was observed in 76% and 82% of the males and females respectively with average differences of 2years between estimated and chronological age

Ajmal et al., 2012 ⁴³	Southern region of the Kingdom of Saudi Arabia.	360 All males	13-23	Demirjian's chart modified by Kasper	Mandibular Third Molars Mean Age (Years) Stage 4: 15.50; Stage 5: 16.52; Stage 6: 17.96; Stage 7: 19.52; Stage 8: 22.02 Range (years) Stage 4: 13-19; Stage 5: 13-20; Stage 6: 15-22; Stage 7: 15-23; Stage 8: 16-24
Karadayi et al., 2012 ⁵	Turkish Caucasian origin and Turkish nationality	768 (374:394)	8-22	Nolla et al.	Mean Age (years) M:F Tooth 28: Stage 6: 14.61:14.49; Stage 7: 16.46:16.40; Stage 8: 17.43:17.87; Stage 9: 19.21:19.23; Stage 10: 20.90:21.02 Tooth 38: Stage 6: 14.89:14.72; Stage 7: 15.93:16.43; Stage 8: 17.03:17.68; Stage 9: 19.06:19.16; Stage 10: 20.91:21.06 Range (Years) M:F Stage 6: 12.45-19.10:11.64-19.81; Stage 7: 12.42-19.94:12.57-21.90; Stage 8: 14.16-22.21:14.56-21.25; Stage 9: 15.47-22.49:16.10-22.22; Stage 10: 19.30-22.45:19.27-22.48 No statistically significant jaw differences was observed
Jung & Cho 2014 ⁴⁴	Korea	2490 (1273:1217) Third molar evaluated: 7081 (3562:3519)	6-24	Modified Demirjian's	Mean Age (years) Stage D: 14.77; Stage E: 16.25; Stage F: 17.58; Stage G: 19.00; Stage H: 21.96 Mean Age (years) M:F Stage D: 14.58:14.87; Stage E: 16.06:16.39; Stage F: 17.17:17.83; Stage G: 18.70:19.35; Stage H: 21.85:22.10
Mathew et al., 2017 ⁴⁵	Kerala, South India	160	9-25	Demirjian et al.	Tooth 38 Mean Age (years) M:F Stage D: 13.2:13.5; Stage E: 13.6:14.6; Stage F: 16.0: 16.0; Stage G: 16.5:17.5; Stage H: 21.2:21.4 Range (years) M:F Stage D: 12-15:12-17; Stage E: 10-17:12-17; Stage F: 14-19:14-19; Stage G: 14-18: 14-22; Stage H: 16-25: 17-25
Firdaus et al., 2018 ⁴⁶	Indonesian	407 (185:222)	8-25	Demirjian et al.	All four Third Molars Mean Age (years) Stage D: 12.26; Stage E: 14.0; Stage F: 16.92; Stage G: 19.61; Stage H: 23.25 Range (years) Stage D: 10.12-16.22; Stage E: 11.32-18.43; Stage F: 14.10-20.94 ; Stage G: 15.77-23.04; Stage H: 20.04-25.73
Branco et al., 2012 ¹³	Portuguese	329 (133:196)	14.0-22.8	Demirjian et al.	Mean Age (Years) M:F Tooth 18: Stage D: 15.1:15.5; Stage E: 15.4:16.3; Stage F: 16.0:17.6; Stage G: 17.6:18.5; Stage H: 19.9:20.0 Tooth 28: Stage D: 15.1:15.3; Stage E: 15.8:16.3; Stage F: 16.0:17.5; Stage G: 17.5:18.9; Stage H: 20.1:20.2 Tooth 38: Stage D: 14.9:15.6; Stage E: 15.6:16.9; Stage F: 17.0:18.1; Stage G: 18.8:19.5; Stage H: 20.3:20.4 Tooth 48: Stage D: 14.7:15.6; Stage E: 15.7:16.2; Stage F: 16.8:18.1; Stage G: 18.5:19.4; Stage H: 20.3:20.5
Widek et al., 2019 ³⁹	Austrian	316; all males	13-25	Demirjian et al., modified by Mincer et al.	Mean Age (years) Tooth 18: Stage D: 13.95; Stage E: 16.03; Stage F: 17.56; Stage G: 20.22; Stage H: 22.39 Tooth 28: Stage D: 13.92; Stage E: 15.81; Stage F: 17.47; Stage G: 20.28; Stage H: 22.36 Tooth 38: Stage D: 14.13; Stage E: 15.8; Stage F: 17.53; Stage G: 20.62; Stage H: 22.15 Tooth 48: Stage D: 14.35; Stage E: 15.89; Stage F: 17.59; Stage G: 20.48; Stage H: 22.61 Range (years) Stage D: 13.01-17.71; Stage E: 13.01-20.96; Stage F: 13.11-23.78; Stage G: 16.19-24.98; Stage H: 17.3-24.96
Ramaswami et al., 2020 ⁴⁷	South Brazilians	1,013 (369:644)	15-23	Demirjian et al.	Mean Age (years) M:F Maxillary Third Molars: Stage D: 16.2:16.2; Stage E: 16.0: 17.2; Stage F: 16.4:17.3; Stage G: 17.0:17.8; Stage H: 20.2:20.5 Mandibular Third Molars: Stage D: 16.0:17.0; Stage E: 16.5:17.8; Stage F: 16.6:17.3; Stage G: 17.3:18.4; Stage H: 20.3:20.7
Arumugam and Doggalli 2020 ¹⁰	Indian	400 (200:200)	14-22	Mincer et al. adapted from Demirjian et al.	All four Third Molars Mean Age (years) M:F Stage D: 14.7:14.8; Stage E: 15.5: 15.8; Stage F: 17:17.1; Stage G: 18.6:18.9; Stage H: 20.7:20.7

Sindi et al., 2023 ⁴⁸	Saudi Arabian	508 (229:279)	6-24	Demirjian's classification modified by Kasper; Moorrees'	All four Third Molars Mean Age ((Range) years Modified Demirjian: Stage D: 13.99 (9-19); Stage E: 15.80 (10-20); Stage F: 16.58 (12-23); Stage G: 17.89 (14-24); Stage H: 20.21 (16-24) Moorrees: Stage 6: 13.20 (9-19); Stage 7:13.70 (9-19); Stage 8: 14.32 (10-21); Stage 9: 15.57(10-20); Stage 10: 17.06 (12-23); Stage 11: 17.06 (13-22); Stage 12: 18.27 (12-24); Stage 13: 19.43 (16-21); Stage 14: 20.42 (17-24)
Sadan et al., 2023 ⁴⁹	Israel	324 (179:145)	8-21	Demirjian et al.	Mean Age (years) Maxillary Third Molars: Stage D: 12.65; Stage E:14.92; Stage F:17.13; Stage G & H:18.28 Mandibular Third Molars: Stage D: 12.90; Stage E:14.77; Stage F:17.49; Stage G & H: 18.23
Shi et al., 2024 ⁴⁰	Croatian	583 (283:300)	10-21.99	Haavikko et al. Demirjian et al.	Tooth 38 Mean Age (years) M:F Haavikko et al. : Crc:13.81:14.16; R1/4: 14.91:15.33; R1/2: 16.45:16.39; R3/4: 16.81:16.69; Rc: 17.50:17.85; Ac: 19.57:19.67 Demirjian et al.: Stage D: 13.74:14.05; Stage E: 15.39:15.56; Stage F: 16.77:16.97; Stage G: 17.51:17.85; Stage H: 19.57:x Range (years) M:F Haavikko et al. : Crc:11.58-16.39:11.76-16.67; R1/4: 10.62-18.08:12.48-18.58; R1/2: 14.11-20.83:12.97-20.92; R3/4: 14.59-19.76:13.85-19.47; Rc: 14.81-19.97:15.06-20.25; Ac: 18.02-20.90:18.09-20.97 Demirjian et al. : Stage D: 11.58-16.39:11.76-16.67; Stage E: 10.62-20.83:12.48-18.85; Stage F: 14.59-19.76:13.85-20.92; Stage G: 14.81-19.97:15.06-20.25; Stage H: 18.02-20.90:x
Sharma et al., 2024 ⁵⁰	Jharkhand, India	720 (370:350)	6-22	Demirjian et al.	All four third molars Mean Age (years) M:F Stage D: 19.00:19.56; Stage E: 19.40:19.63; Stage F: 19.36:20.26; Stage G: 20.45:20.70; Stage H: 21.37:21.69
Present Study	North-Indian	269 (123:146) 995 Third Molars	12-25	Newly proposed method	Mean Age (years) (All four third molars combined) Stage A: 14.729, Stage B: 16.438; Stage C: 17.494; Stage D: 20.787 Tooth-Wise Mean Age (years) Tooth 18: Stage A: 15.015; Stage B: 16.781; Stage C: 17.526; Stage D: 20.606 Tooth 28: Stage A: 14.925; Stage B: 16.864; Stage C: 17.652; Stage D: 20.802 Tooth 38: Stage A: 14.447; Stage B: 16.037; Stage C: 17.366; Stage D: 20.884 Tooth 48: Stage A: 14.436; Stage B: 15.961; Stage C: 17.415; Stage D:20.858 Range (years) M:F Stage A: 12.08-18.71:12.29-23.89, Stage B: 12.90-23.22: 13.14-22.09; Stage C: 14.51-21.19:14.00-24.32; Stage D: 16.00-24.62:12.96-24.59

Is the cervical bone a biological marker for bone age assessment?

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The authors declare that they
have no conflict of interest.

KEYWORDS

Bone Age Assessment,
Cervical Vertebrae,
Cephalometric,
Baccetti's morphological
staging system,
Cameriere's morphometric
method

J Forensic Odontostomatol
2026. Aug; (44): 2-57:66
ISSN :2219-6749
DOI:doi.org/10.5281/zenodo.21849300

ABSTRACT

Age assessment is essential in forensic practice when the chronological age of living individuals is unknown, particularly in legal, civil, migration, and asylum contexts requiring the judicial determination of age-related rights and responsibilities. This study aimed to evaluate the performance of two cervical vertebral methods in a Portuguese reference subadult population (6-23 years), ethnically homogeneous, using the analysis of the fourth cervical vertebra (C4) on lateral cephalometric radiographs. Two approaches were applied: Baccetti's qualitative morphological staging of C2-C4 and Cameriere's morphometric ratio of C4. A total of 528 lateral cephalometric radiographs, balanced by age and sex, were analysed independently by trained observers. Intra- and inter-observer reliability were evaluated using Intraclass Correlation Coefficient (ICC) and weighted Cohen's kappa (κ). Statistical procedures included correlation analyses, multiple linear regression, Classification Trees (CT), and Receiver Operating Characteristic (ROC) curves to assess performance at legally relevant thresholds (12, 14, 16, 18 years).

