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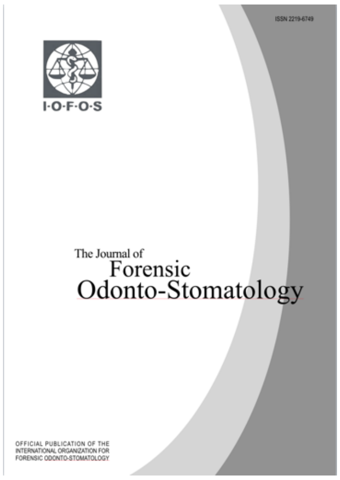

THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

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CONTENTS

Accuracy of four dental age estimation methods in determining the legal age threshold of 18 years among South Indian adolescents and young

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The authors declare that they have no conflict of interest.

KEYWORDS
Dental age estimation;
Third molar maturity index,
Demirjian stages;
Root pulp visibility;
Eruption;
Third molars;
Adult age

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ABSTRACT

The aim of this study was to compare the accuracy, specificity and sensitivity of four commonly used methods of dental age estimation in a sample of south Indian adolescents and young adults aged between 14 and 30 years, with an age threshold of 18 years, using receiver operating characteristic curves (ROC) and the area under the curve (AUC). A total of 1070 orthopantomograms (535 males and 535 females) of adolescents and young adults of south Indian origin were collected retrospectively and interpreted. The effectiveness of each method was evaluated by using sensitivity (Se), specificity (Sp), likelihood ratios (LR+ and LR-) and AUC. Among all methods, I3M< 0.08 resulted in better values of AUC, Se and Sp which were 0.950, 91.5%, 97.8% and 0.950, 88.5% and 98.6% in males and females, respectively. For "stage H" of Demirjian's system, the AUC, Se and Sp were 0.940, 84.9%, 97.7% and 0.930, 79.9% and 98.5% in males and females, respectively. The use of the Olze et al "stage 1 (or higher)" root pulp visibility and "stage D" of third molar eruption were not recommended in the studied population due to the greater percentage of third molars with incomplete mineralization in younger age groups and impaction. Taking into account the values of Se, Sp, both positive and negative LRs, we recommend the use of the cut-off value of I3M< 0.08 to discriminate adults and minors in south Indian adolescents and young adults.

INTRODUCTION

Selection of an appropriate method of age estimation is crucial in forensic and medico-legal settings. It depends on what parameters are present and what general age is represented. Many parameters were examined for age assessment in children and adolescents including the skull bones, long bones, pubic symphysis, hand-wrist bones and permanent dentition. According to the forensic literature, the rate of tooth formation is the better indicator of chronological age than skeletal development, as it is less affected by malnutrition and other factors.[1-3] In most countries, the legal age is 18 years.[4] Forensic experts are often confronted with the conceptually simple medico-legal question of whether an individual is a juvenile (below 18 years) or an adult (18 years or above). This is due to the varied legal consequences in penal and criminal law if a subject of unknown age is judged to be a juvenile or an adult.

Age assessment by dental examination methods is today either the evaluation of the clinical emergence of the teeth or the radiographic evaluation of the mineralization of the crown and root portions of the developing teeth. To answer the legal question pertaining to the attainment of 18 years, one has to rely on the developing third molars. It has been emphasized in the forensic literature for the use of qualified personnel in age assessment who have a clear understanding of the methods in use, who must apply an appropriate method or a proven scientific technique(s) with a known rate of error.[5] Considering the mandibular third molars, their mineralization (Cameriere's third molar maturity index (I3M) and Demirjian's staging system),[6, 7] clinical emergence (Olze et al stages of tooth eruption)[8] and secondary changes (Olze et al stages of root pulp visibility RPV),[9] were extensively studied in the literature to estimate the probability that an individual is over 18 years. All these methods were validated independently in various populations for the evaluation of 18 years of age for forensic purposes.

However, there were no studies that compared these four methods in discriminating adults and minors in south Indian individuals. Therefore, the aim of the present study was to compare the accuracy, specificity and sensitivity of four commonly used methods of dental age estimation i.e. Cameriere's third molar maturity index (I3M< 0.08), Demirjian stages of tooth development (Stage H), Olze's stages of root pulp visibility (stage 1) and Olze's eruption stages of third molars (stage D) in a sample of south Indian adolescents and young adults aged between 14 and 30 years, with an age threshold of 18 years, using receiver operating characteristic curves (ROC) and the area under the curve (AUC).

MATERIAL AND METHODS

Sample

A total of 1070 orthopantomograms (OPGs) of south Indian adolescents and young adults aged between 14 and 30 years were selected retrospectively from the archives of the Department of Radiology, Panineeya Dental College and from Private Dental Clinics in south India. The inclusion criteria were: OPGs from individuals aged between 14 and 30 years, south Indian nationality and good quality images with at least one mandibular third molar. Exclusion criteria were: distorted OPGs, gross pathology affecting the region of interest, rotated third molars that impede the measurements and bilateral absence of the mandibular third molars. Data on the sex, date of birth and the date on which the radiograph was taken were recorded. The chronological age was obtained by calculating the difference between the date of birth and the date the radiograph was taken. All OPGs were initially allotted with consecutive numbers in order to ensure blinding of the examiner to the subjects' details.

Methods

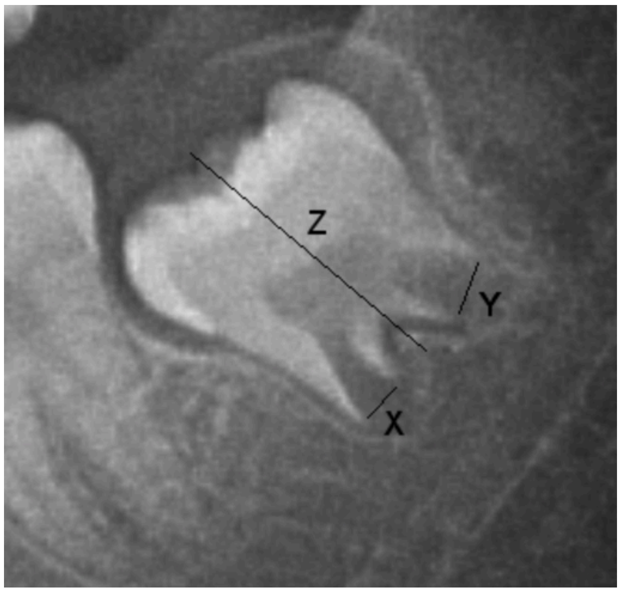
Four methods were adopted to provide the legal age threshold of 18 years, two methods took the maturation of the mandibular third molars into consideration[6, 7], a third method considered eruption of the mandibular third molars[8] and the fourth method studied the changes in the root pulp visibility in the fully formed mandibular third molars[9].

Cameriere et al. third molar maturity index (I3M)

The mineralisation status of the mandibular third molar was calculated using the Cameriere's third molar maturity index (I3M).[6] I3M is evaluated as the sum of the distances between the inner sides of the two open apices (X+Y) divided by the tooth length (Z) (Figure 1). A score of "0" is allocated when the development of the third molar is complete. In the present study, the recommended value of I3M<0.08 was tested to discriminate adults from minors in both sexes.

The tooth length and apical width measurements of mesial and distal roots of mandibular third molars were performed using Image J software (version 1.48, National Institute of Health, USA). All the measurements were carried out by a single examiner.

Figure 1. Measurements of the mandibular third molar for the calculation of the third molar maturity index (I3M)[6]; I3M is evaluated as the sum of the distances between the inner sides of the two open apices (X+Y) divided by tooth length (Z)



Demirjian's stages of tooth development
The mineralisation status of the mandibular third molar was evaluated by using Demirjian's system of tooth development.[7] The discrimination of adults and minors was studied using the Demirjian's final stages of root mineralisation i.e., "stages E to H" (Figure 2). Mincer et al[10] for the first time studied Demirjian's stages of tooth development to answer the medico-legal question pertaining to the legal age threshold of at least 18 years. In the present study, Stage "H" (completed mineralization) was used as a cut-off point to estimate adulthood.

Olze et al stages of third molar eruption
The eruption status of the mandibular third molar was studied using the staging system reported by Olze et al[8] that includes no emergence (stage A), alveolar emergence (stage B), gingival emergence (stage C) and complete emergence (stage D) (Figure 4). Impacted mandibular third molars were excluded from the analysis. In the present study, Stage "D" was used as a cut-off point to predict the attainment of legal age threshold of 18 years.

Olze et al stages of root pulp visibility (RPV)
The prediction of legal age threshold 18 years was also determined using Olze et al[9] stages of RPV that includes stages 0, 1, 2 and 3 where stage "0" has mandibular third molars with root canals visible all the way to apex, while stage "3" has mandibular third molars with the lumen of two root canals virtually invisible for the full length (Figure 3). In this study, we have verified stage "0" of RPV for indicating the major/ minor status.

Analysis of the images by the examiners
Each radiograph was given a unique identification number and was randomly selected for evaluation by the examiner. All the evaluations were made independently by two examiners (A1 and B1), in order to evaluate the inter-examiner variability. Later 50 OPGs were selected randomly and were rescored again by the first examiner (A2) after one month interval to measure the intra-examiner variability.

Figure 2. Demirjian stages of root development of permanent teeth (Stages E to H)

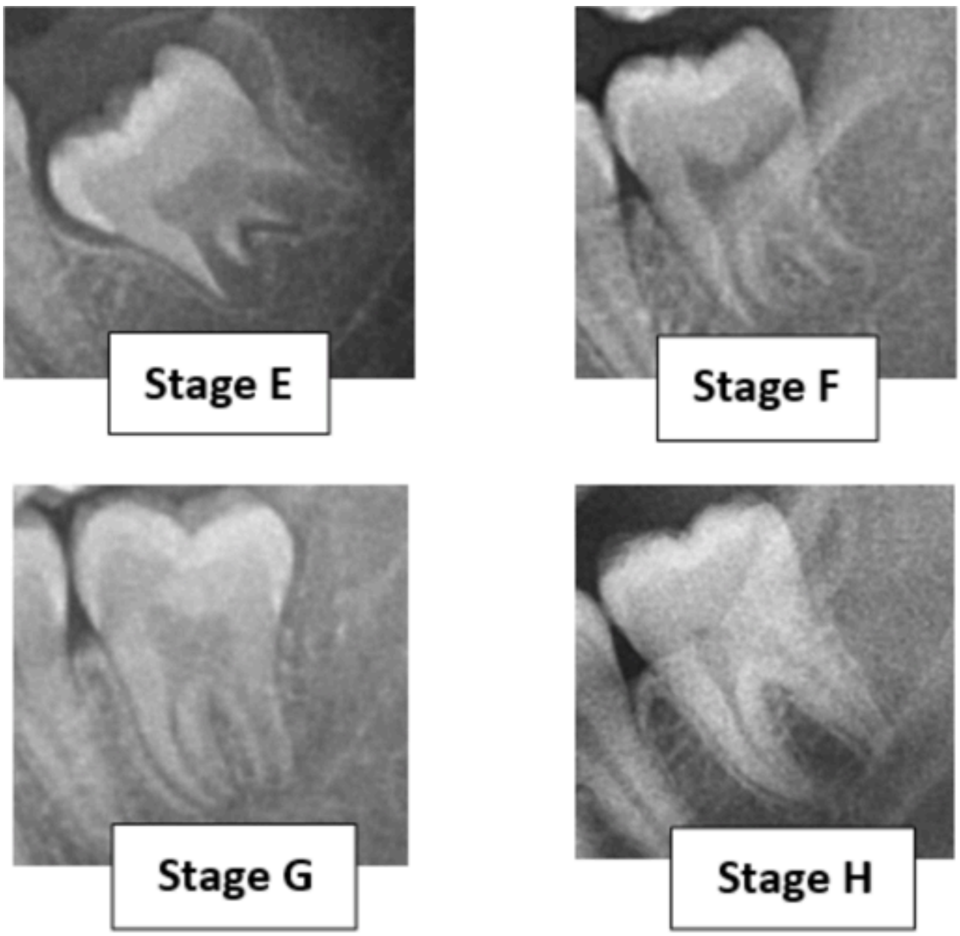


Figure 3. Radiographic images of Olze et al stages of radiographic visibility of root pulp in mandibular third molars