Baccetti's method explained 75.3% (coefficient of determination $R^2=0.753$) of chronological age variability, with a mean error of approximately two years and high accuracy for thresholds of 12-16 years. Cameriere's ratio showed lower explanatory capacity ($R^2=0.568$) and a mean error of 2.7 years, although its ROC curves demonstrated good discriminatory performance across all age cut-offs. The combination of both methods showed a slight increase in predictive performance ($R^2=0.784$), although this combined approach was not formally evaluated as an independent model. Females reached skeletal maturity earlier, although error rates were similar between sexes. Reliability was excellent across methods.

Both approaches proved applicable for forensic age assessment, offering complementary advantages: Baccetti's method provides broader morphological interpretation, whereas Cameriere's morphometric ratio offers objectivity and reproducibility. These population-specific reference data support the forensic use of cervical vertebral analysis as part of a multimodal age estimation framework.

Highlights

•Cervical vertebrae (C4) analysis provides reliable forensic age assessment

- Baccetti's staging shows higher accuracy than Cameriere's morphometric ratio
- Combined use of both methods may enhance predictive performance
- Excellent intra- and inter-observer agreement supports reproducibility
- Useful reference data for multimodal forensic age assessment

INTRODUCTION

Forensic odontology plays an essential role in the medico-legal sciences through the examination of dental and craniofacial evidence for purposes of human identification, trauma evaluation, and biological profiling. One of its most critical applications concerns age assessment in living individuals, a procedure with ethical implications in criminal, civil, asylum and immigration contexts.¹⁻³ In cases where reliable documentation is lacking, age assessment contributes to determining whether a person has reached legally relevant thresholds, such as 12, 14, 16, or 18 years of age.^{4,5}

In forensic odontology, biological markers-including dental and skeletal indicators-are the most accurate of age assessment methodologies. These markers reflect biological maturation rather than chronological age and are influenced by genetic, environmental, and socioeconomic factors.^{6,7} Dental and skeletal development have long been recognized as the two most reliable systems for assessing biological age.^{1,8} In the Portuguese context, multiple studies have validated dental atlases⁹⁻¹² and dental quantitative models derived from morphometric radiological parameters.¹³⁻¹⁶ These works collectively established the population-specific reference studies in dental age assessment.

Over the last decade, Portuguese forensic odontology has expanded from single-modality assessments toward integrated, multimodal approaches, combining dental and skeletal parameters to improve diagnostic precision. Studies by Pereira and collaborators have contributed substantially to this evolution-ranging from ROC-based model comparisons¹⁷, pulp/tooth ratio analysis via CBCT¹⁸, and combined dental scoring systems¹⁹, to multidisciplinary research on bone and dental age assessment in judicial contexts.²⁰ Moreover, recent investigations have incorporated machine learning and deep learning models for automated dental evaluation^{21,22}, reinforcing the transition

toward AI-assisted forensic decision-support systems.²²

Despite these technological advances, manual multimodal assessment-based on measurable and morphological criteria-remains essential to establish validated reference datasets and ensure methodological transparency before AI implementation.

Two manual methods for assessing skeletal maturation in lateral cephalometric radiographs are Cameriere's morphometric approach, which quantifies dimensional ratios of the fourth cervical vertebra (C₄), and Baccetti's morphological Cervical Vertebral Maturation (CVM) method, which classifies the developmental stages of the second to fourth cervical vertebrae (C₂-C₄) based on shape and contour characteristics.²³⁻²⁵

Originally developed for orthodontic growth assessment, both methods have since been adapted and validated for forensic age assessment, offering possible alternatives to hand-wrist radiographs while minimizing radiation exposure-an ethical requirement when evaluating living individuals.²⁰

In this context, the present study applies these cervical vertebral indicators within a manual multimodal biological marker framework, exploring their medico-legal applicability in a Portuguese subadult population aged 6 to 23 years. The study aims to establish population-specific reference data and to assess the accuracy of the cervical vertebral biological indicator for age assessment in the judicial context, integrating its performance with established dental and hand-bone methods within a multimodal assessment framework.

OBJECTIVES

Main Objectives

The primary objective of this study was to evaluate the performance of the fourth cervical vertebra (C₄) in a Portuguese child and subadult population aged 6-23 years, using two independent methods applied to lateral cephalometric radiographs:

- (1) Baccetti's morphological staging system, based on qualitative assessment of vertebral shape, and
- (2) Cameriere's morphometric method, which quantifies the ratio between the anterior and posterior heights of C₄.

Specifically, the study aimed to:

1. Evaluate the correlation between chronological age and bone maturation estimated by each method.
2. Assess the accuracy of both methods in classifying individuals below legally relevant age thresholds (12, 14, 16, and 18 years).

Secondary Objectives

Secondary objectives included examining sex-related differences in the performance of both methods, in order to determine whether male and female developmental patterns significantly influence vertebral maturation scores and bone age assessment.

Final Objective

Finally, this study sought to verify the accuracy and forensic applicability of Baccetti's morphological and Cameriere's morphometric methods for assessing biological age from the fourth cervical vertebra (C₄), contributing population-specific reference data to support their integration into forensic odontology practice.

MATERIALS AND METHODS

Experimental Design

This study was conducted jointly at the Department of Stomatology of the Local Health Unit of Santa Maria Hospital (ULS Santa Maria) and the Faculty of Dental Medicine, University of Lisbon (FMDUL). The target population comprised patients who received dental or stomatological care at these institutions and whose clinical records included a lateral cephalometric radiograph as part of routine diagnostic documentation.

Ethical approval for the study was obtained from the Ethics Committee of ULS Santa Maria (approval no. 217/23). The study protocol and all supporting documents were reviewed and approved, including authorization from the Director of the Department of Stomatology and the Clinic Director of FMDUL. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Sample and Data Collection

The dataset consisted of 528 lateral cephalometric radiographs from healthy Portuguese individuals aged 6-23 years. Radiographs were sourced from the archives of the ULS Santa Maria Stomatology Department

and FMDUL. These radiographs were originally acquired for clinical diagnostic purposes (orthodontic assessment), and not for forensic use. Only individuals without known growth disorders or craniofacial abnormalities were included, representing a generally healthy population. Only images of diagnostic quality, free from distortions or artifacts, were included to ensure measurement precision.

To maintain statistical validity, radiographs were selected to achieve balanced distribution by age and sex across the sample. All data were retrieved from existing clinical records with no direct patient contact, and full anonymisation was ensured. Case identifiers were removed after linkage to project codes, and all variables (sex, birth date, examination date and age at the date of examination) were stored in a structured Excel® file designed to maintain data traceability and confidentiality.

For intra- and inter-observer reliability, a randomly selected subsample of 114 radiographs (more than 20%) was re-evaluated.

Baccetti's Method

Baccetti's method is a non-metric, morphological approach that evaluates the maturation of the second, third, and fourth cervical vertebrae (C₂-C₄) using lateral cephalometric radiographs.

It assesses two main features:

1. The presence or absence of concavity along the inferior border of each vertebral body.
2. The shape transformation of the vertebral bodies, which progress from trapezoidal to rectangular forms as skeletal maturation advances.

Skeletal development follows a predictable pattern characterized by increasing concavity of the inferior margins and a gradual change in the vertebral height-to-width ratio.²⁶ Baccetti's classification defines six cervical maturation stages (CVM I-VI), ranging from flat inferior borders and trapezoidal vertebrae (CVM I) to pronounced concavities and vertically rectangular shapes (CVM VI). These stages provide a morphological scale of skeletal maturity that can be consistently identified on cephalometric radiographs.

Cameriere's Method

Cameriere's method adopts a morphometric quantitative approach applied specifically to the fourth cervical vertebra (C₄). The method

involves measuring the anterior (A) and posterior (B) heights of the C4 vertebral body and computing the Vba ratio (A/B). This ratio reflects the degree of vertebral body development: younger individuals typically show trapezoidal shapes ($B > A$ and, consequently, $Vba < 1$), while progressive skeletal maturation leads to a rectangular configuration ($A \approx B$ and, therefore, $Vba \approx 1$)². Hence, Vba increases towards 1 with age. Measurements were obtained using ImageJ® software (National Institutes of Health, Bethesda, MD, USA) for accuracy and reproducibility.

Special attention was given to ensure that the anterior height was measured along the straight segment of the anterior surface, excluding any curvature near the superior border to avoid measurement bias. Only radiographs showing clear visualization of the C4 vertebral body were analyzed. This method is indicated for subadult individuals without developmental or traumatic anomalies affecting the cervical region.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics, version 30.0 (IBM Corp., Armonk, NY, USA). The significance level was set at $\alpha = 0.05$, with results considered significant at $p < 0.05$. Confidence intervals (CI) will be displayed using 95% confidence.

Reliability Analysis

Intra- and inter-observer reliability were evaluated using the Intraclass Correlation Coefficient (ICC) (two-way mixed-effects, absolute-agreement model) and weighted Cohen's kappa (κ). A random subsample of at least 20% was re-measured after two weeks for intra-observer consistency.

Descriptive and Inferential Statistics

Descriptive statistics (mean, standard deviation (SD), range) were calculated for all variables. Correlation and multiple linear regression models were developed to assess the relationship between chronological age and morphological (Baccetti) and morphometric (Cameriere) parameters of the C4 vertebra. Predictive performance was expressed as the coefficient of determination (R^2), the mean error of estimate (MEE), the standard error of

estimate (SEE), and the mean absolute error of estimate (MAE).

Classification Accuracy

To test the discriminatory ability of both methods for legally relevant age thresholds (12, 14, 16, and 18 years):

1. Classification Tree (CT) analyses were performed with 10-fold cross-validation.
2. Receiver Operating Characteristic (ROC) curves were generated, and the Area Under the Curve (AUC) was computed to quantify classification accuracy and diagnostic performance.

RESULTS

Reliability Analysis

Baccetti's Method

To assess reliability of vertebral staging, a random subsample of at least 20% of the dataset (114 cephalometric radiographs) was re-evaluated:

- Intra-observer agreement: weighted Cohen's $\kappa = 0.914 \rightarrow$ almost perfect concordance.
- Inter-observer agreement: weighted Cohen's $\kappa = 0.804 \rightarrow$ substantial/good concordance.

Although the two-week interval may be considered relatively short, it was deemed sufficient to minimise recall bias given the sample size and randomisation of cases.

These findings indicate high repeatability, particularly within-observer (Supplementary Table S1).

Cameriere's Method

Using the same $n = 114$ subsample, replicability of Vba was quantified with ICC (two-way mixed, absolute agreement) Table 1:

- Intra-observer ICC = 0.999 $\rightarrow$ excellent.
- Inter-observer ICC = 0.946 $\rightarrow$ excellent.

Table 1. Intra- and inter-observer concordance coefficients for Cameriere's Method

Type of validation	ICC	Interpretation
Intra-observer	0.999	Excellent
Inter-observer	0.946	Excellent

Thus, Cameriere's linear measurements and the derived Vba (A/B) ratio show very high measurement reliability).

Sample Description

All 528 radiographs from subjects aged 6–23 years were classifiable: 267 males (50.6%), 261 females

(49.4%) (Supplementary Table S2; Figure S1). The sampling strategy achieved an approximately uniform distribution per age and sex, with small shortfalls at 6, 22, and 23 years due to limited archives.

Baccetti's Method

Distribution of Cervical Maturation Stages

Stage frequencies were: CS5 = 31.4% (n = 166), CS4 = 18.4% (n = 97); least frequent CS1 (n = 56) and CS6 (n = 55). Mean chronological age increased monotonically from CS1: 8.05 ± 1.66 years (throughout the article, $\pm$ will be used to represent mean $\pm$ standard deviation) to CS6: 20.32 ± 2.56 years (range minima/maxima detailed in Supplementary Table S3). This pattern confirms the expected temporal association between vertebral morphology and maturation.

Linear Regression Model for Age Estimation

A dummy-coded regression using CS2–CS6 as predictors yielded $R^2 = 0.753$ and SEE = 2.55 years ($p < 0.001$ for model and for each coefficient):

Estimated Age (years) = $8.05 + 1.80 \times \text{CS2} + 3.37 \times \text{CS3} + 7.75 \times \text{CS4} + 11.19 \times \text{CS5} + 12.26 \times \text{CS6}$.

A statistically significant linear relationship between chronological age and CVM stages was observed ($p < 0.001$).

The model was internally validated using cross-validation procedures applied in classification analyses.

Sex-specific models showed strong fits (Males: $R^2 = 0.785$, SEE ≈ 3.19 years; Females: $R^2 = 0.726$, SEE ≈ 2.68 years) (Supplementary Tables S4–S5).

Estimation Error

For the full sample (N = 528): MEE = 0.00 years (median error of 0.06 years; SD = 2.54 years) and MAE = 2.04 ± 1.51 years (median absolute error of 1.78 years).

The mean estimation error (MEE) of 0.00 years reflects the symmetrical distribution of positive and negative residuals rather than perfect prediction accuracy.

By sex:

- Males: MEE = -0.20 years (SD = 2.41); MAE = 1.92 ± 1.46 years.
- Females: MEE = +0.20 years (SD = 2.66); MAE = 2.17 ± 1.56 years.

No systematic over/under-estimation was observed (Supplementary Tables S4–S5).

Classification at Legal Age Thresholds

Performance at thresholds 12, 14, 16, and 18 years is summarized below Table 2 (full CT/ROC in Supplementary Tables S6–S9; Supplementary Figures S3–S4):

Table 2. Legal Age Thresholds 12, 14, 16 and 18 years old by Baccetti's Method

Legal age (years)	Accuracy (%)	Sensitivity (%)	Specificity (%)	AUC (95% CI)
12 years	88.8%	86.5%	93.6%	0.949 (0.932–0.966)
14 years	93.0%	97.6%	87.1%	0.979 (0.970–0.988)
16 years	87.5%	83.0%	91.1%	0.934 (0.913–0.955)
18 years	83.0%	87.4%	80.7%	0.889 (0.862–0.916)

Sex-stratified results remained high (e.g., 14 years males: accuracy 94.8%, AUC 0.985; 12 years females: accuracy 89.7%, AUC 0.951), with a modest drop at 18 years, more pronounced in females (Supplementary Table S7). However, it should be noted that the classification is carried out in the same way for both sexes in all age groups (DT presented in Supplementary Figures S3–S4).

Cameriere's Method

Sample Vba Distribution

In the N = 528 dataset, Vba (A/B) ratio for C4 ranged 0.54–1.09; mean 0.83 ± 0.09 overall (Males 0.82 ± 0.09 ; Females 0.83 ± 0.08). The ratio increased with age (children < adolescents < at least 16 years), reflecting the transition from trapezoidal to rectangular vertebral body morphology (Supplementary Table S8).

Linear Regression for Age Estimation

A model including A, B, and Vba showed $R^2 = 0.568$; SEE = 3.37 years ($p < 0.001$). The simplified model (A + Vba) performed identically ($R^2 = 0.568$; SEE = 3.37 years), confirming Vba as the dominant predictor.

Pooled equation: Age (years) = $-18.70 + 0.040 \times A + 39.04 \times V_{ba}$ (both coefficients $p < 0.001$).

Sex-specific fits: $R^2 = 0.621$ (males) vs 0.533 (females); SEE ≈ 3.37 years in both (Supplementary Tables S9–S10).

Estimation Error

Overall: MEE = 0.00 years (median error of -0.005 years; SD = 3.36 years) and MAE = 2.67 ± 2.05 years (median absolute error of 2.33 years).

By sex:

- Males: EE = +0.29 years; MAE = 2.84 ± 2.16 years.
- Females: EE = +0.09 years; MAE = 2.72 ± 2.20 years (Supplementary Tables S9–S10).

Classification at Legal Age Thresholds

Performance at thresholds 12, 14, 16, and 18 years is summarized below (table 3). CT and ROC analyses (Supplementary Tables S11–S14; Supplementary Figures S5–S8):

Table 3. Legal Age Thresholds 12, 14, 16 and 18 years old by Cameriere's Method.

Threshold	Accuracy	Sensitivity	Specificity	AUC (95% CI)
12 years	82.6%	89.3%	68.8%	0.886 (0.857–0.915)
14 years	85.6%	90.8%	78.9%	0.921 (0.899–0.944)
16 years	80.5%	89.8%	73.0%	0.904 (0.879–0.928)
18 years	83.5%	73.1%	88.7%	0.899 (0.874–0.925)

Performance peaked at 14 years (AUC 0.921). At 18 years, specificity was high (88.7%) but sensitivity declined (73.1%), indicating a conservative classification above majority.

Sex-stratified results clustered accuracy 80.5–85.6% with AUC ≥ 0.889 up to 16 years; at 18 years, specificity stayed very high (>92%) while sensitivity dropped (notably males 60.9%) (Supplementary Table S15). On the other hand, it should be observed that the classification is carried out using different classification thresholds in the two sexes (DT presented in Supplementary Figures S9–S12).

Comparative Performance of Baccetti's and Cameriere's Methods at Legal Age Thresholds

At 12 years, Baccetti outperformed Cameriere on overall accuracy (88.8% vs 82.6%) and

specificity (93.6% vs 68.8%), while Cameriere showed slightly higher sensitivity (89.3% vs 86.5%).

At 14 years, Baccetti achieved 93.0% accuracy and AUC 0.979 vs 85.6% and 0.921 for Cameriere.

At 16 years, Cameriere had higher sensitivity (89.8% vs 83.0%), but Baccetti retained higher specificity (91.1% vs 73.0%) and overall accuracy (87.5% vs 80.5%).

At 18 years, overall accuracy was comparable (83.0% vs 83.5%). Baccetti favored sensitivity (87.4%), whereas Cameriere favored specificity (88.7%). AUCs were similar (0.889 vs 0.899).

(Full comparison in Supplementary Table S16)

Resume from the main results:

- Reliability was high/excellent for both methods (κ up to 0.914; ICC up to 0.999).
- Predictive strength for age was higher with Baccetti ($R^2 \approx 0.75$; SEE ≈ 2.55 years) than with Cameriere ($R^2 \approx 0.57$; SEE ≈ 3.37 years).
- Classification near legal thresholds was excellent at 12–16 years for both methods; Baccetti consistently delivered higher accuracy/AUC up to 16 years.
- At 18 years, methods were comparable overall, with complementary profiles (Baccetti exhibits greater sensitivity; Cameriere exhibits greater specificity).
- Sex effects were modest; patterns were broadly consistent between males and females.

DISCUSSION

Reliability analysis

Both approaches showed high reproducibility. For Baccetti's CVM staging, weighted Cohen's κ reached 0.914 (intra-observer; almost perfect) and 0.804 (inter-observer; substantial), in line with reports that CVM can be reliably scored after calibration and with standardized viewing conditions^{10,25,27}. For Cameriere's morphometric method, ICCs were 0.999 (intra-observer) and 0.946 (inter-observer), indicating excellent repeatability and consistent with published recommendations from AGFAD (Schmeling et al., 2008) and IOFOS (International Organization for Forensic Odonto-Stomatology) guidelines for forensic age estimation in living individuals.^{1,28,29} As expected, metric measurements (A, B, A/B) tend to attenuate subjective variability relative to visual staging, a

trend also observed in national validations using vertebral and dental indices.^{5,13,19}

Importantly, all radiographs used in this study were originally acquired for clinical diagnostic purposes, avoiding additional radiation exposure. This is particularly relevant in forensic age estimation of living individuals, where ethical guidelines recommend the use of existing medical images whenever possible.^{1,4}

Baccetti's method

Sample characteristics

The dataset (N = 528; 267 males, 261 females; 6–23 years) was broadly balanced by age and sex, with only modest under-representation at 6, 22 and 23 years—sufficient for robust modelling and sex-stratified analyses. This mirrors prior Portuguese cohorts and facilitates comparability across studies.^{9,10,20}

Stage distribution and biological plausibility

Chronological age increased monotonically from CS1 (8.05 ± 1.66 years) to CS6 (20.32 ± 2.56 years), reproducing the expected maturation trajectory described in classical skeletal/dental research^{32,33,34} and recent CVM literature^{25,35}. Wider age dispersion at advanced stages (CS5–CS6) reflects known interindividual variability around and after the pubertal growth spurt. Females presented lower mean ages per stage, consistent with sexual dimorphism in skeletal timing.^{19,36}

Regression performance

CVM stages predicted age with $R^2 = 0.753$; SEE = 2.55 years ($p < 0.001$), aligning with Portuguese/European validations that report strong effect sizes for CVM.^{10,15,22,25} Sex-specific models preserved high fit (males $R^2 = 0.785$, females $R^2 = 0.726$). These findings support CVM as a dependable predictor for forensic purposes when scored under standardized criteria and with examiner training, as advocated by AGFAD.¹

The predictive performance observed in this study ($R^2 \approx 0.75$) is consistent with previous studies in European populations, which report values ranging between 0.70 and 0.80, supporting the robustness of this method across different cohorts.^{25,35}

Classification at legal thresholds

CVM showed excellent discrimination between 12–16 years (accuracy $\geq 87\%$; AUC 0.934–0.979), peaking at 14 years (accuracy 93.0%, sensitivity

97.6%, AUC 0.979). As widely reported, performance declined modestly at 18 years (accuracy 83.0%, AUC 0.889) due to increased biological heterogeneity near adulthood^{32,33}. Sex-stratified metrics remained high, with slightly different sensitivity/specificity balances across sexes—patterns consistent with earlier maturation in females.