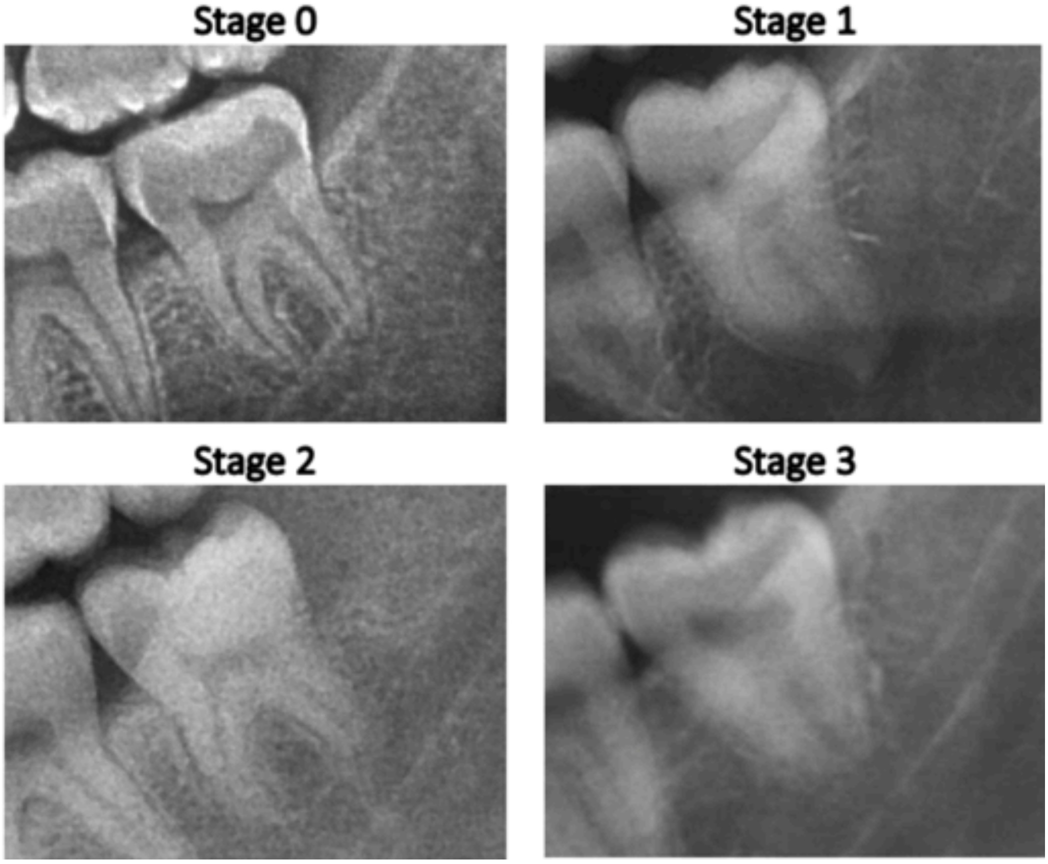
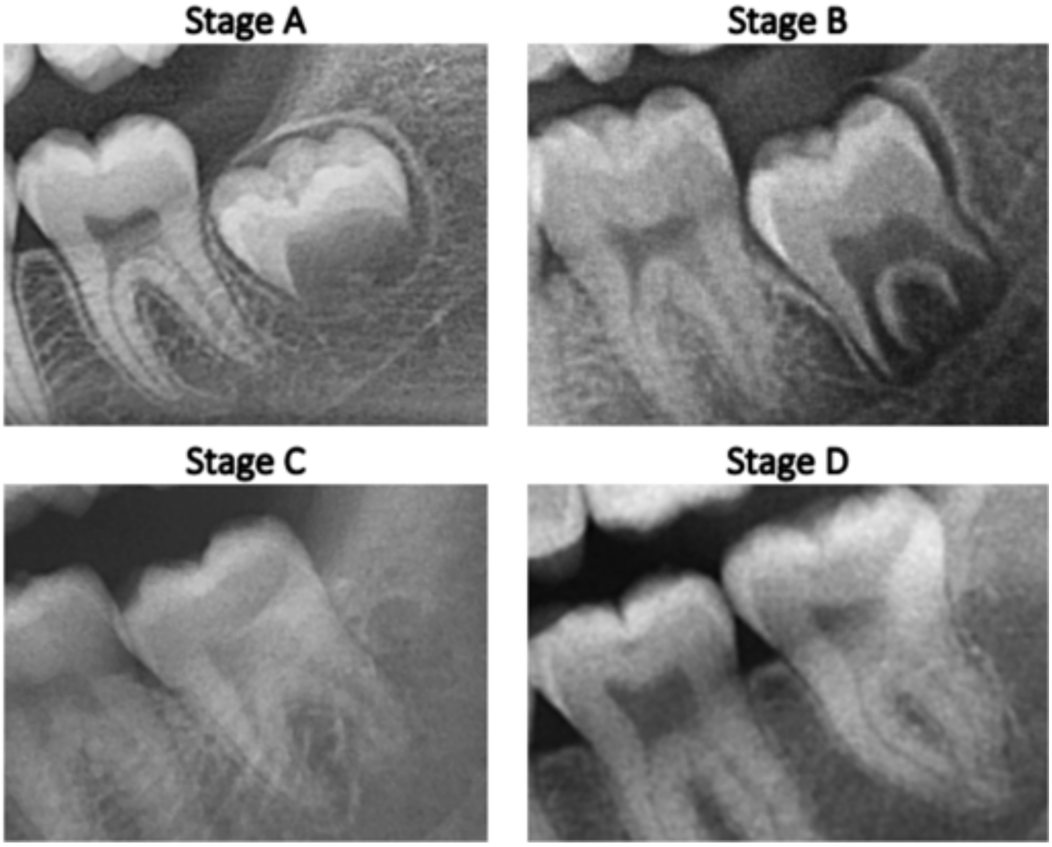


Figure 4. Radiographic images of Olze et al stages of mandibular third molar eruption



Statistical analysis

Statistical analysis was performed using SPSS 22.0 for Windows (IBM Corp, Chicago, IL, USA). The significance level for the analysis was set at 5%. Intra-examiner agreement was calculated for each method using the intra-class correlation coefficient, rescoring 50 OPGs one month after the initial evaluation. Inter-examiner agreement was calculated using another examiner using the intra-class correlation coefficient for each method.

The overall effectiveness of all four methods of age estimation were evaluated by plotting the ROC curve. Using the age threshold of 18 years, the area under the ROC curve (AUC) was calculated for each method and both sexes.[11] Additionally, the performance of all the methods was tested by 2×2 contingency table. A 2×2 contingency table is a tabular representation of categorical data used to summarize the relationship between two categorical variables. It generally displays the number of participants, who are true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN) in the overall sample. The performance of each method was further evaluated by; accuracies (Ac), sensitivities (Se), specificities (Sp) and positive and negative likelihood ratios (LR+, LR-). Accuracy represents the overall performance of the method in terms of discriminating between minors and adults. The sensitivity (Se) represents the proportion of individuals aged 18 years and older who had I3M<0.08 or those who attained the stage "H" of DSS or stage "1" or above (≥ stage 1) of RPV or those who reached the stage "D" of eruption of mandibular third molars. The specificity (Sp) represents the proportion of individuals aged 18 years and older who had I3M≥0.08 or those who were ranked as stage "G" or below of DSS or stage "0" of RPV or those who ranked as stage "C" or below of mandibular third molar eruption. Both LR+ and LR- ratios indicate how many times more or less likely the individuals to be a major or a minor based on the cut-off value used. They in turn help rule in or rule out diagnoses respectively in most situations. An LR+ value of above 10 increases the likelihood of being an adult, while an LR- value under 0.1 decreased the likelihood of being adult.[12]

RESULTS

Age and sex distribution of the individuals who took part in this study were presented in table 1. The mean chronological age of males and females was 21.1 ± 4.44 and 21.09 ± 4.49 years, respectively. Tables 2 and 3 present the total number of evaluated samples in both sexes using the Olze et al methods of root pulp visibility and mandibular third molar eruption. Olze et al RPV method could not be applied to a sample of 32.5% males and 35.9% females due to incomplete mineralization (open apices) of the mandibular third molars examined. On the other hand, in 23.6% of males and 26.7% of females, the Olze et al method of mandibular third molar eruption could not be applied due to the impacted third molars. Both intra- and inter-examiner agreement values were presented in table 4.

Table 1. Age and Sex distribution of the overall sample

Age groups	Male n (%)	Female n (%)
14- 14.9	40 (7.5)	40 (7.5)
15- 15.9	40 (7.5)	40 (7.5)
16- 16.9	40 (7.5)	40 (7.5)
17- 17.9	40 (7.5)	40 (7.5)
18- 18.9	40 (7.5)	40 (7.5)
19- 19.9	40 (7.5)	40 (7.5)
20- 20.9	40 (7.5)	40 (7.5)
21- 21.9	40 (7.5)	40 (7.5)
22- 22.9	40 (7.5)	40 (7.5)
23- 23.9	25 (4.7)	25 (4.7)
24- 24.9	25 (4.7)	25 (4.7)
25- 25.9	25 (4.7)	25 (4.7)
26- 26.9	25 (4.7)	25 (4.7)
27- 27.9	25 (4.7)	25 (4.7)
28- 28.9	25 (4.7)	25 (4.7)
29- 29.9	25 (4.7)	25 (4.7)
Total	535 (100)	535 (100)

Table 2. Descriptive table of the total sample according to each stage of Olze et al root pulp visibility in both sexes

Stages of Root pulp visibility	Males n (%)	Females n (%)
Stage 0	22 (4.1)	20 (3.7)
Stage 1	142 (26.5)	124 (23.2)
Stage 2	158 (29.5)	147 (27.5)
Stage 3	39 (7.3)	52 (9.7)
Incomplete mineralization*	174 (32.5)	192 (35.9)
Total	535 (100)	535 (100)

*Indicates third molars with incomplete mineralization which were excluded from analysis.

Table 3. Descriptive table of the total sample according to each stage of Olze et al tooth eruption in both sexes

Eruption Stages	Males n (%)	Females n (%)
Stage A	29 (5.4)	23 (4.3)
Stage B	80 (15)	81 (15.1)
Stage C	18 (3.4)	27 (5)
Stage D	282 (52.7)	261 (48.8)
Impacted*	126 (23.6)	143 (26.7)
Total	535 (100)	535 (100)

*Indicates impacted third molars which were excluded from analysis.

Table 4. Intra-class correlation coefficients (ICC) for intra- and inter-examiner agreements

Method	ICC	95% Confidence Interval	
		Lower limit	Upper limit
Intra-examiner agreement			
Cameriere's method of I3M	0.931	0.878	0.962
Demirjian's staging system	0.895	0.853	0.921
Olze's Root pulp visibility	0.815	0.776	0.838
Olze's stages of tooth eruption	0.846	0.808	0.869
Inter-examiner agreement			
Cameriere's method of I3M	0.887	0.824	0.926
Demirjian's staging system	0.847	0.811	0.883
Olze's Root pulp visibility	0.792	0.767	0.829
Olze's stages of tooth eruption	0.828	0.793	0.844

Intra-class correlation coefficient values of approximately 0.8 and higher were recorded for all methods in both sexes indicating good agreement between the repeated measurements for within and between examiners. Figures 5 to 7 presented the best performance for discrimination between adults and minors using the area under the curve for different methods of age assessment in both sexes.
Tables 5 to 8 present the contingency data showing the discrimination performance of all the methods, according to the sex. Overall performance measures of the cut-off values for all the methods used in our sample are presented in table 9 for both sexes, separately.

DISCUSSION

Assessing the probability of at least 18 years of age is the commonest practise of medico-legal physicians and forensic odontologists. They are often under pressure (for legal reasons) by police personnel to quickly estimate the age of a young person in conflict with the law. Therefore, it is common for them to rely on the simple and reproducible methods to assess dental age. Various authors have used different perspectives of dental age assessment using third molars that mainly involves development/maturation of third molars and their stages of eruption. It has been stated in previous studies that it is easier to learn the few stages of eruption than to study the eight stages of the Demirjian et al,[7] that allows medico-legal experts to quickly provide an answer for legal reasons.[13] However, we believe that it is important to choose an age estimation method based on their specificity index (rate of false positives), especially in a criminal context where an age misclassification could result in significant legal and ethical consequences.[14] In 2012, Pinchi et al[15] tested the accuracy of four methods of dental age estimation to predict the legal age threshold of 14 years in an Italian population. To the best of our knowledge, no study is available in the literature testing the accuracy of four different methods of dental age estimation to predict the legal age threshold of 18 years. In the present study, we compared the accuracy, sensitivity and specificity of the commonly used methods of dental age estimation in forensic practice in estimating the legal age of 18 years in a sample of south Indian adolescents and young adults.