Cameriere's method

Morphometric trends

The C4 Vba ratio rose progressively with age (overall mean 0.83 ± 0.09 ; males 0.82 ± 0.09 , females 0.83 ± 0.08), corroborating Cameriere's original observations and subsequent validations that the vertebral body evolves from trapezoidal to rectangular geometry during adolescence.^{2,26}

Regression performance

Models including A, B, and Vba achieved $R^2 = 0.568$; SEE = 3.37 years ($p < 0.001$). A reduced model (A + Vba) performed equivalently, confirming Vba as the dominant predictor. Sex-specific fits were $R^2 = 0.621$ (males) and 0.533 (females). Although precision was lower than CVM, residuals and MAE = 2.67 ± 2.05 years remained within accepted forensic ranges for radiographic indicators^{14,18,37}. Similar levels of performance ($R^2 \approx 0.50$ –0.60; SEE ≈ 3 –4 years) have been reported in previous validations of Cameriere's method, indicating that the present results fall within the expected range for morphometric skeletal indicators^{2,4,13}.

Classification at legal thresholds

Accuracy ranged 80.5–85.6% with AUC 0.886–0.921 for 12–16 years, with highest performance at 14 years (AUC 0.921). At 18 years, overall accuracy was 83.5%, with high specificity (88.7%) and lower sensitivity (73.1%), mirroring the general decline in discriminative power near majority.^{26–33} Sex-stratified analyses confirmed consistent patterns, with females showing very high specificity at 18 years but reduced sensitivity.

Comparative appraisal of methods

Across models, adjusted R^2 spanned ~ 0.53 –0.75. CVM provided higher predictive precision ($R^2 = 0.753$; SEE = 2.55 years; MAE ≈ 2.0 years) and stronger AUC/accuracy at 12–16 years. Cameriere offered greater measurement

reproducibility and transparent quantification, with MAE $\approx$ 2.7 years, making it a robust, objective complement. At 18 years, both methods converged in overall accuracy (approximately 83%) but with distinct profiles: CVM favored sensitivity, Cameriere favored specificity. Such complementarity supports combined use to balance false-positive/false-negative risks at legally critical thresholds.

4,22,34-37

Where ethical considerations apply-e.g., protection of minors in asylum/justice contexts-approaches that minimize over-classification as adult may be preferred, consistent with EU guidance.^{38,39}

Reference value of the study

From a practical perspective, lateral cephalometric radiographs offer an important advantage over hand-wrist radiographs, as they involve relatively low radiation doses and are frequently available in routine orthodontic records. This supports their use in forensic contexts without the need for additional imaging, in line with radiation protection principles.^{1,20}

This work provides population-specific reference data for Portugal (6-23 years; N = 528) for both CVM stages and C4 morphometrics, extending earlier national studies.^{9,10,13,15,22} The dataset contributes to the development of regression and classification models that are broadly consistent with AGFAD and IOFOS recommendations, although further external validation is required^{40,41}, emphasizing transparency, proportionality, and accountability in medico-legal evaluations. Methodological limitations inherent to 2D cephalometry (e.g., projection geometry) underline the value of standardized acquisition and calibration (e.g., ISO/IEC 23053 frameworks) to reduce geometric bias.

CONCLUSION

Conclusions related to the main objectives

Group I – Baccetti's Method

The application of Baccetti's Cervical Vertebral Maturation (CVM) method yielded consistent and statistically robust results:

- There was no significant difference between chronological and estimated biological ages, confirming the method's accuracy for the studied population.
- The regression model explained approximately 75% of the variability in chronological age

(Adjusted $R^2 \approx 0.75$) with a mean absolute error of almost 2.0 years, demonstrating high predictive precision.

- The model achieved excellent classification performance below the 12-, 14-, and 16-year legal thresholds (AUC > 0.93), indicating strong discriminative power for forensic purposes.
- At the 18-year threshold, classification accuracy and AUC decreased, reflecting the reduced sensitivity of morphological indicators once skeletal maturation nears completion. Consequently, the method should not be used as a stand-alone criterion for adulthood determination.

Group II – Cameriere's Method

For the Cameriere's morphometric model, the following conclusions were established:

- A statistically significant correlation was observed between chronological and estimated biological ages. The regression model explained approximately 56% of the chronological age variability (Adjusted $R^2 \approx 0.56$) with a mean absolute error of approximately 2.7 years, indicating good, but lower predictive accuracy compared to Baccetti's method.
- The method showed satisfactory classification capacity below the 12-, 14-, and 16-year thresholds (AUC range: 0.886-0.921).
- Performance declined at the 18-year threshold, consistent with the increased biological variability typical of late adolescence and the transition to skeletal maturity.

Conclusions related to the secondary objectives

Group I – Baccetti's Method

- No statistically significant sex differences were detected in biological age estimation using the CVM method.
- Nonetheless, the analysis confirmed the biological pattern of earlier skeletal maturation in females, a well-documented form of sexual dimorphism.

Group II – Cameriere's Method

- Similarly, no statistically significant sex differences were observed in Cameriere's method.
- The regression model explained a slightly higher proportion of chronological age variability in males, whereas females reached

skeletal maturity earlier, confirming known developmental trends.

Conclusions related to the final objective

Both Baccetti's morphological staging and Cameriere's morphometric model exhibited strong correlations with chronological age and demonstrated practical applicability in forensic age estimation.

When directly compared, Baccetti's method provided greater precision and predictive power, while Cameriere's method offered enhanced objectivity and reproducibility, reducing observer-related variability.

From a forensic standpoint, the two techniques are complementary rather than competing:

- CVM captures the morphological progression of skeletal maturation;
- Cameriere's ratio quantifies it objectively.

Their combined use may improve diagnostic confidence, minimize estimation bias, and align forensic evaluations with AGFAD and IOFOS recommendations for multi-parameter age estimation in living individuals.

This study also provides population-specific reference standards for Portuguese adolescents and young adults, contributing to the establishment of nationally validated scoring systems and supporting the international effort to harmonize forensic age assessment protocols under ethical, transparent, and standardized frameworks.

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FUNDINGS

This work is partially financed by national funds through FCT – Fundação para a Ciência e a Tecnologia under the project UID/00006/2025. This work is supported by the Dental Forensic Sciences Research Group (FORENSEMED) integrated part of the Oral and Biomedical Sciences Research Unit (UICOB) at Faculdade de Medicina Dentária da Universidade de Lisboa. This work is partially financed for the project 2024.07444.IACDC, supported by measure 'RE-Co5-io8.Mo4 – "RE-Co5-io8.Mo4 – "Apoiar o lançamento de um programa de projetos de I&D orientado para o desenvolvimento e implementação de sistemas avançados de cibersegurança, inteligência artificial e ciência de dados na administração pública, bem como de um programa de capacitação científica", of the Recovery and Resilience Plan (Plano de Recuperação e Resiliência – PRR), under the funding contract signed between the Recovering Portugal Mission Structure (EMRP)) and the Fundação para a Ciência e a Tecnologia I.P. (FCT), as intermediary beneficiary. <https://doi.org/10.54499/2024.07444.IACDC> This work is related to the CA24147 - Artificial Intelligence Driven Dental AgeEstimation Network (AID-AgE).

ACKNOWLEDGMENTS

The authors express their gratitude for the valuable collaboration of researchers Inês Marques, Beatriz Serra and Ana Rodrigues.

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SUPPLEMENTARY MATERIAL

Table S1. Intra- and inter-observer concordance coefficients for Baccetti's method

Type of validation	Cohen's Kappa (κ)	Interpretation
Intra-observer	0.914	Almost perfect
Inter-observer	0.804	Substantial/Good

Table S2: Intra- and inter-observer ICC coefficients for Vba of Cameriere's method

Type of validation	ICC	Interpretation
Intra-observer	0.999	Excellent
Inter-observer	0.946	Excellent

Table S3. Table of the sample distribution by age and sex

		Sex		
		Male	Femal	Tota
Age	6	15	7	22
	7	15	16	31
	8	15	15	30
	9	15	15	30
	10	15	15	30
	11	15	15	30
	12	15	15	30
	13	15	15	30
	14	16	15	31
	15	14	15	29
	16	15	15	30
	17	15	15	30
	18	15	15	30
	19	15	14	29
	20	15	16	31
	21	15	15	30
	22	15	13	28
	23	12	15	27
Total		267	261	528

Table S4. Minimum, maximum, mean, and standard deviation of age for each Baccetti stage

Baccetti stage	N subjects	Minimum age (years)	Mean (years)	Standard deviation	Maximum age (years)
1	56	6.15	8.05	1.66	13.94
2	80	6.24	9.85	2.04	13.32
3	74	7.26	11.42	2.14	18.36
4	97	9.09	15.81	3.36	23.75
5	166	14.16	19.25	2.64	23.95
6	55	14.23	20.32	2.56	23.87

Table S5. Regression model summary and ANOVA for chronological age prediction based on Baccetti's cervical vertebral maturation stages

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate			
1	.868 ^a	0.753	0.751	2.55400			
a. Predictors: (Constant), Baccetti_classification=Stage 2 Baccetti's method, Baccetti_classification=Stage 3 Baccetti's method, Baccetti_classification=Stage 4 Baccetti's method, Baccetti_classification=Stage 5 Baccetti's method, Baccetti_classification=Stage 6 Baccetti's method							
b. Dependent Variable: Age							
ANOVA ^a							
Model		Sum of Squares	df	Mean Square	F	Sig.	
1	Regression	10386.260	5	2077.252	318.455	<.001 ^b	
	Residual	3404.956	522	6.523			
	Total	13791.216	527				

a. Dependent Variable: Age
 b. Predictors: (Constant),
 Baccetti_classification=Stage 2 Baccetti's method,
 Baccetti_classification=Stage 3 Baccetti's method,
 Baccetti_classification=Stage 4 Baccetti's method,
 Baccetti_classification=Stage 5 Baccetti's method,
 Baccetti_classification=Stage 6 Baccetti's method