Figure 5. Receiver Operating Characteristic curves for Cameriere's I3M< 0.08 & Stage H of Demirjian's method for discriminating adults and minors in both sexes

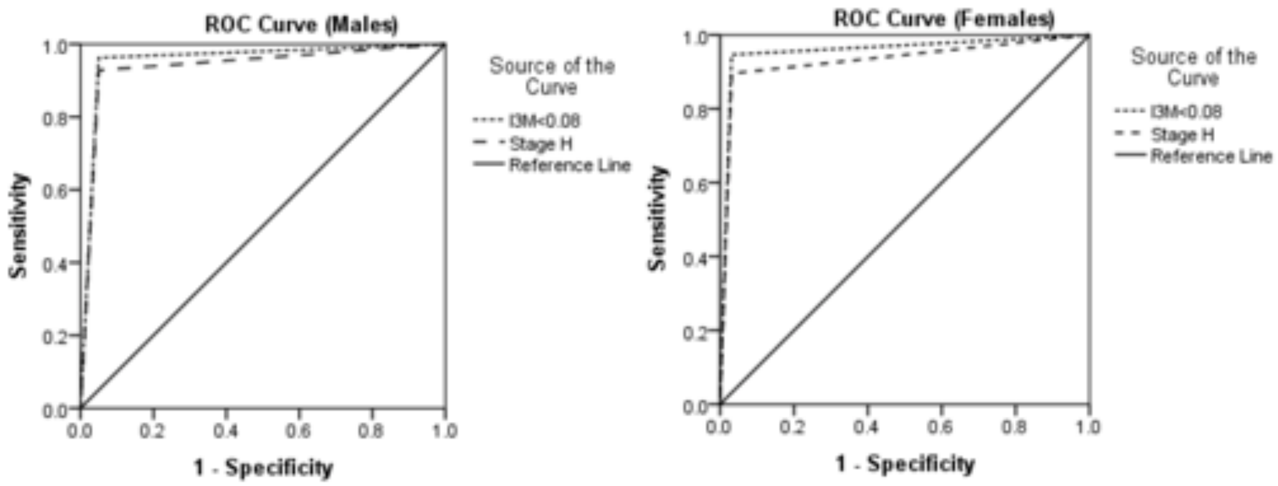


Figure 6. Receiver Operating Characteristic curves for Stage 1 of Olze et al root pulp visibility for discriminating adults and minors in both sexes

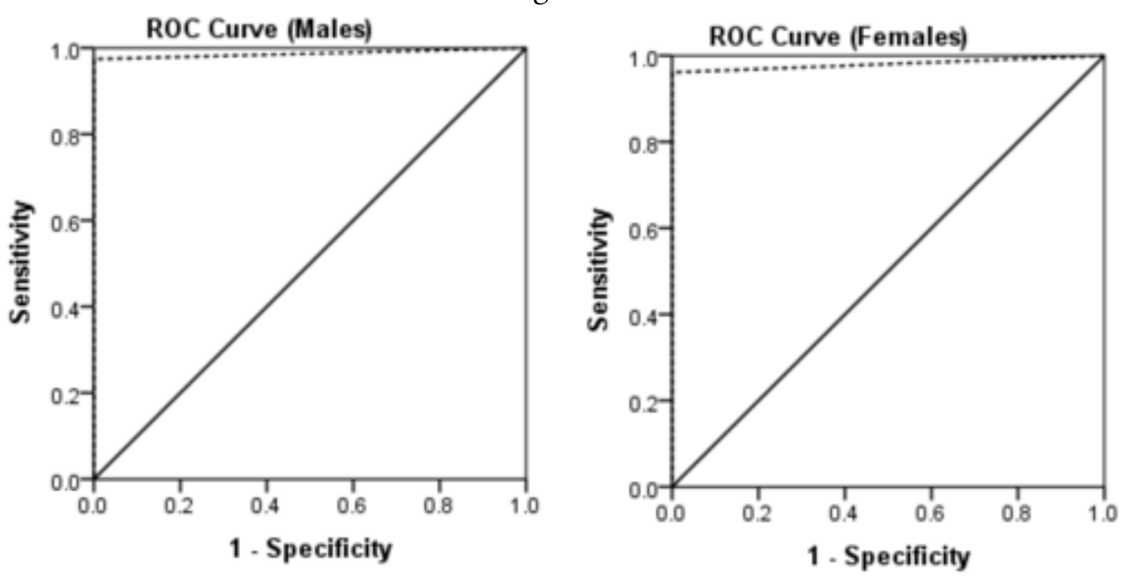


Figure 7. Receiver Operating Characteristic curves for Stage D of Olze et al third molar eruption for discriminating adults and minors in both sexes

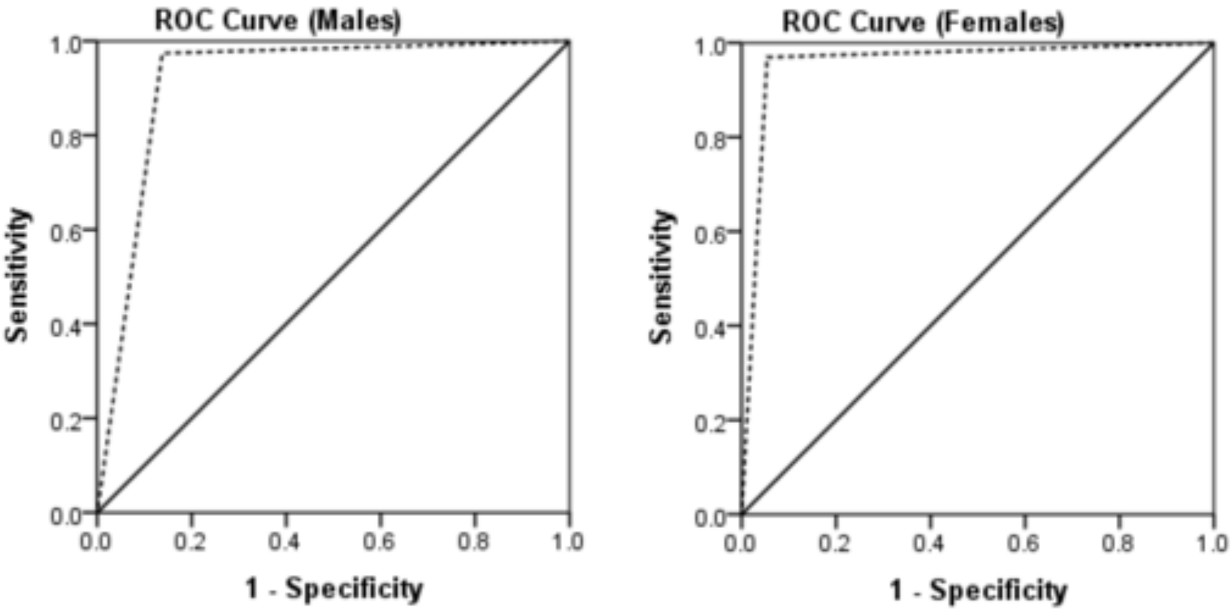


Table 5. Contingency tables describing discrimination performance of the test on being adult (≥18) or minor (<18) for third molar maturity index (I3M< 0.08)

Males			
	Age status		**Total**
	<18 years	**≥18 years**	
≥0.08	152^{TP} (95)	14^{FN} (3.7)	166 (31)
<0.08	8^{FP} (5)	361^{TN} (96.3)	369 (69)
Total	160 (100)	375 (100)	535 (100)
Females			
	<18 years	**≥18 years**	**Total**
≥0.08	155^{TP} (96.9)	20^{FN} (5.3)	175 (32.7)
<0.08	5^{FP} (3.1)	355^{TN} (94.7)	360 (67.3)
Total	160 (100)	375 (100)	535 (100)

Note: TP; True positive, FP; False positive, TN; True negative, FN; False negative

Table 6. Contingency tables describing discrimination performance of the test on being adult (≥18) or minor (<18) for "stage H" of Demirjian's method

Males			
	Age status		**Total**
	<18 years	**≥18 years**	
≤ Stage G	152^{TP} (95)	27^{FN} (7.2)	179 (33.5)
Stage H	8^{FP} (5)	348^{TN} (92.8)	356 (66.5)
Total	160 (100)	375 (100)	535 (100)
Females			
	<18 years	**≥18 years**	**Total**
≤ Stage G	155^{TP} (96.9)	39^{FN} (10.4)	194 (36.3)
Stage H	5^{FP} (3.1)	336^{TN} (89.6)	341 (63.7)
Total	160 (100)	375 (100)	535 (100)

Note: TP; True positive, FP; False positive, TN; True negative, FN; False negative

Table 7. Contingency tables describing discrimination performance of the test on being adult (≥18) or minor (<18) for "stage D" of Olze et al third molar eruption

Males			
	Age status		**Total**
	<18 years	**≥18 years**	
≤ Stage C	119^{TP} (86.2)	7^{FN} (2.6)	126 (30.8)
Stage D	19^{FP} (13.8)	264^{TN} (97.4)	283 (69.2)
Total	138(100)	271 (100)	409 (100)
Females			
	<18 years	**≥18 years**	**Total**
≤ Stage C	123^{TP} (94.6)	8^{FN} (3.1)	131 (33.4)
Stage D	7^{FP} (5.4)	254^{TN} (96.9)	261 (66.6)
Total	130 (100)	262 (100)	392 (100)

Note: TP; True positive, FP; False positive, TN; True negative, FN; False negative

Table 8. Contingency tables describing discrimination performance of the test on being adult (≥18) or minor (<18) for "stage 1" of Olze et al radiographic visibility of root pulp

Males			
	Age status		**Total**
	<18 years	**≥18 years**	
Stage 0	13^{TP} (100)	9^{FN} (2.6)	22 (6.1)
≥ Stage 1	0^{FP} (0.0)	339^{TN} (97.4)	339 (93.9)
Total	13(100)	348 (100)	361 (100)
Females			
	<18 years	**≥18 years**	**Total**
Stage 0	7^{TP} (100)	13^{FN} (3.9)	20 (5.8)
≥ Stage 1	0^{FP} (0.0)	323^{TN} (96.1)	323 (94.2)
Total	7 (100)	336 (100)	343 (100)

Note: TP; True positive, FP; False positive, TN; True negative, FN; False negative

Table 9. Output measures from 2x2 contingency tables (95% confidence interval) to test the performance of cut-off value of I3M< 0.08, Demirjian "stage H", "Stage 1 (or higher)" of root pulp visibility (RPV) and "stage D" of third molar eruption to discriminate subjects as ≥ 18 years and <18 years of age in males and females.

Males	**I3M< 0.08**	**Stage H**	**Stage 1 (or higher) of RPV**	**Stage D of eruption**
Accuracy	95.8 (93.8- 97.4)	93.4 (91.1- 95.4)	97.5 (95.3- 98.8)	93.6 (90.8- 95.8)
Sensitivity	91.5 (86.2- 95.3)	84.9 (78.8- 89.8)	59.1 (36.3- 79.2)	94.4 (88.9- 97.7)
Specificity	97.8 (95.7- 99.1)	97.7 (95.6- 99.1)	100 (98.9- 100)	93.2 (89.7- 95.9)
LR+	42.23 (21.25- 83.95)	37.79 (18.99- 75.18)	--	14.07 (9.09- 21.76)
LR-	0.09 (0.05- 0.14)	0.15 (0.11- 0.22)	0.41 (0.25- 0.68)	0.06 (0.03- 0.12)
AUC	0.95 (0.93- 0.97)	0.94 (0.91- 0.96)	0.98 (0.97- 0.99)	0.92 (0.88- 0.95)
Females	**I3M< 0.08**	**Stage H**	**Stage 1 (or higher) of RPV**	**Stage D of eruption**
Accuracy	95.3 (93.1- 96.9)	91.7 (89.1- 93.9)	96.2 (93.6- 97.9)	96.2 (93.7- 97.8)
Sensitivity	88.5 (82.9- 92.8)	79.9 (73.5- 85.3)	35 (15.3- 59.2)	93.9 (88.3- 97.3)
Specificity	98.6 (96.7- 99.5)	98.5 (96.6- 99.5)	100 (98.8- 100)	97.3 (94.5- 98.9)
LR+	63.77 (26.6- 152.5)	54.49 (22.76- 130.4)	--	35.01 (16.84- 72.8)
LR-	0.12 (0.08- 0.18)	0.20 (0.15- 0.27)	0.65 (0.47- 0.9)	0.06 (0.03- 0.12)
AUC	0.95 (0.93- 0.97)	0.93 (0.91- 0.95)	0.98 (0.96- 0.99)	0.96 (0.93- 0.98)

Note: LR+, positive likelihood ratio; LR+, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value; Bayes PTP, Bayes post-test probability; AUC, Area under the curve; RPV, Root pulp visibility

Cameriere's third molar maturity index (I3M<0.08)

Cameriere's method of third molar maturity index (I3M<0.08)[6] is a widely used method in recent times for estimating adult age. When tested in various populations, it showed different results with varying accuracy, Se and Sp values. The results of the present study showed that the I3M< 0.08 provides the AUC value of 0.95 both for males and females. This indicates that a randomly selected individual (either male or female) from the older age category (≥18 years) will have I3M index less than 0.08 compared to a randomly chosen individual from the younger category (<18 years) approximately 95% of the time. The Se and Sp of this test was 91.5% and 97.8% for males and 88.5% and 98.6% for females. The proportion of the correctly classified individuals was 95.8% and 95.3% in males and females. These results were in line with the conclusions of the previous studies where higher Se and Sp values of greater than 90% were reported.[16-28] When these results were compared with studies within the same country, results differed.[29] Our study showed better accuracy, Se and Sp compared to the findings of Sharma et al.[29] The possible differences might be due to the age range studied, the sample size and the variation in the third molar development even within the same country.

As regards the results of likelihood ratio are concerned, the diagnostic test of I3M< 0.08 has a significant contribution to the prediction of legal age threshold of 18 years, due to the balanced values of LR+ and LR-. The cut-off value of 0.08 provided higher LR+ values (42.23 in males and 63.77 in females) meaning that there was excellent prediction of the probability of majority; and lower LR- (0.09 in males and 0.12 in females), indicating that the test is very good at identifying subjects younger than 18 years of age. As for the obtained results, the cut-off value of I3M< 0.08 successfully predicted age over 18 years in 96.3% of the males and in 94.7% of the females. In addition, 95% and 96.9% of predictions were accurate for the group younger than 18 years, for males and females respectively. This means that the cut-off value of I3M< 0.08 is suitable as an age marker for 18 years and it resulted in 5% and 3.1% of ethically unacceptable errors in males and females.

Demirjian's staging system

Unlike Cameriere's method, Demirjian's staging system is a subjective method of assessment that allows the examiner to determine the stage of tooth development. In this method, the mineralization of the root portion was categorised into four stages (E to H), where the last stage (stage H) indicated the closed apex or finished maturation of the tooth.[7] The more advanced the stage or final phase of the root development, the better it was for the discrimination between adults and minors.[30] From our study findings, we observed that the "stage H" of root maturity as a reliable marker to indicate that an individual was quite likely to be at least 18 years of age. The values of Se for "stage H" were 84.9% and 79.9% respectively, while the values of Sp were 97.7% and 98.5% in males and females, respectively. In the original study[6], the authors also obtained lower Se values (58%) and higher Sp values (98%) when "stage H" was used as a marker for adult age. In order to increase the Se of the test, they recommended the use of "stage G". However, it resulted in a significant decrease of the specificity to 90% and also the probability that the individual has reached 18 years. In another study by Quispe- Lizarbe et al[21], the authors reported a significant decrease of the specificity i.e., from 98% to 85% in males and 98% to 93% in females when "stage G" was used instead of "stage H" for indicating the age of majority. Similar results were reported by Costa et al[31] in Mexicans and Columbians and Sharma P et al in Indians.[32] According to Meinl et al[33] when "stage H" was determined in an Austrian individual, it was 99% certain that the individual was an adult. Our results concerning Se and Sp are fairly similar to those obtained in above-mentioned studies.[6, 21, 31] One difference between these studies and our study findings was better "Se" values when "stage H" was used as a cut-off value, which we believe is due to the extension of upper age limit to 30 years.

In the present study, the "stage H" showed 5% and 3.1% incorrect classifications (false positives) in males and females, with a better specificity, which is ethically mandatory for criminal proceedings. However, it has resulted in increased false negatives/ technically unacceptable errors (7.2% in males and 10.4% in females). The ROC curves demonstrated that "stage H" of Demirjian's method is able to properly discriminate adults from minors with AUC values of 0.94 and 0.93 in males and females, respectively. Regarding the aggregated estimation of the diagnostic performance, it is 37.79 times in males and 54.49 times in females, that "stage H" is more likely to occur in an individual at least 18 as opposed to someone younger than 18 (LR+).

In 2001, Solari and Abramovitch[34] added intermediate sub-stages (F1 and G1) to the later stages of root development of third molars in order to improve the accuracy of third molars to calculate the probability of an individual being under age 18. Future research should address the use of "stage G1" in the prediction of 18 years in the studied population.

Olze et al stages of root pulp visibility

From our study findings, stage 1" of RPV was chosen as a cut-off value for the 18 year old threshold of both sexes. The AUC, Se and Sp were 0.980, 59.1% and 100% in males; and 0.980, 35% and 100% in females, respectively. In the original study by Olze et al,[9] the authors studied the RPV in the OPGs of individuals aged between 15 and 40 years. According to their results, an age under 18 years (being a minor) can easily be excluded when individuals were classified as stage 1 and above of RPV in mandibular third molars. In the present study, few individuals (2.6% males and 3.9% females) who were marked as "stage 0" were adults (≥18 years) resulting in false negatives. However, all minors with completely matured third molars were presented with "stage 0" of RPV, indicating 100% specificity.

The results from this study, especially the higher specificity values and lower sensitivity values of "stage 1 (or higher)" of root pulp visibility may be partially explained by different age distributions. It is to be noted that the samples were skewed towards the younger ages with a higher number of samples in the older age range in the studied population. This uneven age distribution among the samples resulted from the selection criteria that only the mandibular third molars showing complete root formation should be analyzed. In the present study, we have analyzed OPGs of individuals aged between 14 and 30 years, following the age criteria similar to that of the original study[8] and the other studies[35-41] with age ranging from 15 to 40 years. It was reported in the literature that very few mature third molars i.e., approximately 5% to 9% were from the 16 year old age group. In the present study, all third molars were not fully matured until 17 years, and only 25% of them exhibited fully matured mandibular third molars in the age group of 17 years. Unfortunately, other studies[35-41] on the radiographic visibility of root pulp did not report these statistics on the number of third molars with incomplete mineralization below 18 years.

Despite the higher specificity and AUC values, one significant limitation of this method was the skewed age distribution due to the lack of young individuals (<18 years) with fully matured mandibular third molars. Therefore, this method should not be used in the studied population, especially to predict whether a male or a female has reached 18 years of age. In future studies, the authors must locate and evaluate an additional sample of equal and possibly higher number of younger individuals with fully matured third molars.

Olze et al stages of mandibular third molar eruption

Studies on the chronology of the third molar eruption are scarce. Age assessment based on the eruption of the teeth are simple, reliable, can be performed easily by medico-legal physicians who are not experts in forensic odontology.[42, 43] There is a need to understand the eruption of third molars and their use in forensic age assessment. Even though, it was mentioned in the literature that the emergence of third molars does not occur before the 17th year of life[44], few reports have indicated that they might emerge as early as 13 years,[45-47] that raised concerns regarding their use for forensic age assessment in a court of law. Later Olze et al[8] presented a classification comprised of four stages of third molar eruption, investigated and compared the chronological course of third molar eruption in Black South African[8], Japanese[48], German[49] and Canadian[50, 51] males and females. They concluded that the data based on the third stage of eruption can be utilized for forensic estimation of the minimum and most probable ages of investigated persons.

From our study findings, "stage D" of third molar eruption was chosen as a cut-off value for the 18 year old threshold of both sexes. The AUC, Se and Sp were 0.920, 94.4% and 93.2% in males; and 0.960, 93.9% and 97.3% in females, respectively. According to the likelihood ratio, it is 14.07 times in males and 35.01 times in females, that "stage D" of third molar eruption is more likely to occur in an individual at least 18 as opposed to someone younger than 18 (LR+). Our study results showed that 100% of individuals older than 18 years of age had attained stages C and D of third molar eruption. However, stage C was reached by 7.5% and 12.5% of males and females younger than 18 years. On the other hand, stage D was attained by 12% and 4.3% of males and females younger than 18 years, respectively. Therefore, when "stage D" was used to predict the legal age threshold 18 years, it has resulted in 13.8% and 5.4% of false positives or ethically unacceptable errors in males and females, respectively.

Our study findings indicated that the prediction of the age of majority using the eruption of the mandibular third molars is possible in the studied population, however with some important limitations. Third molars are by far the most commonly impacted teeth in the oral cavity.[52] It has been reported that 72% of a Swedish population and 68.8% of a Chinese population of young adults had at least one impacted wisdom tooth.[53, 54] In our study, we have observed that the rate of mandibular third molar impaction was 23.6% and 26.7% in males and females, respectively. Due to the non- inclusion of the impacted teeth in the scoring system by Olze et al[9] those teeth which were disturbed by impaction were excluded from the analysis, which further limited the use of this approach.

Influencing factors

Sex is considered to be one of the factors that influences the biological maturation of the skeletal and dental parameters. It is widely accepted that the maturation of skeletal parameters occurs earlier in females than their male counterparts. Concerning the dental maturation, unlike other teeth, third molar maturation occurs earlier in males than females. Our study results corroborated the findings of the literature.[10, 34, 55] The second factor to consider is the difference in the maturation/development between the right and left sides. It was clearly highlighted in the literature that there were no side differences with respect to dental maturation.[56, 57] No side differences were reported in the studied population from previous studies,[22, 58] and therefore the maturation/eruption/secondary dentine formation of lower left mandibular third molars were studied. Another factor to consider is the representativeness of the sample. In the present study, we could not take the socio-economic status into consideration due to the retrospective nature of the study. In forensic and legal scenarios, children are typically from impoverished environments with poor nutritional status during growth.[59] Therefore, we believe that these may not perform accurately, and would likely increase the number of false negatives (appear younger than they are) in individuals who were malnourished. However, considering the context of criminal proceedings, it is more important to avoid false positives (appear older than they are), these methods can perhaps be considered as a conservative approach to this age estimation problem. Considering secondary dentine formation, future research should investigate the influence of dietary habits and modern dental health care on the radiographic visibility of the root pulp in third molars.

CONCLUSIONS

According to our search of the forensic literature, this is first radiographic study to compare the accuracy of four dental age estimation methods to indicate the legal age over 18 years in south Indian adolescents and young adults. The following conclusions can be drawn from our study findings:

1. The specific cut-off value of I3M< 0.08 showed better accuracy, higher sensitivity and specificity values of >90% for discriminating adults from minors in the studied population.
2. "Stage H" of the Demirjian staging system could be useful in answering the medico-legal question on whether a subject is at least 18 years of age. However, it has resulted in decreased sensitivity values (false negatives).
3. "Stage 1(or higher)" of RPV has resulted in 100% specificity suggesting the strong possibility that the individuals are aged over 18 years. However, the skewed age distribution towards younger age groups and greater percentage of third molars with incomplete mineralization has limited the applicability of this method.
4. "Stage D" of tooth eruption i.e., complete emergence of the third molars in the occlusal plane could be useful in predicting the age equal to or over 18 years. Although, it is easy to use in a daily clinical context, increased percentage of third molar impactions has limited the use of this approach.

Despite the limitations, these determinations can be seen as preliminary and tentative attempts at improving age of majority (18 years) determinations in the living individuals of south Indian origin from the maturation, eruption and the secondary dentine formation in mandibular third molars.

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Evaluation of third molar maturity index (I3M) in assessing the legal age of subjects in an Indian Goan population

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KEYWORDS
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ABSTRACT

India affords special laws and exemptions to minors under the criminal, marriage, labour and administrative laws. Many perpetrators claim to be a minor in the hope of a lenient trial and verdict. The authorities often rely upon forensic experts to provide evidence-based reports. The third molar can be relied upon in the assessment of legal age as it continues developing into the early twenties. The method established by Cameriere et al in 2008 provides an objective method for the accurate evaluation of legal age. Our study was designed to analyze and validate the efficacy of Third Molar Maturity index (I3M) in an Indian Goan population and compare it to published literature. 542 panoramic radiographs of subjects aged between 14 and 24 years were evaluated. The chronologic age increased as I3M reduced. There was no evidence of sexual dimorphism in third molar development across various I3M classes ($p>0.05$). Receiver Operator Characteristic Curve was plotted for males and females which showed an Area Under Curve of 0.95 (95% CI, 0.92-0.97) and 0.93 (95% CI, 0.90-0.96) respectively. 2x2 contingency tables were used to test the performance of various I3M cut-off values ranging from I3M=0.02 to 0.14. I3M = 0.08 showed the most promising results for the assessment of legal age. Our study achieved a high degree of accurate classification of 0.90 and 0.88 for males and females respectively. Results demonstrate a sensitivity of 0.899 and 0.854 and specificity of 0.90 and 0.93 for males and females respectively. The positive likelihood ratios were 9.88 and 12.44 while negative likelihood ratio was 0.11 and 0.15 for males and females respectively. A favourable Bayes Post Test Probability of 0.95 was noted for both males and females. These results allow us to strongly recommend the use of I3M for the assessment of legal age in an Indian Goan population.