Table S6. Model summary and Statistic by sex

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
Male	.885 ^a	0.785	0.780	3.19
Female	.852 ^a	0.726	0.721	2.68

a. Predictors: (Constant),
 Baccetti_classification=Stage 2 Baccetti's method,
 Baccetti_classification=Stage 3 Baccetti's method,
 Baccetti_classification=Stage 4 Baccetti's method,
 Baccetti_classification=Stage 5 Baccetti's method,
 Baccetti_classification=Stage 6 Baccetti's method
 b. Dependent Variable: Age

Table S7. Classification results (confusion matrix and AUC) of the CT model for the 12-year threshold based on Baccetti's CVM

	Observed	Predicted			Area Under the ROC Curve			
		No	Yes	Percent Correct	Area	Std. Error ^a	Asymptotic Sig. ^b	Asymptotic 95% Confidence Interval
12 years	No	162	11	93.6%	0.949	0.009	0.000	Lower bound 0.932
	Yes	48	307	86.5%				Upper Bound 0.966
	Overall Percentage	39.8%	60.2%	88.8%				
14 years	No	203	30	87.1%	0.979	0.005	0.000	Lower bound 0.970
	Yes	7	288	97.6%				Upper Bound 0.988
	Overall Percentage	39.8%	60.2%	93.0%				
16 years	No	267	26	91.1%	0.934	0.011	0.000	Lower bound 0.913
	Yes	40	195	83.0%				Upper Bound 0.955
	Overall Percentage	58.1%	41.9%	87.5%				
18 years	No	285	68	80.7%	0.889	0.014	0.000	Lower bound 0.862
	Yes	22	153	87.4%				Upper Bound 0.916
	Overall Percentage	58.1%	41.9%	83.0%				

Table S8. Accuracy, sensitivity, specificity, and AUC for legal age thresholds by sex (Baccetti's method)

Legal age (years)	Sex	Accuracy (%)	Sensitivity (%)	Specificity (%)	AUC
12	F	89.7	89.3	90.4	0.95
	M	88.0	83.6	96.7	0.94
14	F	91.2	98.6	81.4	0.97
	M	94.8	96.6	92.5	0.98
16	F	85.1	80.5	88.8	0.91
	M	87.3	100.0	77.3	0.95
18	F	80.5	84.1	78.6	0.86
	M	85.4	90.8	82.8	0.91

Table S9. Mean, standard deviation, minimum and maximum of the Vba by sex (Cameriere's method)

	N	Mean	SD	Min	Max
Males	267	0.82	0.09	0.54	1.00
Females	261	0.83	0.08	0.60	1.09
Total sample	528	0.83	0.08	0.54	1.09

Table S10. Regression results of Cameriere's method for age estimation.

Variables Entered/Removed ^a				
Model	Variables Entered	Variables Removed	Method	
1	Ratio Vba, Posterior		Enter	
2		Posterior side	Backward (criterion: Probability of F-to-remove >= ,100).	
a. Dependent Variable: Age				
b. All requested variables entered.				
Model Summary ^c				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.754 ^a	0.568	0.566	3.37193
2	.753 ^b	0.568	0.566	3.36966
a. Predictors: (Constant), Ratio Vba, Posterior side, Anterior side				
b. Predictors: (Constant), Ratio Vba, Anterior side				
c. Dependent Variable: Age				

Table S11. Regression results of Cameriere's method for age estimation by sex (Cameriere's method) Model Summary^b by sex

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
Male	.788 ^a	0.621	0.617	3.370
Female	.730 ^a	0.533	0.528	3.370

a. Predictors: (Constant), Ratio Vba, Posterior side, Anterior side b. Dependent Variable: Age

Table S12. Classification results (confusion matrix and AUC) of the CT model for the 12-year threshold based on Cameriere's Vba ratio

Observed		Predicted		
		No	Yes	Percent Correct
No		119	54	68.8%
Yes		38	317	89.3%
Overall Percentage		29.7%	70.3%	82.6%
Area Under the ROC Curve				
			Asymptotic 95% Confidence Interval	
Area	Std. Error	Asymptotic Sig	Lower Bound	Upper Bound
0.886	0.015	0.000	0.857	0.915

Table S13. Classification results (confusion matrix and AUC) of the CT model for the 14-year threshold based on Cameriere's Vba ratio

Observed		Predicted		
		No	Yes	Percent Correct
No		184	49	78.9%
Yes		27	268	90.8%
Overall Percentage		40.0%	60.0%	85.6%
Area Under the ROC Curve				
			Asymptotic 95% Confidence Interval	
Area	Std. Error	Asymptotic Sig.	Lower Bound	Upper Bound
0.92	0.012	0.000	0.899	0.944

Table S15. Classification results (confusion matrix and AUC) of the CT model for the 18-year threshold based on Cameriere's Vba ratio

Observed	Predicted			
	No	Yes	Percent Correct	
No	313	40	88.7%	
Yes	47	128	73.1%	
Overall Percentage	68.2%	31.8%	83.5%	
Area Under the ROC Curve				
			Asymptotic 95% Confidence Interval	
Area	Std. Error	Asymptotic Sig.	Lower Bound	Upper Bound
0.89	0.013	0.013		

Table S14. Classification results (confusion matrix and AUC) of the CT model for the 16-year threshold based on Cameriere's Vba ratio

Observed	Predicted			
	No	Yes	Percent Correct	
No	214	79	73.0%	
Yes	24	211	89.8%	
Overall Percentage	45.1%	54.9%	80.5%	
Area Under the ROC Curve				
			Asymptotic 95% Confidence	
Area	Std. Error ^a	Asymptotic Sig. ^b	Lower Bound	Upper Bound
0.904	0.012	0.000	0.000	0.000

Table S16. Accuracy, sensitivity, specificity, and AUC for legal age thresholds by sex (Cameriere's method)

Legal age	Sex	Accuracy (%)	Sensitivity (%)	Specificity (%)	AUC
12 years	M	82.6	89.3	68.8	0.88
	F	85.6	90.8	78.9	0.912
14 years	M	80.5	89.8	73.0	0.93
	F	83.5	73.1	88.7	0.928
16 years	M	82.4	76.9	86.7	0.927
	F	82.4	75.4	88.1	0.90
18 years	M	82.0	60.9	92.2	0.919
	F	84.3	62.5	95.4	0.90

Table S17. Comparison of Baccetti's and Cameriere's methods at legal age thresholds (12, 14, 16, and 18 years)

Legal age	Method	Accuracy (%)	Sensitivity (%)	Specificity (%)	AUC
12 years	Baccetti	88.8	86.5	93.6	0.949
	Cameriere	82.6	89.3	68.8	0.886
14 years	Baccetti	93.0	97.6	87.1	0.979
	Cameriere	85.6	90.8	78.9	0.921
16 years	Baccetti	87.5	83.0	91.1	0.934
	Cameriere	80.5	89.8	73.0	0.904
18 years	Baccetti	83.0	87.4	80.7	0.889
	Cameriere	83.5	73.1	88.7	0.899

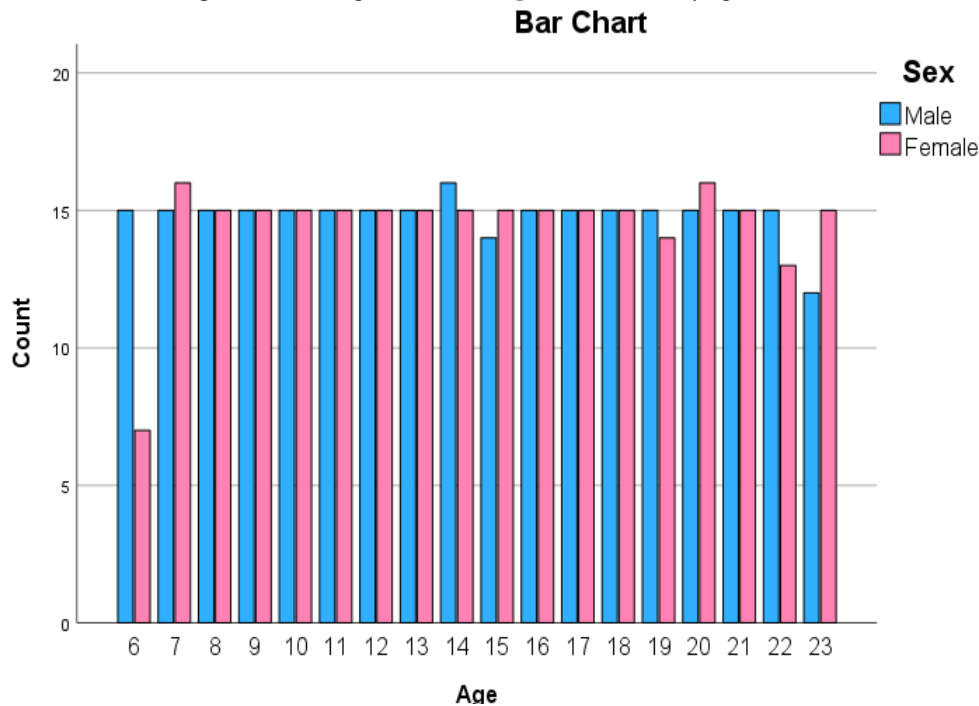
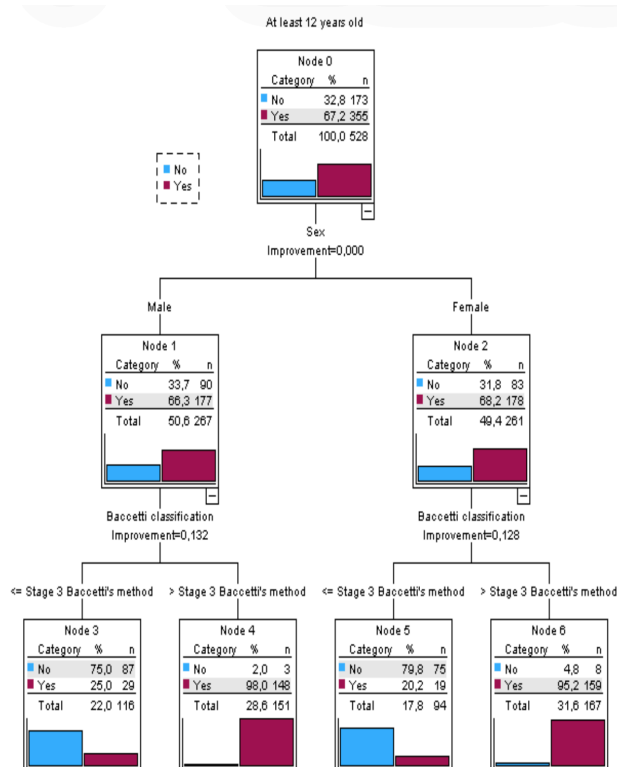
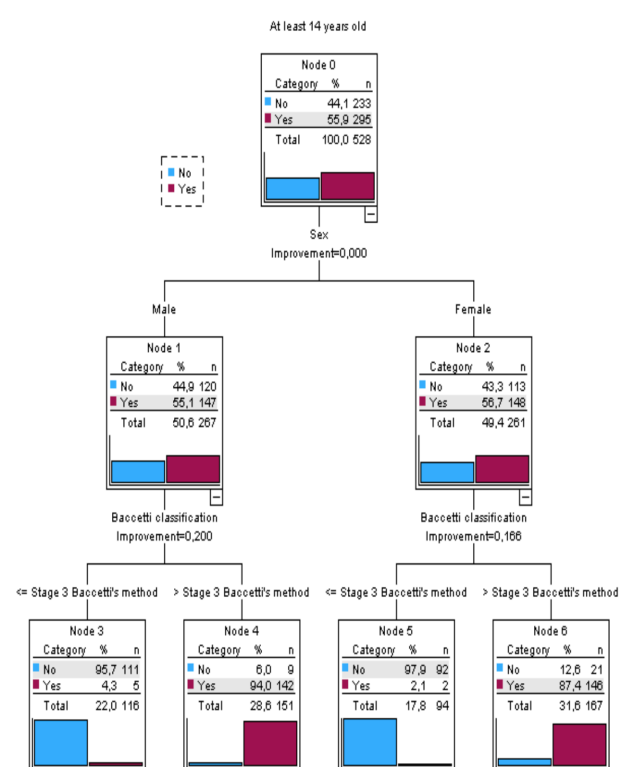
Figure S1. Histogram of the sample distribution by age and sex**Figure S2.** Classification tree (CT) model illustrating the influence of sex and Baccetti's classification stages on the probability of being at least 12 years old**Figure S3.** Classification tree (CT) model illustrating the influence of sex and Baccetti's classification stages on the probability of being at least 14 years old