INTRODUCTION

It is common to find undocumented minors facing civil or criminal charges. In all cases of suspicion and in accordance with Indian criminal, marriage, and administrative laws, the prosecutor or corresponding administrative authority is obliged to establish the age of the purported minor[1]. This necessitates accurate and reliable estimation of legal adulthood of a subject. The application of the most widely used age estimation techniques such as Demirjan's[2] and Willems[3] in assessment of legal adulthood have some drawbacks. They are subjective, rely on evaluation of parameters influenced by environmental and nutritional factors or provide an age range as opposed to an

accurate age. Thus, these approaches may not be applicable while attempting to assess legal adulthood.
Dental age estimation using the third molars could be ideal for estimation of legal age as they are the last teeth to develop and they can offer information with regard to this critical age[4].
Cameriere's third molar maturity index (I3M) offers a technique which uses objective analysis of the third molar development to assess legal age[5]. This potentially overcomes the drawbacks previously stated. The method uses the ratio of inter-pulpal distance between the open apices of the third molar and the height of the third molar. A threshold (cut-off) value of 0.08 was identified and used to discriminate between individuals who are above or below legal age.
Multiple studies have been published in recent literature evaluating the efficacy of the I3M cut-off of 0.08 in assessing legal age of 18 in their populations[6,8-20]. It has been established that the development of the dentition can vary with race[21]. Thus, it is important to verify the third molar maturity index of the local population and to compare them to published literature from other populations. The aim of this study was to evaluate the cut-off value of I3M=0.08 previously recommended by Cameriere in an Indian Goan population.

MATERIAL AND METHODS

Sample Collection
Panoramic radiographs (OPGs) of 576 Indians between 14 – 24 years of age were collected from the database of the Department of Oral Medicine and Radiology, Goa Dental College. The inclusion criteria ensured images of good quality, complete patient records and of known age (14 – 24 years) when the OPG was obtained. The samples with missing third molars, incomplete records, developmental anomaly or pathology involvement were excluded. The final sample size used for evaluation was 542. (Table 1)
The OPG number, chronologic age, gender and date of radiography was recorded using Microsoft Excel while maintaining anonymity of the patient. Institutional Research Ethical Committee approval was grated and the study was conducted in accordance with the ethical standards of the Declaration of Helsinki.

Table 1. Number of samples collected within various age groups where number in parenthesis represents samples with closed apices of Left Third Molar (I3M=0)

Number of Samples for I3M			
Age (Years)	**Males**	**Females**	**Total**
14 – 14.9	35	27	**62**
15 – 15.9	31	22	**53**
16 – 16.9	31	29	**60**
17 – 17.9	24 (3)	24 (3)	**48**
18 – 18.9	33 (14)	29 (8)	**62**
19 – 19.9	33 (19)	30 (12)	**63**
20 – 20.9	31 (28)	34 (21)	**65**
21 – 21.9	28 (23)	31 (23)	**59**
22-24	23 (20)	47 (41)	**70**
Total	**269**	**273**	**542**

Measurements
The sample was obtained and stored as a JPEG. The chronologic age of the subject was calculated by subtracting the date of birth from the date on which OPG was obtained. Adobe Photoshop CS7 was used to adjust colour, contrast, grey scale to improve the quality of image and to carry out the measurement of I3M. The left third molar of males and females was evaluated separately in accordance with previously published literature for the purpose of standardization. I3M was determined as previously described by Cameriere et al.[3] Briefly, if the third molar has root development complete, i.e., apical ends of the roots completely closed, then I3M = 0, otherwise, I3M is evaluated as the inter-pulpal distance (A) divided by the tooth length (L). In case of a multi-rooted tooth the inter-pulpal distance is measured at the apex as the sum of the distances between the inner sides of each root (A1 + A2). Both impacted and non-impacted third molars were included in the study.

Statistical Analysis and Data Management
SPSS Statistics 22.0 for Windows (IBM® SPSS®) and MS Excel 2016 (Microsoft® Office 2016) were used for all statistical analyses and data

management. All measurements and calculation of I3M were performed after blinding of the OPG records by the first observer. Inter-observer and intra-observer reliability was calculated using Inter-class Correlation Coefficient (ICC) on 56 random OPG samples (10% total sample size) to assess the correlation between the first and second observers respectively. To calculate intra-observer error, the first observer repeated the measurements of OPGs after one month of recording the first set of observations. Mann Whitney U test was performed to evaluate sexual dimorphism within I3M classes.

A Receiver Operator Characteristic Curve (ROC Curve) was used to assess the reliability of the test. It is a graphic representation of the relationship between sensitivity (Se) and specificity (Sp) in a test. The curve is confined in a unit square. The left-lower corner (Se = 0, Sp = 1) corresponds to the highest possible test cut-off value. As the cut-off value decreases, the test Se increases and Sp decreases, moving on the curve from the left-lower corner up and to the right to ultimately reach the right-upper corner of the square where Se = 1 and Sp = 0, corresponding to the lowest possible test cut-off value. The area under the curve (AUC) is indicative of the reliability of the test. An AUC of 0.5 would mean a worthless test, on the other hand an AUC of 1 depicts a perfect test.[21] A 2x2 contingency table was plotted to test the performance of other I3M cut-off levels. The results showed the number of participants who were 18 and above with I3M < the cut-off value, those who were less than 18 years with I3M ≥ cut-off value, those who were under 18 with I3M < the cut-off and those who were older than 18 with I3M ≥ the cut-off. A participant who is 18 and above with I3M< cut-off value was defined to have a positive test.

Using the 2x2 contingency table, measurements for Accurately Classified (AC) individuals, the Sensitivity (Se), Specificity (Sp), Youden's Index (J-Index), Positive Predictive Value (PPV), Negative Predictive Value (NPV), Positive Likelihood Ratio (LR+), Negative Likelihood Ratio (LR-) and Bayes Post Test Probability (Bayes PTP) was calculated for various cut-off values. J-index is a function of the Se and Sp and captures the performance of a diagnostic test. The PPV is the proportion of the participants who were true positives. The NPV is the proportion of the participants who were true negatives. The LR+ is the ratio of the true positive rate (sensitivity) to the false positive rate (1 −Se). This likelihood ratio statistic measures the value of the test for increasing certainty about a positive diagnosis. The LR- is the ratio of the false negative rate to the true negative rate (Sp). The likelihood ratios indicate how many times more or less likely adults are to have I3M<0.08 than minors and minors are to have I3M≥0.08 than majors.

Bayes Post Test Probability (Bayes PTP) may be written as

$$P = \frac{P1P0}{(1-P2)(1-P0)+P1P0}$$

where P is post-test probability, P1 is Sensitivity, P2 is Specificity and P0 is the probability that the participant in question is 18 years or older given that he or she is aged between 14 and 24 years, which represents the target population. In this study, probability P0 was calculated as the proportion of participants between 18 and 24 years of age and those between 14 and 24 years who live in Goa. This data was obtained from the 2011 census for India. The P0 was found to be 0.65 and 0.63 in males and females respectively.

RESULTS

The mean age of the total sample among males and females was found to be 18.31 and 19.01 years respectively. The first case of apical closure was noted at the age of 17 years for males and females. As expected, the number of cases showing apical closure increased steadily as the age increased (Table 1). It was observed that a greater proportion of males achieved apical closure at a younger age compared to females (Table 1). The highest I3M value noted was 1.48. A box plot showing the relationship between chronologic age and I3M demonstrated that the I3M ratios increased as the mean ages decreased. (Figure 1)

The ICC results yielded 0.992 (95% CI, 0.987 to 0.996) and 0.994 (95% CI, 0.987 to 0.997) for the intra-observer and inter-observer tests respectively. There was no sexual dimorphism observed within the I3M classes (Table 2).

The ROC curves for the cut-off value of 0.08 (Figure 2) showed excellent results with an AUC of 0.952 (95% CI, 0.927 to 0.978) and 0.934 (95% CI, 0.902 to 0.966) for males and females respectively. A further indication of an excellent test result can be observed as the ROC curves demonstrate an initial rise vertically from the lower left corner and then moves horizontally along the upper line[22].

Figure 1. Boxplot Graph showing relation of Chronologic Age & I3M Classes.

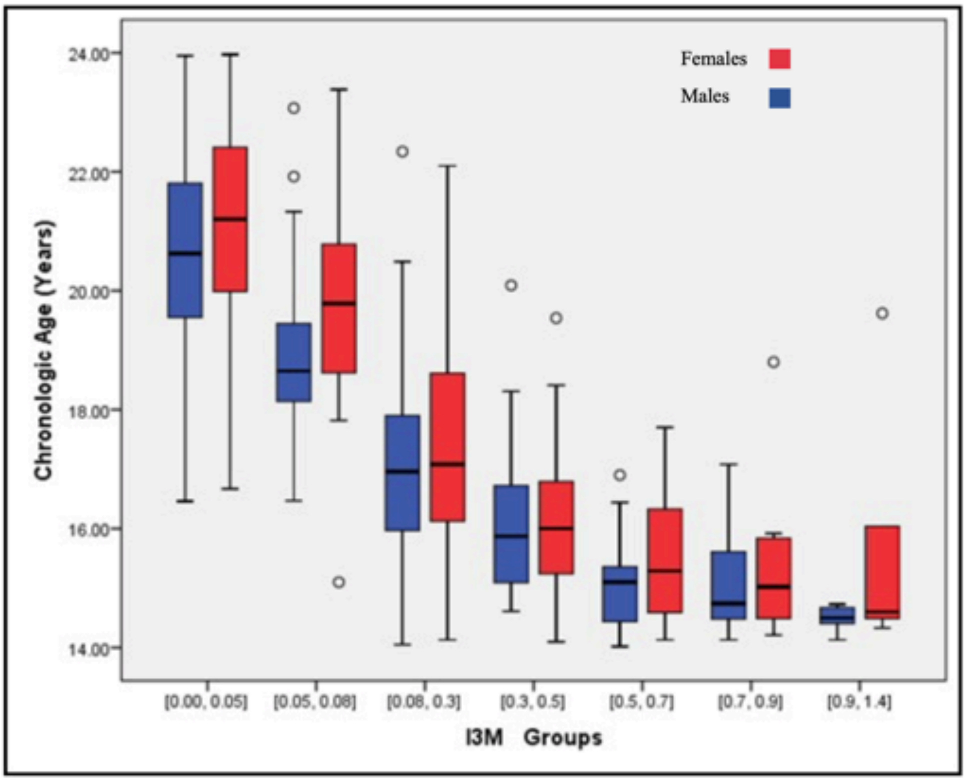

Table 2. Summary of descriptive statistics of chronological age according to third molar maturity index (I3M)

I3M	Males								Females								
	N	Mean	SD	Min	Q1	Median	Q3	Max	N	Mean	SD	Min	Q1	Median	Q3	Max	P*
[0.00,0.05]	117	20.54	1.60	16.46	19.52	20.61	21.81	23.95	123	21.10	1.66	16.67	19.94	21.20	22.45	23.97	0.349
[0.05,0.08]	27	18.84	1.57	16.47	18.05	18.65	19.53	23.07	30	19.88	1.68	15.10	18.61	19.79	20.81	23.38	0.259
[0.08, 0.3]	58	17.00	1.58	14.05	15.91	16.96	17.91	22.34	67	17.32	1.87	14.13	16.12	17.08	18.65	22.10	0.795
[0.3, 0.5]	21	16.08	1.36	14.61	15.03	15.87	16.78	20.09	21	16.12	1.31	14.10	15.12	16.00	16.80	19.54	0.302
[0.5, 0.7]	17	15.12	0.83	14.02	14.37	15.10	15.67	16.90	17	15.58	1.21	14.13	14.51	15.29	16.59	17.70	0.642
[0.7, 0.9]	22	15.03	0.76	14.13	14.48	14.74	15.61	17.08	9	15.41	1.42	14.21	14.42	15.02	15.88	18.80	0.223
[0.9, 1.4]	7	14.50	0.21	14.13	14.35	14.50	14.69	14.73	6	15.61	2.06	14.33	14.45	14.60	16.94	19.62	0.317

Legend : * Mann Whitney U Test, Number of individuals (N), Mean, standard deviation (SD), minimum value (Min), 1st quartile (Q1), median, 3rd quartile (Q3) and maximum value (Max) of age distribution for each I3M class, for females and males.