Figure S4. Classification tree (CT) model illustrating the influence of sex and Baccetti's classification stages on the probability of being at least 16 years old

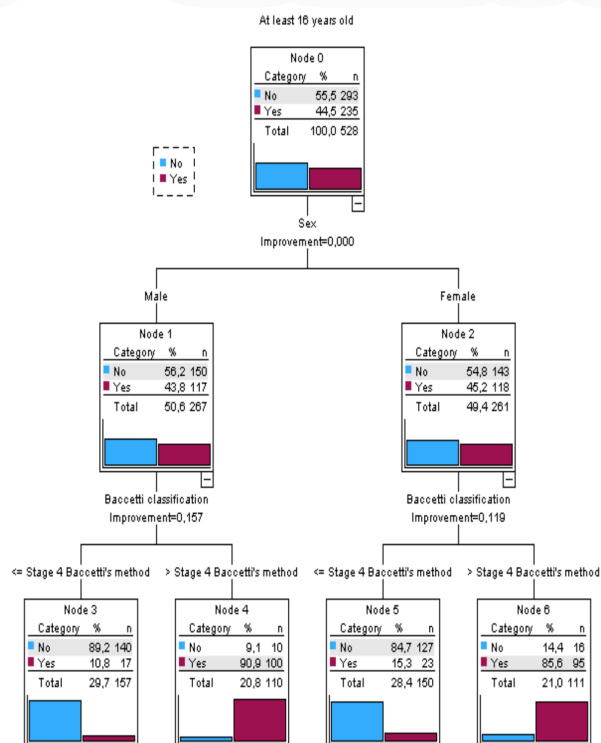


Figure S6. Classification tree (CT) for predicting whether subjects are at least 12 years old based on Ratio Vba

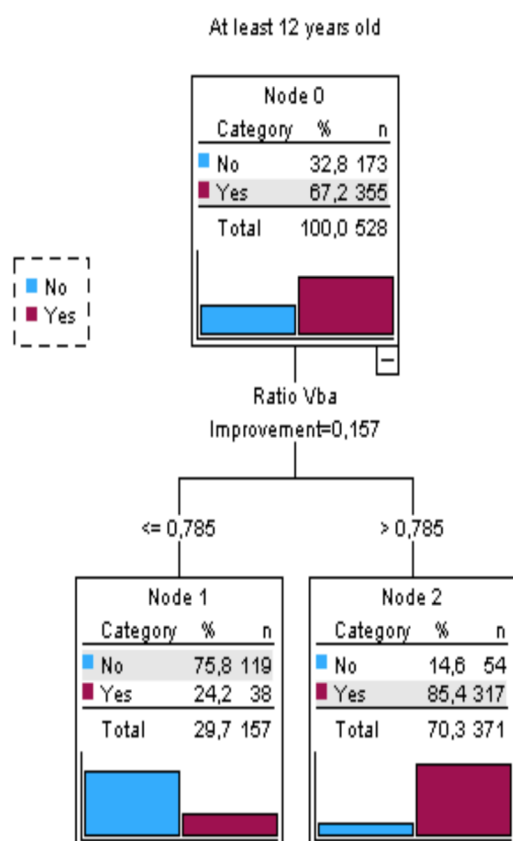


Figure S5. Classification tree (CT) model illustrating the influence of sex and Baccetti's classification stages on the probability of being at least 18 years old

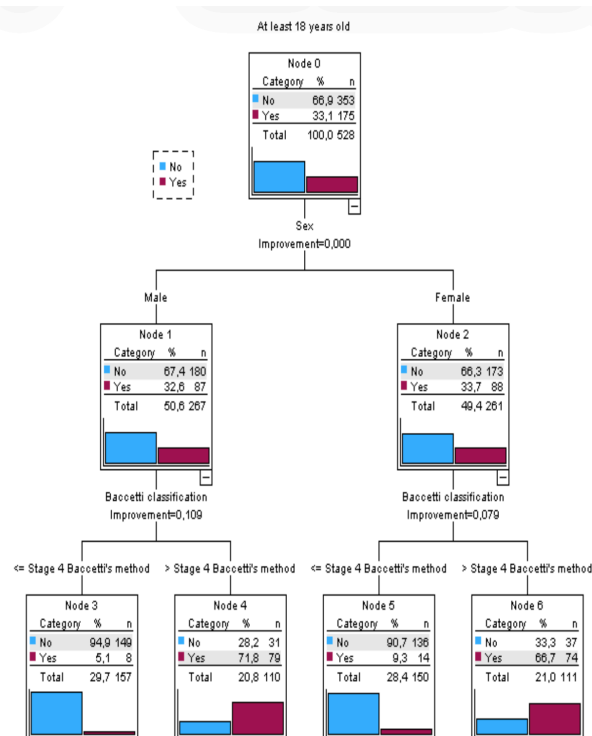


Figure S7. Classification tree (CT) for predicting whether subjects are at least 14 years old based on Ratio Vba