Figure 2. Receiver Operator Characteristic Curve for I3M for males & females

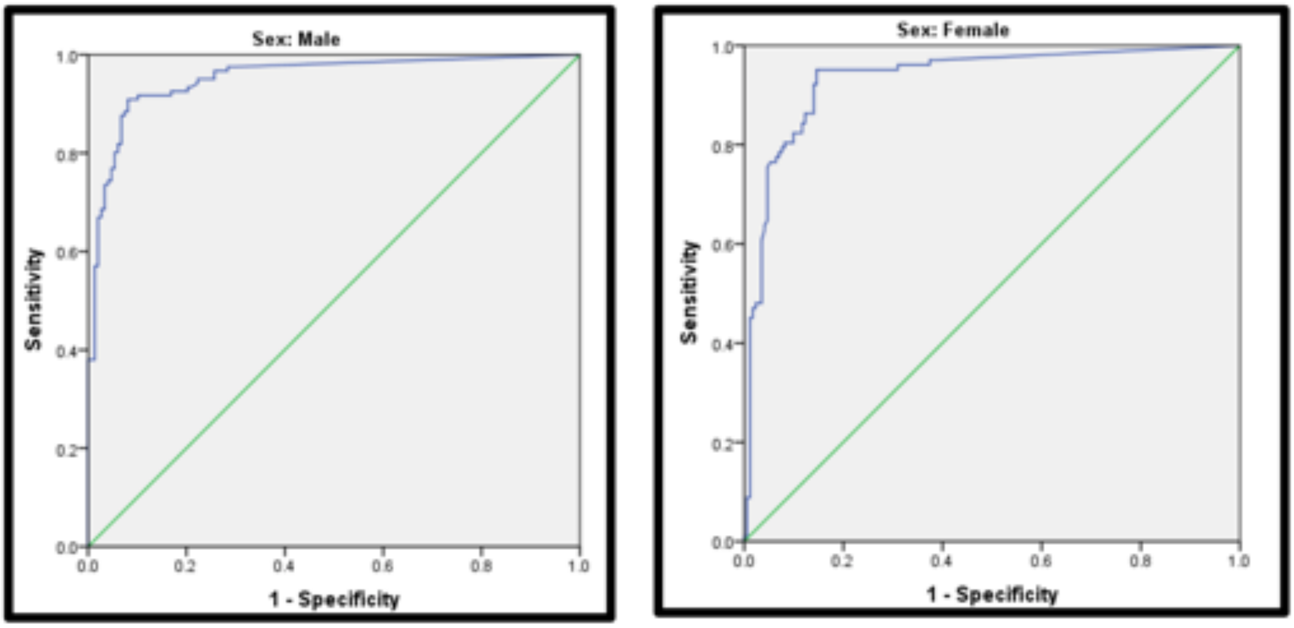

Table 3. Contingency table describing discrimination performance of the test for different cut-off values of third molar maturity index (I3M) in males and females.

I3M (Males)				I3M (Females)			
	Age				Age		
Test	≥18	<18	Total	Test	≥18	<18	Total
I3M < 0.02	106	4	110	I3M < 0.02	107	3	110
I3M ≥0.02	42	117	159	I3M ≥0.02	64	99	163
I3M < 0.04	110	4	114	I3M < 0.04	112	4	116
I3M ≥ 0.04	38	117	155	I3M ≥ 0.04	59	98	157
I3M < 0.06	118	8	126	I3M < 0.06	134	5	139
I3M ≥ 0.06	30	113	143	I3M ≥ 0.06	37	97	134
I3M < 0.08	133	11	144	I3M < 0.08	146	7	153
I3M ≥ 0.08	15	110	125	I3M ≥ 0.08	25	95	120
I3M < 0.1	136	14	150	I3M < 0.1	147	13	160
I3M ≥ 0.1	12	107	119	I3M ≥ 0.1	24	89	113
I3M < 0.12	138	16	154	I3M < 0.12	150	15	165
I3M ≥ 0.12	10	105	115	I3M ≥ 0.12	21	87	108
I3M < 0.14	138	17	155	I3M < 0.14	150	16	166
I3M ≥ 0.14	10	104	114	I3M ≥ 0.14	21	86	107
Total	**148**	**121**	**269**	**Total**	**171**	**102**	**273**

Table 3 shows a consolidated 2x2 contingency table for various age groups. Tables 4 and 5 are comparative tables showing derived values for various I3M cut-off values for males and females respectively.

In males, as observed in Table 4, for the cut-off value 0.08 the AC, Sp, J-Index and Bayes PTP were 0.903, 0.909, 0.808 and 0.949 respectively. I3M cut-off of 0.06 performs better when Sp and Bayes PTP are considered but the AC and J index are significantly lower at 0.859 and 0.731 respectively. The cut-off of 0.1, 0.12 and 0.14 shows marginally better performance when Ac and Se are considered but under-performs in other parameters.

In females, as observed from the findings of Table 5, for the I3M cut-off of 0.08, the AC, Sp, J Index and Bayes PTP was 0.883, 0.931, 0.785, 0.955 respectively. The I3M ratio of 0.06 shows better Sp of 0.951 but also has a lower Ac and J Index of 0.846 and 0.735 respectively. The I3M cut-off of 0.08 out-performs the cut-off of 0.1 in most other parameters.

From a practical standpoint, medico-legal tests used to estimate legal adulthood, must give appropriate weighting to the cut-off value with higher specificity while maintaining adequate results of other parameters. It is important to ensure that the test shows a low proportion of false positives (minors identified as adults). The gradual decrease in the PPV, LR+ and LR- values and an increase in the NPV values is an expected result as the cut-off value increases. Therefore, the I3M cut-off of 0.08 shows the best accuracy and reliability for both males and females.

Table 4. Comparative chart showing values derived from contingency table for various I3M cut-off values to determine legal age in Indian Goan population

	I3M Male						
	0.02	**0.04**	**0.06**	**0.08**	**0.1**	**0.12**	**0.14**
AC	0.829	0.844	0.859	0.903	0.903	0.903	0.900
Sensitivity	0.716	0.743	0.797	0.899	0.919	0.932	0.932
Specificity	0.967	0.967	0.934	0.909	0.884	0.868	0.860
J-index	0.683	0.710	0.731	0.808	0.803	0.800	0.792
PPV	0.964	0.965	0.937	0.924	0.907	0.896	0.890
NPV	0.736	0.755	0.790	0.880	0.899	0.913	0.912
LR+	21.666	22.483	12.059	9.885	7.942	7.052	6.637
LR-	0.293	0.266	0.217	0.111	0.092	0.078	0.079
Bayes PTP	0.976	0.977	0.958	0.949	0.938	0.930	0.926

Legend: AC accurate classification, Se sensitivity, Sp specificity, J-index Youden index, PPV positive predictive value, NPV negative predictive value, LR+ positive likelihood ratio, LR-, negative likelihood ratio, Bayes PTP Bayes post-test probability

Table 5. Comparative chart showing values derived from contingency table for various I3M cut-off values to determine legal age in Indian Goan population.

	I3M Female						
	0.02	**0.04**	**0.06**	**0.08**	**0.1**	**0.12**	**0.14**
AC	0.755	0.769	0.846	0.883	0.864	0.868	0.864
Se	0.626	0.655	0.784	0.854	0.860	0.877	0.877
Sp	0.971	0.961	0.951	0.931	0.873	0.853	0.843
J-index	0.596	0.616	0.735	0.785	0.732	0.730	0.720
PPV	0.973	0.966	0.964	0.954	0.919	0.909	0.904
NPV	0.607	0.624	0.724	0.792	0.788	0.806	0.804
LR+	21.275	16.702	15.986	12.441	6.745	5.965	5.592
LR-	0.386	0.359	0.228	0.157	0.161	0.144	0.146
Bayes PTP	0.973	0.966	0.965	0.955	0.920	0.911	0.906

Legend: AC accurate classification, Se sensitivity, Sp specificity, J-index Youden index, PPV positive predictive value, NPV negative predictive value, LR+ positive likelihood ratio, LR-, negative likelihood ratio, Bayes PTP Bayes post-test probability.

DISCUSSION

In India the Juvenile Justice Act exempts minors from criminal liability and they are subject to special criminal standards[23]. Many perpetrators claim to be juveniles in the hope of a lenient trial and verdict when arrested and presented before the court. The authorities and courts have often relied upon forensic odontologists for age estimation to offer an evidence-based methodology for this issue. In such cases the fundamental question that needs to be answered, is the subject a major or a minor rather than the exact age.

Age estimation of non-adults can be performed by using skeletal indicators or dental age estimation. There are reports that question the accuracy of skeletal indicators due to variations in bone development, which can be influenced by nutritional and environmental factors[24]. Furthermore, ossification of hand and wrist bones is completed by 18 years and thus cannot be used to evaluate legal age[25]. Tooth development on the other hand, is controlled more by genetics rather than by environmental and nutritional factors[24]. Dental development is independent of the exogenous factors such as nutrition[26], disease[26-28], mental stress[26,27], pathology [29] and environmental factors[30]. Among subjects over the age of 14 years, radiographic evaluation of mandibular third molars is often relied upon as it is the only tooth continuing to develop at this age[31]. Cameriere's study on the third molar maturity index provides us with an objective and reliable technique to determine legal age. It was found that geographical differences between third molar development are of negligible clinical influence and thus can be considered and applied beyond geographic boundaries[29,32].

The studies on I3M have been carried out on a wide demography which includes North America[12], South America[17], Europe[7,9,13,16], Africa[4,11,15], Asia[25] and Australia[14]. Balla S.B. et al carried out the first Asian study on South Indian population[25]. Our study was the second study carried out in an Indian population and the first study in a Goan population.

Our sample included Indian Goans ranging from 14 to 24 years of age. The first case of apical closure was noted at 17 years in both males and females which is consistent with findings of other studies[15,16]. Hence, it could be stated that the minimum age for apical closure is 17 years for both sexes.

Many studies show evidence that there is an overall lower degree of third molar development in females compared to males[15,16]. The same can be observed in the present sample (Table 1) which could indicate a possible male precedence in the development of third molars. However, there was no statistical difference noted on comparison of mean ages within I3M classes for both sexes.

For the cut-off value of 0.08, our study noted an AUC of 0.95 and 0.93 for males and females respectively which is comparable with the results obtained by other studies[15,17]. Comparatively, 90% males were accurately classified as compared to 88% for the females. The Se and Sp for males (0.89, 0.90) and females (0.85, 0.93) were comparable with other studies[7-10,12,15-17].

The PPV, NPV and LRs are useful in forensic odontology to analyse the probability of an individual being at least 18 years of age. The lesser false positives (higher specificity) signify a higher PPV in the observed population and lesser false negatives (higher sensitivity) will have a higher NPV in the observed population. We noted PPV values of 0.92 and 0.95 for males and females respectively.

The LR values combine sensitivity and specificity into a single figure that indicated by how much the test result will reduce the uncertainty of a given diagnosis. The LRs allow the researcher to study the ability of a test to alter a pre-test probability of being a major into a post-test probability. A LR+ > 10 and a LR- < 0.1 are considered to exert highly significant changes in probability.[16] If the LR value is close to 1 it indicates a worthless test. Studies by Cavric J. et al[15], Zelic K et al[16] and Quispe- Lizarbe Roselhy, J. et al[17] recorded high LR+ values ranging from 13 - 37. The lowest LR+ was noted by Franklin D et al[14] at 6.04. In the present study the LR+ was 9.88 in males and 12.4 in females. This would indicate that the likelihood of being at least 18 years, if the mandibular third molar had achieved apical closure was at least 9 and 12 times more likely in males and females respectively. The LR- was recorded at 0.11 and 0.15 for males and females respectively. A LR- of less than 0.1 was noted in some studies[13,16,17]. A majority of the studies noted LR- between 0.1 – 0.15 8,14,15.

The Bayes PTP indicates the probability of an individual who was classified as a major to factually being a major. This calculation factors in the proportion of individuals who are above 18 years in the representative demography. Bayes PTP for our study was 0.95 for both males and females.

The J-Index captures the performance of a dichotomous diagnostic test. It is a function of sensitivity and specificity. It denotes the maximum distance of the ROC Curve from the diagonal line. The value ranges from 0 to 1. A value of 1 indicates a perfect test whereas, 0 indicates a worthless test. Cavric J et al[15] noted a value of 0.82 and 0.84 for males and females respectively. The present study showed comparable results 0.8 and 0.78 for males and females respectively.

On comparison of various cut-off values (Tables 4 and 5), I3M cut-off value of 0.08 showed the best performance I3M with respect to AC, J index, Sp and Bayes PTP. The cut-off value of 0.1 showed marginally higher Se for both sexes. This increase in Se does not justify selecting 0.1 as a cut-off value over 0.08, as the Sp and post-test probability is higher for the cut-off value of 0.08. A similar inference was made by Cavric J. et al[15].

In conclusion, the third molar maturity index as described by Cameriere et al[5] offers a reliable and objective method for estimation of legal adulthood. According to Corradi et al[33] 51% of correct classification may be sufficient for civil cases with "more probable than not" evidence while very high levels, at least 90 %, was needed for criminal cases which require "beyond all reasonable doubt" evidence. Hence, the I3M cut-off of 0.08 can be utilised reliably for forensic application in criminal and judicial courts.

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Age estimation from dry bone measurements: evidence from a sample of soldiers exiled in two concentration camps in Bari

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ABSTRACT

The mandible undergoes remodelling and morphological alterations throughout the life of an individual, and it is subjected to sex- and age-related structural changes. Personal identification from skeletal remains represents one of the most difficult challenges for a forensic anthropologist. The study of mandibular morphology is an important aid in determining the sex and age of skeletal remains. The objective of this study was to evaluate the age-related changes of three mandibular dimensions through dry bone measurements: bigonial width, ramus height and gonial angle. A total of 93 skeletal remains were included in this study, from a group of soldiers of Yugoslav origin who lived in two concentration camps in Bari (southern Italy) during World War II. These are included in the collection of the Forensic Anthropology Laboratory of the Institute of Forensic Medicine of Bari. The measurements were recorded after comprehensive examination by a forensic anthropologist and a forensic odonto-stomatologist. The data obtained were analysed statistically using a bivariate test and a multivariate linear regression model, using the Statal 13MP software. The results indicate that the bigonial width and gonial angle vary significantly according to age. In conclusion, this study confirms that the mandible is useful for age estimation in the identification of skeletal remains using these specific mandibular measurements when performed on dry bone without radiological distortion.

INTRODUCTION

A key objective of the practice of forensic anthropology is to ensure identification of recovered human remains in a medico-legal context. To determine the species, sex, age at death, stature, time since death, and estimate specific morphometric characteristics provide opportunities to identify missing people.[1] The identification of human remains may be necessary as a result of accidents and in criminal investigations or ethnic studies. In such cases, the estimation of age and sex is one of the first objectives sought by the medical examiner. The study of age and sex based on the analysis of skeletal remains can be of particular difficulty for anthropologists, especially when such studies need to be carried out on small skeletal fragments.

Evaluation of the morphological characteristics of the skull and mandible constitutes a commonly used sex-estimation approach for forensic anthropologists. In particular, analysis of the mandible can be used for sex estimation when it is not possible to assess the skull or the pelvis. The bone and tooth tissues that can be extracted from human remains can thus be used in the age-estimation process.

The mutual dependence that is recognized between the passing of time and the morphological changes of the human body may be useful in the estimation of age at death. Such ageing patterns can be detected by both microscopic examination and macroscopic observation. Furthermore, several studies have shown that measurements made directly on bones are more likely to provide true information, which is why the methods using this technique are the most reliable in determining sex and age.[2]

Recently, studies have highlighted that the anatomy and shape of the mandible undergo changes according to both age and sex.[3-9] The morphology of the mandibular base depends on the occlusal state and the contractile strength of the masticatory muscles; i.e., the medial pterygoid and masseter muscles.[10] With age, there are modifications to the structure of the masticatory muscles that are demonstrated in reduced contractile strength. Indeed, in edentulous subjects, there is frequently a variation in mandibular shape, with reduction in the contractile strength of the masticatory muscles secondary to the loss of the teeth.[11]

Sex differences in the morphology of the mandible are a consequence of different genetic patterns, whereby in male subjects the mandible grows exponentially during puberty, unlike for females. Furthermore, the masticatory muscles are less voluminous in females than males, and this results in differences between the sexes in the shape of the mandible.[12] Several anthropological analyses have been carried out on these dimorphic characteristics. Different mandibular dimensions have been studied, such as the gonial angle, ramus length, bicondylar breadth, bigonial breadth and mandibular base length.[8]

The present study focuses on three of these mandibular dimensions as determinants of age: gonial angle, bigonial width and ramus height. Although there is some information in the literature about age- and sex-related changes of these measurements, the findings of various studies have not been consistent. Some studies have shown variations in the bigonial width and ramus height with age and sex,[9,10] while other studies have not confirmed these.[3,13] In the same way, age- and sex-related alterations in the gonial angle have been demonstrated in some studies,[14,15] while other studies highlight different results.[7,9,12]

The literature shows how these correlations have been studied through radiological investigations, such as with panoramic radiographs, computed tomography and cephalograms. To further examine skull age-related dimensional variations, the present study instead recorded the three mandibular measurements of gonial angle, bigonial width and ramus height directly from the skulls of a skeletal collection.

MATERIAL AND METHODS

To demonstrate these variations in the mandibular morphology, the sample group consisted of the skeletons of 93 soldiers from the Royal Yugoslavian Chetnik Army who were in exile in Bari in 1941 (89 men, 4 women), which form part of the skeletal collection of the Institute of Legal Medicine at the University of Bari (southern Italy). The samples were from Caucasian Mediterranean subjects who had lived in two concentration camps in the Bari area during the Second World War, and who were aged from 1 year to 75 years. All of the mandibles used were intact or not excessively fragmented, with no proportional discrepancies and no bone disease, and with clear and accessible surfaces for the measurements that were deemed suitable for this study. All distorted, incomplete, or fractured mandibles were excluded from this study. From an original population of 93 individuals (100%), 37 (39.8%) were removed from this study after application of these exclusion criteria. The remaining 56 (54 men, 2 women) were included in this analysis, and were aged between 18 years and 62 years (mean ±SD, 33.08 ±11.54 years).

A single forensic anthropologist recorded the three mandibular measurements. To ensure greater reliability and reproducibility, the measurements obtained were also reassessed by a forensic odonto-stomatologist at a later date. The mandibles underwent measurements of the bigonial width, the mandibular ramus height and the gonial angles using a goniometer and callipers. The angle formed by the intersection of the lower margin of the body of the mandible and the rear margin of the ramus was measured as the gonial angle. The bigonial width was measured as the distance between the two opposite gonial angles. The ramus height was measured as the

distance from the gonion to the upper margin of the mandibular condyle.
The descriptive statistics for each set of mandibular measurements were recorded, including the means ±standard deviation and ranges (minimum to maximum) in the males and females. The initial analyses were performed using an Excel database, and the statistical analysis was performed using the Statal 13MP software (StataCorp LLC, College Station, Texas).
Bivariate analysis was used to compare the study parameters with age. Then all of the independent variables with a p-value ≤0.25 were considered suitable for the multivariate analysis. Thus, a multivariate linear regression model was used to correlate the independent variables with age. The results of the multivariate analysis are expressed with 95% confidence intervals (CIs), with statistically significant taken as a p-value <0.05.

RESULTS

The descriptive statistics reported in Table 1 show the sex differences for the bigonial width, ramus height and gonial angle. Males have higher values for the bigonial width compared to the females. The mean ramus height and gonial angle of the male population were slightly lower than those of the female population.

Table 1. Mandibular values obtained for the whole sample analysed

Mandibular measure	Males				Females			
	Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Bigonial width (cm)	10.13	0.72	8.9	11.9	9.7	0.2	9.5	9.9
Ramus height (cm)	6.48	0.71	4.0	8.1	6.5	0.2	6.3	6.7
Gonial angle (°)	118.72	5.76	106	132	122	6	116	128

Bivariate analysis provided an estimate of the existing associations between each single independent variable (i.e., bigonial width, ramus height, gonial angle) and the outcome of interest, as age. As shown in Table 2, all three of these parameters were considered suitable for the subsequent multivariate analysis (p-value ≤0.25).

Table 2. Summary of the significance from the bivariate analysis for the comparison of the study parameters with age

Mandibular measure	*p*
Bigonial width	0.007
Ramus height	0.19
Gonial angle	0.005

Finally, each of these variables was inserted into the multivariate linear regression model that was developed to assess the association between the variables and the dependent variable, as age. The results from the multivariate analysis are summarised in Table 3, which shows significant association between age and bigonial width and gonial angle (p-value <0.05).

Table 3. Summary of the significance from the multivariate linear regression analysis for the comparison of the study parameters with age

Mandibular measure	*p*	95% Confidence interval	
Bigonial	0.025	0.666	9.538
Ramus height	>0.05	/	/
Gonial angle	0.016	-0.423	-0.045

DISCUSSION AND CONCLUSIONS

The published literature indicates that the mandibular dimensions have been considered as one of the most useful parameters to determine sex and age in human remains as accurately as possible. From a medico-legal point of view, odontology is commonly used to identify human remains, as skulls and teeth can give accurate information about the age of an individual. This is why there are many age assessment criteria based on dental methods.[16]
Forensic odontology can have a useful role in determining the age and sex of subjects with unrecognizable body structures. There are differences in dental characteristics between the

two sexes, such as morphology, crown size, and root lengths.[17] There are also other characteristics of the teeth that are linked to changes that occur over time, and are thus also useful in determining age.

Previous morphometric analyses using panoramic radiographs have demonstrated significant results for age- and sex-related alterations to the jaw.[5] In this way, radiography has become a useful tool in studies that are designed for forensic age and sex estimation. In contrast, the present study was designed to determine age-related changes in the mandible through direct measurements from the bone, without the aid of radiographs or other imaging modalities.

The parameters assessed here were bigonial width, ramus height and gonial angle. Bigonial width and ramus height represent the horizontal and the vertical dimensions of the mandible, respectively. Furthermore, the gonial angle is formed by the intersection of an anteroposterior tangent with a vertical line. The implication of these three parameters is that they represent the mandible across all of the planes, which is important to assess mandibular shape and to demonstrate the effects of ageing in the process of modification of the mandible. Many investigations have studied age and sex alterations according to other parameters,[6] but the relation between these three parameters has been studied by very few.[3,10]

This study investigated these changes in the mandibular dimensions in adult soldiers who lived in exile in two concentration camps in Bari during World War II, with dry bone measurements included for 56 male and female individuals. The mean age of all of these subjects was 33.08 years.

In the present study, the bigonial width dimension was greater in the males, while the ramus height and gonial angle were higher in the females compared to the males. These findings are similar to those of other studies,[3,5,9,11,14] although they are also partly contrary to others.[10,18] This difference is related to the greater contractile force of the masticatory muscles of the male subjects compared to the muscles of the females.

Statistically significant differences in the bigonial width and gonial angle were noted in the present study in terms of the age of the individuals. These findings are similar to some previous studies where significant differences in these dimensions have been noted.[3,4,10] Our findings are, however, also in contrast to some other studies that found that the differences in these parameters were not statistically significant.[6,9]

The ramus height was not statistically meaningful in terms of the age variation. This finding is similar to some studies,[4] although in other studies this difference has been statistically significant.[10] These data are probably the result of a change in the mandibular shape that would have resulted in a reduction in the contractile strength with ageing.

A limit of this study is represented by the sample, because it was composed of a small number of Caucasian Mediterranean subjects with a limited age range and, in particular, few females. Future studies should include a larger study population with a wider age range, and should evaluate another population, because mandibular parameters can vary across different human populations. The greatest strength of this study, however, is the absence of geometric distortion as a result of the radiography that has been used in other studies, due to the direct measurements recorded here on the dry skull.

In conclusion, this study confirms that the mandible is important for age estimation, and our findings show significant differences in bigonial width and gonial angle measurements according to the age of these individuals from samples of Caucasian Mediterranean skeletal remains. However, the changes in the ramus height were not statistically significant according to the age variation. As indicated, the greatest strength of this study is due to the absence of geometric distortion during radiography, as has been seen in other studies. Thus, knowledge of these different patterns provides further essential information in the determination of the sex and age of human skeletal remains, whereby the morphometric analysis of these mandibular dimensions represents a helpful tool for forensic science.

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Applicability of forensic facial approximation in the recognition process of unclaimed victims

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ABSTRACT

Background: Identifying bodies in a state of putrefaction, skeletonization or mutilation is often difficult. In these cases, it is possible to use auxiliary methods such as forensic facial approximation, considering the possibility of recognition by a relative or acquaintance, helping to obtain ante-mortem data for the identification process. The aims of the present study were to evaluate the capacity of recognition of individuals from digital facial approximation and to verify the association between the level of understanding of the issue by evaluators and the recognition success index. **Methods:** 16 skulls with previous photographic records were selected and then utilized for three-dimensional approximation using the digital technique, scanned by photogrammetry, and reconstructed by computerized method using open-source software. Twenty evaluators tried to recognize the facial approximation performed from images present in the photospreads. **Results:** The mean overall score was 23.75%, and it was observed that in only five approximations (31.24%) the option of correct recognition of the victim was the one that obtained the highest number of selections. False positives and negatives corresponded, respectively, to 11.56% and 12.5%. **Conclusions:** It can be concluded that the methodology can provide recognition albeit in low numbers, and permitting the acquisition of ante-mortem data for the proper process of human identification through primary methods.