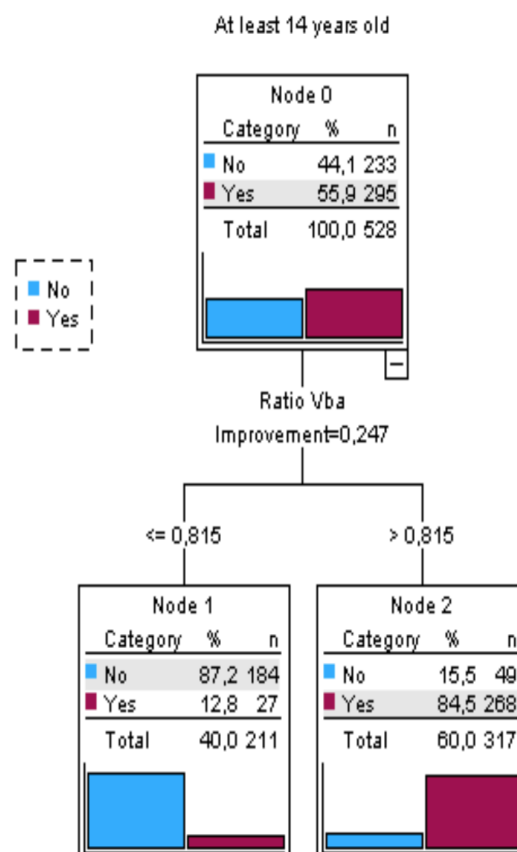


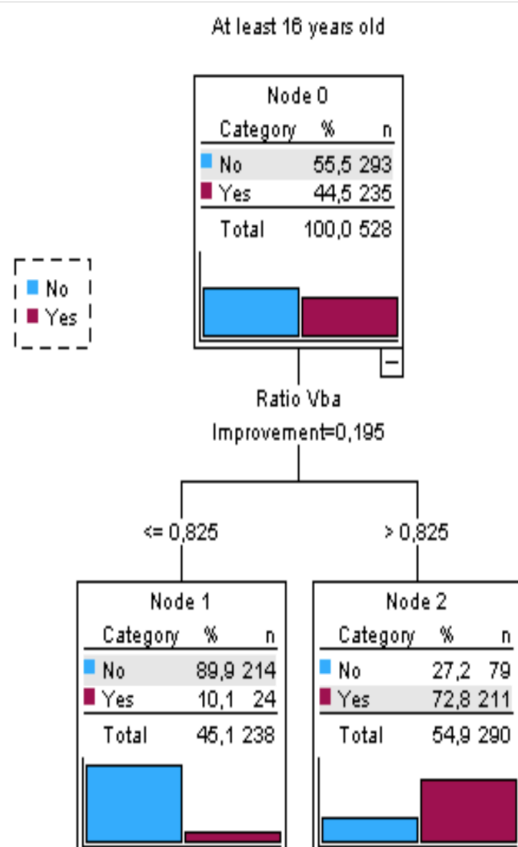
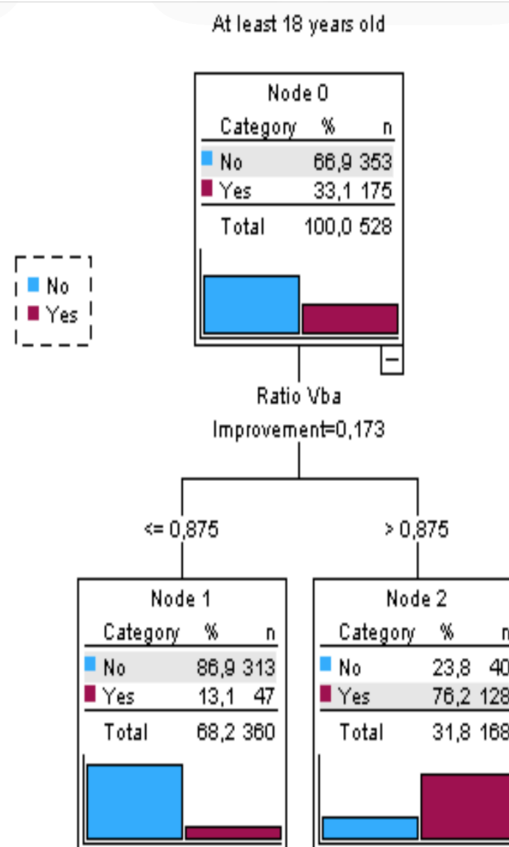
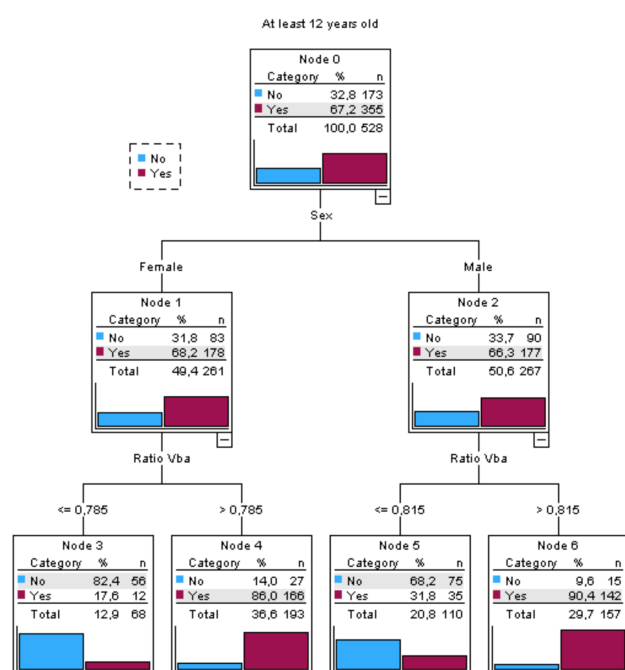
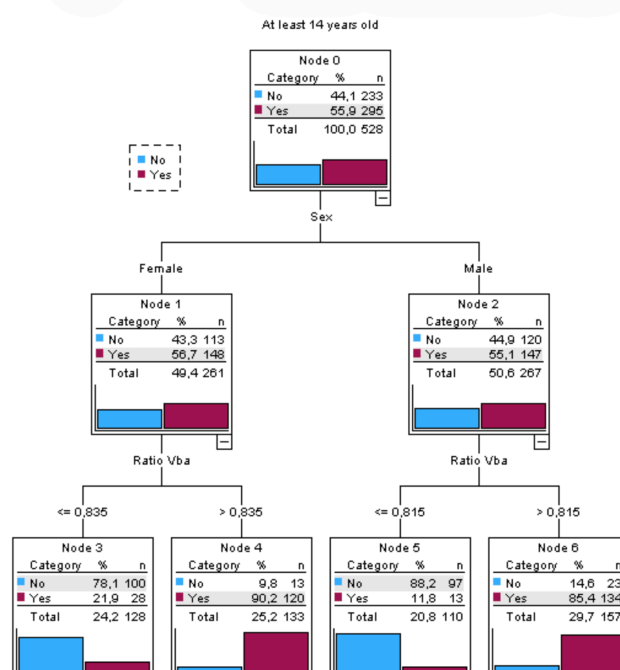
Figure S8. Classification tree (CT) for predicting whether subjects are at least 16 years old based on Ratio Vba**Figure S9.** Classification tree (CT) for predicting whether subjects are at least 18 years old based on Ratio Vba**Figure S10.** Classification tree (CRT) model illustrating the influence of sex and Cameriere's Vba ratio on the probability of being at least 12 years old**Figure S11.** Classification tree (CRT) model illustrating the influence of sex and Cameriere's Vba ratio on the probability of being at least 14 years old

Figure S12. Classification tree (CRT) model illustrating the influence of sex and Cameriere's Vba ratio on the probability of being at least 16 years old

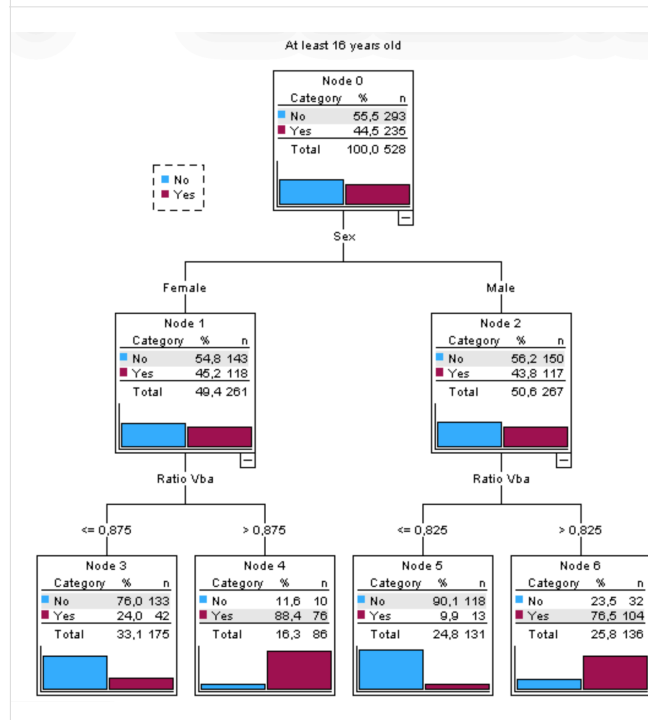
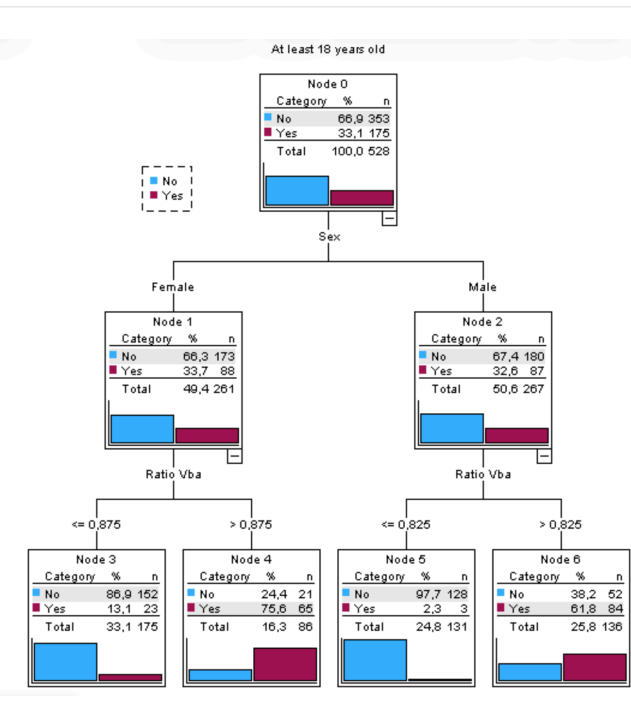


Figure S13. Classification tree (CRT) model illustrating the influence of sex and Cameriere's Vba ratio on the probability of being at least 18 years old



Population affinity estimation of an unidentified individual using nonmetric dental traits: a case report

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The authors declare that they
have no conflict of interest.

KEYWORDS

Biodistance,
Forensic anthropology,
Dental morphology,
Human identification,
Legal identity

J Forensic Odontostomatol
2026. Aug; (44): 2-67:72
ISSN :2219-6749
DOI: doi.org/10.5281/zenodo.21849339

ABSTRACT

Due to their strong correlation to heredity, nonmetric dental traits are deemed useful to estimate an individual's population affinity. This case report demonstrates the use of the rASUDAS2 application for estimating a decedent's population affinity when other primary identification methods proved insufficient. Initial examinations showed that the decedent (referred to as KF230565 or Mr X) was a 42-year-old male whose death was caused by non-homicidal drowning. Due to slight deviations from the typical Western Indonesian craniofacial characteristics, his nonmetric dental traits were graded to confirm his population affinity. After grading the dental traits according to the Arizona State University Dental Anthropology System (ASUDAS), the data were entered into the rASUDAS2 software. Based on the analysis, the decedent was estimated to be of Western Eurasian (46.24%) or Southeast Asian (20.58%) affinity. This information was then passed to forensic artists and law enforcement, and a facial approximation sketch was constructed using the appropriate population-specific soft tissue thickness dataset. The sketches were then disseminated to the public to aid recognition. After receiving additional reports from the public, positive identification of the decedent was confirmed through primary identifiers. This report encourages the use of multidisciplinary methods in forensic identification, as well as further studies on dental morphology.

INTRODUCTION

Nonmetric dental traits refer to unique morphologies of the human tooth concerning its orientation, absence or presence, and its position within the oral cavity. These morphological characteristics can be found in several dental anatomies, such as the cusps, grooves, tubercles, and ridges. Dental morphology is mostly affected by genetics (up to 80%) and does not experience significant post-eruptive alteration, hence its potential use in biodistance analysis of both living and deceased individuals.¹⁻³

Studies have documented differences in the frequencies of dental traits among biogeographic populations.⁴ For example, Carabelli's trait and protostylid are more common in Western Eurasian populations and not in Asian and African populations.^{5,6} Meanwhile, bilaterally winged and shovel-shaped incisors displayed higher frequencies in Asian and Asian-derived populations, unlike Western Eurasian populations.⁷⁻⁹

These variations were possibly related to evolutionary factors that affect gene flow and adaptation across and within populations.^{10,11}

Given its potential forensic value, an application based on the Arizona State University Dental Anthropology System (ASUDAS) was developed. Called the rASUDAS2 (available at <https://osteomics.com/rASUDAS2/>), the software utilised naïve Bayesian methods to predict the biogeographic affinity of an individual, which can be useful for forensic cases. When used on all seven populations, it correctly assigned 51.8% of individuals, confirming its validity.^{12,13}

The following case report aims to display the use of rASUDAS2 to help the investigators in estimating an unidentified individual's population affinity. This report highlights a multidisciplinary approach for constructing an accurate biological profile of the decedent. As of this report's publication, this report is the only documented case involving rASUDAS2 in a forensic case in Indonesia. Therefore, this case calls for further investigations and explorations on dental morphology and its applicability in actual forensic cases.

CASE

A body was found in the Rolak River, Gunungsari, Jambangan, Surabaya City, by an excavator operator. The witness discovered a floating body on November 23, 2023, at 2:30 PM (GMT+7) when he was cleaning up water hyacinths. He reported the discovery to 112 (Indonesia's emergency hotline) and retrieved the body from the river using his excavator.

The body was handled by the Sectoral Police Force of Jambangan District and coded as "KF230565"—also referred to as "Mr X". The body arrived at Dr Soetomo General Hospital's morgue later that day, precisely at 6:05 PM (GMT+7). The Letter of Visum et Repertum Request (No. VER/17/XI/2023/SPKT) was received by the medical examiners along with the body. An external examination was conducted immediately, and a further internal examination was conducted the next day (November 24, 2023, 5:30 PM).

The cause of death was determined as non-homicidal drowning due to positive diatom results, sand particles found in the respiratory system, as well as no corresponding trauma. Primary identification efforts were unsuccessful because of incomplete dental records, destroyed

eyeballs, and wrinkled fingers and toes. Therefore, the Police and Hospital requested the consultancy of the corresponding author under Request Letter No. B/224/XI/2023/Reskrim to construct Mr X's biological profile and conduct a two-dimensional (2-D) facial approximation using forensic anthropological methods. Since the craniodental region was the focus of the analysis, maceration and decapitation were performed to facilitate detailed observation and measurement (Figs. 1 and 2).

Figure 1. Maxillary dental arch of Mr X



Figure 2. Mandibular dental arch of Mr X



Based on the robust nuchal line, pronounced glabella, large mastoid processes, robust mandibular morphology, and external genitalia, we concluded that Mr X was assigned male at

birth (AMAB). Cranial suture scores estimated Mr X's age at 42.35 ± 12.05 years. His stature was estimated to be about 165–175 centimetres. Mr X possessed an elongated face, a narrow nasal aperture, and a high nasal spine, which are quite atypical for Western Indonesians. Since population affinity is an important factor in facial approximation, confirmatory analysis using his dental morphology was performed.

Mr X's dental morphology was assessed based on the

ASUDAS. Even though both sides were examined, only the right side was reported for further analysis. Since no radiological images or loose focal teeth were obtained, only the crown traits were graded (Table 1). The rASUDAS2 software was utilised for further analysis. The grades were entered into the software and then analysed according to their naïve Bayesian statistics. Based on the analysis, Mr X was most likely of Western Eurasian (46.24%) or Southeast Asian (20.58%) affinity (Table 2).

Table 1. Nonmetric dental traits of Mr X

Trait	Description	Tooth*	Range	Grade
Winging	Rotated maxillary central incisors.	11	0–3	0
Shovelling	Presence of marginal ridges on the lingual side of maxillary incisors.	11 12	0–7 0–7	3 1
Double shovelling	Presence of ridges on the labial side of maxillary incisors.	11	0–6	1
Tuberculum dentale	Presence of enamel elevation on the lingual surface of the anterior teeth.	11 13	0–6 0–7	3 2
Interruption grooves	Distinct grooves interrupting the normal course of the mesial or distal marginal ridges or even the basal cingulum.	12	0, M, D, MD, med	M
Bushman canine	Hypertrophied mesial ridge and a small tubercle that join together on the maxillary canine.	13	0–3	0
Carabelli's trait	Cingular derivative on the lingual surface of the mesiolingual cusp of maxillary molars.	16	0–7	3
Cusp 5	Presence of a fifth cusp on the distal fovea of the maxillary molars.	16 17	0–5 0–5	0 1
Enamel extensions	Enamel extends beyond the cemento-enamel junction towards the apex of the roots.	16 46	0–3 0–3	0 0
Hypocone	Size of the distolingual cusp on maxillary molars.	17	0–6	3
Pegged, reduced, or missing	A reduction in size to a missing third molar.	18	0–3	3
Distal accessory ridge	Accessory ridge on the distal portion of the canine.	43	0–5	1
Premolar multiple lingual cusps	Lingual cusp variation of the mandibular premolars.	44 45	0–3 0–3	1 2
Cusp 6	Presence of a sixth cusp on the distal portion of the mandibular molars.	46 47	0–5 0–5	0 0
Cusp 7	Presence of additional lingual cusp of the mandibular molar.	46	0–4	0
Deflecting wrinkle	Incidental ridge on the mesiolingual cusp that changes course as it runs toward the central groove.	46	0–3	0
Protostylid	An extra cusp found on the buccal surface of the mesiobuccal cusp of the mandibular first molar.	46	0–7	1
Groove pattern	Pattern of contact of major cusps of lower molars.	47	X, +, Y	+
Four-cusped molar	Presence of the distal cusp of mandibular molars.	46 47	0–5 0–5	3 2

Upper premolar root number	Root number of the anterior upper premolar.	14	1-3	N/A
Upper second molar root number	Root number of the maxillary second molar.	16	1-3	N/A
Lower canine root number	Root number of the mandibular canine.	43	0-1	N/A
Tome's root	Mesiolingual groove or separation of the roots of the mandibular first premolar.	44	0-5	N/A
Three-rooted lower first molar	Presence of accessory distolingual root of the mandibular first molar.	46	1-3	N/A
One-rooted lower second molar	Presence of root fusion of the mandibular second molar.	47	1-2	N/A

Table 2. Estimated population affinity of Mr X

Biogeographic population	Prior	Posterior	Most likely	Consider
American Arctic and Northeast Asia	0.1429	0.0705		
Australo-Melanesia and Micronesia	0.1429	0.1298		
East Asia	0.1429	0.0341		
North and South America	0.1429	0.0040		
Southeast Asia and Polynesia	0.1429	0.2058		Yes
Sub-Saharan Africa	0.1429	0.0935		
Western Eurasia	0.1429	0.4624	Yes	Yes

Information on Mr X's population affinity was then reported to the assigned forensic artist of this case so that facial approximation sketches could be created using a population-appropriate soft tissue thickness dataset. The sketch was then distributed to the public in the hope of obtaining recognition. After its public dissemination, recognition was achieved. Later, law enforcement was able to confirm the legal identity of Mr X using primary identifiers; however, the specific identifiers and confirmatory documentation were not accessible to the authors. Law enforcement further stated that Mr X was of Indonesian and Pakistani descent and had resided in Surabaya, confirming the rASUDAS2 analysis.

DISCUSSION

The forensic value of nonmetric dental traits is attributable to their ability to accurately estimate the population affinity of an individual when only craniodental remains are available.¹⁴ Previous reports describing the forensic application of nonmetric dental traits have often focused on isolated traits, such as cusp 6,¹⁵ shovel-shaped incisors,¹⁶ hypodontia,¹⁷ and taurodontism.¹⁸ Despite their scientific importance, these pieces of evidence might not hold sufficient value in court. Instead of isolated assessments, constellated analysis of these traits might offer a

more statistically probable and medicolegally accepted outcome.¹⁹ This concept underlies the development of the analytical tool rASUDAS2 as a decision support system for biological anthropology.¹²

Results from the rASUDAS2 analysis showed that Mr X was possibly of Western Eurasian (46.24%) or Southeast Asian (20.58%) populations, while others were deemed unlikely by the system. Along with his atypical craniofacial features, this finding further highlights careful interpretation of individual variations that may occur in forensic cases. This case illustrates that a region such as Western Indonesia may be genetically and morphologically heterogeneous. Since nonmetric dental traits are polygenic in nature, slight deviations from the typical can be caused by multiple factors, such as possible admixture, historical gene flow, and individual mutations.^{20,21} A study showed that despite falling in the same category of Southeast Asia, Javanese dental patterns show a more similar frequency to Australo-Melanesian (e.g., interruption grooves, cusp 6, and deflecting wrinkle) and East Asian (e.g., cusp 5 and Y-groove patterns) populations.^{7,22}

Similar to other forensic anthropological methods, the population affinity of Mr X should be interpreted as a probabilistic affinity instead of

a positive identity.²³ In principle, nonmetric dental traits and rASUDAS2, by extension, provide a statistical possibility instead of absolute group classification. The sole use of nonmetric traits, therefore, is not recommended regardless of the strength of the statistical support.²⁴ Rather, it should be accompanied by findings from other biological indicators, such as cranial measurements and stable isotopes.²⁵

Despite these challenges, nonmetric dental traits remain a robust tool in forensic anthropology and odontology due to their strong genetic basis and resistance to postmortem degradation.³ When combined with standardised scoring systems such as the ASUDAS and analytical tools like rASUDAS2, they offer a reproducible and relatively objective approach to affinity estimation.¹² In this case, the results were operationally useful, as they informed the selection of population-appropriate soft tissue thickness data for facial approximation, ultimately contributing to public recognition and successful identification.

Nevertheless, several limitations must be acknowledged. The analysis was based on a single individual, and the number of observable traits may have been constrained due to preservation or methodological issues, such as the lack of a medical imaging device. Additionally, the accuracy of rASUDAS2 depends on the representativeness of its reference samples, which may not adequately capture the full spectrum of Southeast Asian dental variations, particularly Indonesian populations.²⁶ Observer-related variation in trait grading may also introduce bias, although adherence to ASUDAS standards helps mitigate this issue.²⁷

Future research should prioritise the expansion of regionally relevant dental morphological

databases, particularly for underrepresented populations in Southeast Asia and Indonesia. Studies on dental morphology remain scarce in Indonesia, highlighting the need for further research to establish reliable forensic reference datasets. Integrating nonmetric traits with other lines of evidence, such as dental measurements and genetic phenotyping, would further enhance the reliability of population affinity estimation.

In conclusion, this case demonstrates that a multidisciplinary approach that involves forensic pathology, dental anthropology, and forensic arts can significantly improve identification outcomes in forensic contexts. This case also highlights how estimating a decedent's biological profile serves as a practical tool for police investigations. While the results provide probabilistic estimates rather than definitive identifications, they act as an important filter for narrowing down long lists of missing persons. By identifying specific physical characteristics, law enforcement can focus its efforts on a more relevant group of candidates and speed the recovery of the decedent's legal identity.

ACKNOWLEDGEMENTS

This case report was presented on February 16, 2024, in Surabaya. The authors thank the Final Report (*Referat*) Defence Committee of the Installation of Forensic Medicine and Medicolegal of the Dr Soetomo General Hospital and the Department of Forensic Medicine and Medicolegal (Sp-1) of Universitas Airlangga. The authors also thank Universitas Airlangga's Department of Forensic Science (MA) as well as the Social Biology and Human Cultural Adaptation Study Centre for their collaboration.

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