INTRODUCTION

The performing of identification tests is very frequent[1,2] in forensic practice. However, it is sometimes difficult to identify deceased bodies, due to advanced stages of decomposition, as well as mutilation and carbonization. In these cases, to reduce the possibilities of who this person could be, and increase the chances of a positive identification, an auxiliary method can be applied, such as the forensic facial approximation (FFA)[3,4]. This method recreates the appearance of an unknown skull and then this image is publicized, in the attempt to recognize the person by friends and family, making *ante mortem* data possible to obtain. [5–8]

The chances for identification can be increased when the recognition brings information that enable primary methods of identification to be applied, such as DNA profiling or dental methods. [2,5-9] Despite some limitations on obtaining a positive identification,[10] FFA is very useful in reinforcing the identificatory process, especially when the bodies can be categorized as mentioned above.[4,10]

It is easier to recognize familiar faces, therefore the recognition of people without prior contact is different to that with prior contact.[11-13] For that reason, the unfamiliar face matching has been the target of many previous studies.[11,12,14-16] Some studies show that even people familiarized with the subject are not infallible, sometimes even giving values below average,[14,17–19] therefore Herrera et al.[20] emphasized the need to investigate the evaluator's experience in identifying faces, as it may influence the facial recognition.

The aims of this study were to evaluate a capacity of unfamiliar face matching by facial approximation in skulls whose faces are known, and to verify the existence of an association between level of understanding of the subject by the evaluators and the recognitions matches.

MATERIAL AND METHODS

This project was approved by the Research Ethics Committee (CAAE: 69052017.8.0000.5440) and it was conducted in Ribeirão Preto, São Paulo – Brazil using skulls from southeast region. Sixteen skulls of unclaimed individuals with prior photographic registration from a Forensic Anthropology Laboratory were selected. The laboratory is a section of the legal/forensic medical institute (CEMEL), from Ribeirão Preto Medicine School of University of São Paulo (FMRP/USP). The coroner provided the anthropological profile, including age, sex and ancestry and these aspects were confirmed by their real identification. In addition, the skulls should have an *ante mortem* photo or image before necropsy procedure at morgue. Since the skull should have this photograph, the number of skulls included in the research were limited. Furthermore, skulls with trauma in the facial portion of the head, and photographs of the face in advanced decomposition were excluded.

To perform the digital facial approximation, the skulls were digitalized using the photogrammetry technique, which consists of several photographs around the skull in different angles following a standardized process.[21,22] The photographs were taking using a camera Canon, PowerShot SX1IS (Canon U.S.A Inc, United State of America), focal length of 60mm and images size of 3648 x 2736 pixels. A photographic scale was used to maintain accurate the skull and structures size. A total of 120 photographs of each skull was taken to realize the reconstruction of the images in a 3D model, using the software Autodesk Recap Studio (Autodesk Inc™, United States). This methodology shown accuracy in measures and surface appearance without distortion [21,22]. The digital replica of the skull was imported into the Blender digital program (Blender Foundation™, Netherland), and an open-source 3D digital facial approximation protocol[5] was applied, performing steps used in the manual method, adapted for the virtual environment. The person who performed the facial approximation was graduated in Forensic Odontology for 3 years, having knowledge and ability to perform this methodology.

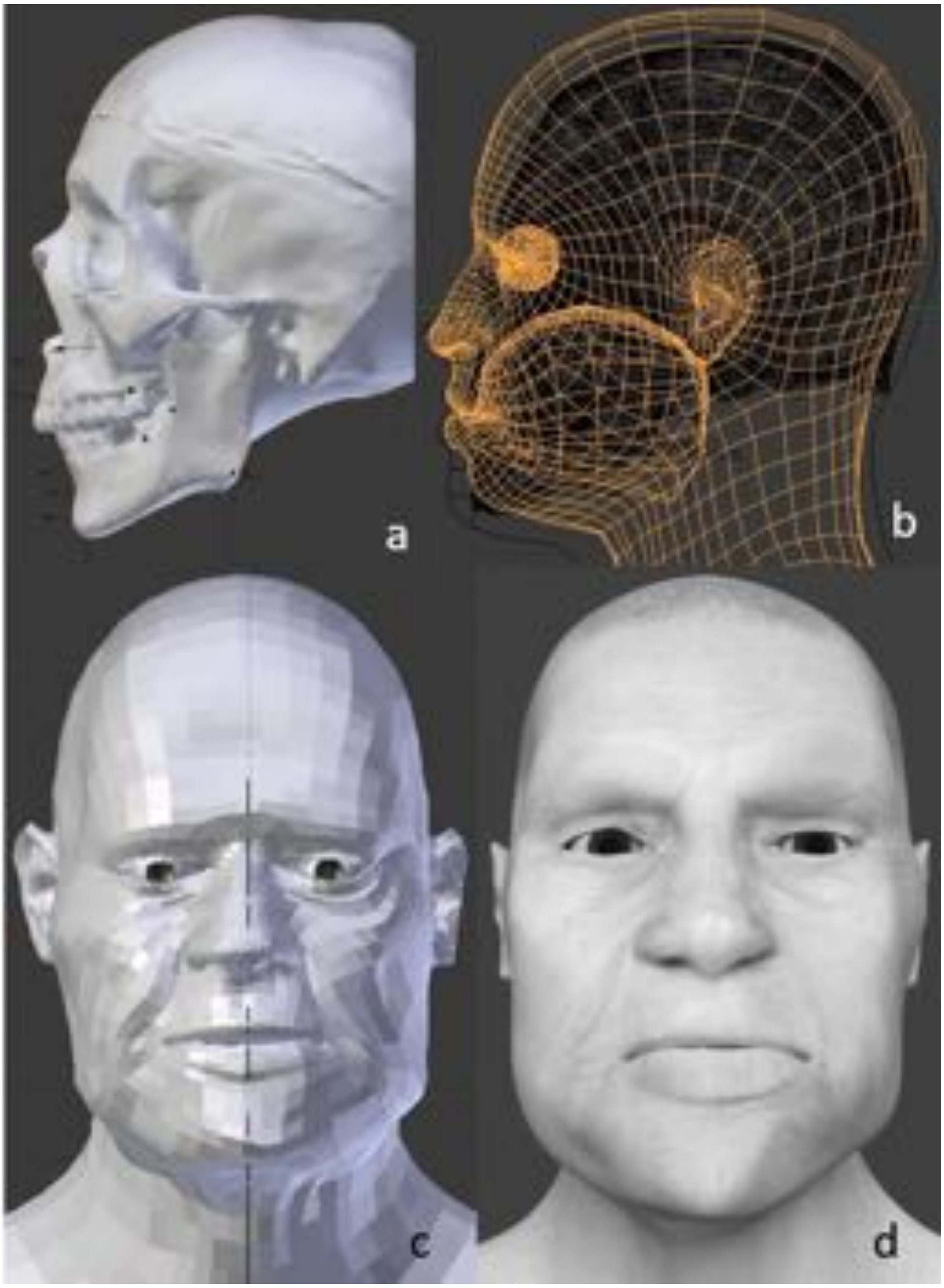
This protocol[5] determines the steps and the sequence to recreate a face. First, the anthropological profile of the skull needed to be provided. These results, such as age, sex and ancestry, will guide all of the reconstruction process. Then, the virtual soft tissue pegs (arrows) are positioned in 21 different landmarks in the skull (Table 1) with the size of the peg according to the anthropological profile. The soft tissue table used was developed by Beaini[23] in a Brazilian sample, using a sample from southeast region, the same region of the skulls from this paper.

Table 1. Name of the twenty-one locations/ landmarks in the skull used to position the virtual soft tissue pegs in the forensic facial reconstruction.

Locations - landmarks	
Midline	**Lateral (right, left)**
Supraglabella	Frontal eminence
Glabella	Supraorbital
Nasion	Suborbital
End of nasal	Inferior malar
Mid-philtrum	Lateral orbits
Upper lip margin	Zygomatic arch
Lower lip margin	Supraglenoid
Chin-lip fold	Occlusal line
Mental eminence	Gonion
Beneath chin	Upper second molar
	Lower second molar

After this step lines are placed to guide the location/shape of the eyes, nose and mouth, then a generic face is developed (automatically with the anthropological profile) and adapted through these markers, creating a unique face to that skull (Figure 1). The finalized version of the face was automatic rendered with the same program using a specific tool, in basic shades without hair, and for the creation of the photospreads both reconstructions and photographs of each individual were placed in shades of gray.

Figure 1. a – Digital skull with facial tissue markers (black arrows) with specific thickness for this given bioanthropological profile (male, older than 41 years, miscegenation – white and black); b – the general face (orange) that will be adapted in the outline created with the connection of the markers (black line); c – face after modifications required and ready to be rendered; d – face rendered and in shades of gray to compose the photospread.

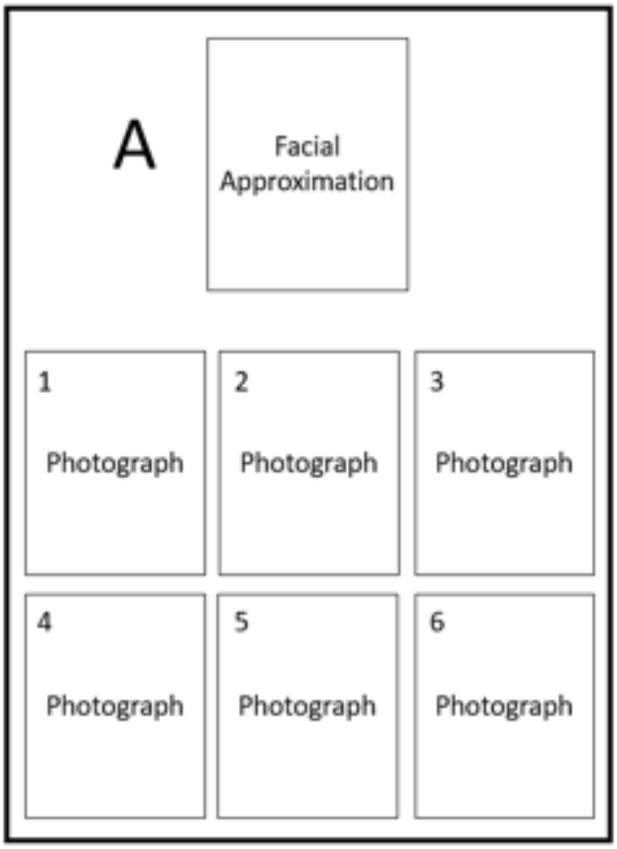


The facial approximations were analyzed by 20 evaluators, separated in equal numbers, into four different groups (according to their level of knowledge on the subject): Forensic Odontologists (group 1), Criminal Experts (group 2), last year undergraduate students (group 3), and people without previous knowledge on the subject (laymen) (group 4). The objective of the evaluators was to try to match faces to the facial approximations in a photospread with photographs of possible individuals. The photospread consisted of six photos, along with each digital facial approximation performed (A to P), randomly ordered on each sheet, totaling 16 sets of photos (one for each facial approximation). In order to standardize the photospreads, all images were placed in shades of gray and photographs of people in similar age groups, population affinity and head shape, both among the individuals themselves and in relation to the facial approximation, were selected as recommended [24].

In total 18 photographs were used, separated in groups of six in the 16 different facial approximations. Of these, only 14 had photographs that could be recognized and thus two approximations should not have matched faces by the evaluators, since the real photography of the FFA was not available. Four more photographs were placed that did not have their facial approximations done, so they should not be chosen, as they do not have a corresponding FFA. The correct photographs were randomly placed at each position.

The evaluators observed each facial approximation separately and indicated which of the six photographs the given approximation belonged (Figure 2). The evaluators should say if the photograph for the specific facial approximation was present or not. For that, they give a number in each set of photograph/approximation, from 0 to 6. Being 0 for non-correspondence (not present), and, once the evaluators recognize the approximation in one of the photographs, they should choose the number (position) 1 to 6, correspondent of the photograph. The evaluators were oriented explicitly, as recommended [24], that the specific person might not be in the photospread, so they could choose the non-correspondence in those cases.

Figure 2. Draft of the photospread analyzed by the evaluators. One image from the corresponding facial approximation (A-P) was placed above than six photographs that could be the person to be or not recognized (1-6). If the evaluators did not recognize any person for the respectively facial approximation, they should mark the number 0.



Descriptive statistics performed from the absolute and relative frequency, as well as the average of correct recognitions for each digital approximation and the chosen options, and false positive and false negative analysis. To verify the association between the approximation and the level of knowledge, categorized according to the different groups of evaluators, the Chi-square test was applied.

RESULTS

It can be verified that there was no relationship between the level of knowledge measured according to the group of evaluators, and the correct answer index, once the values were close

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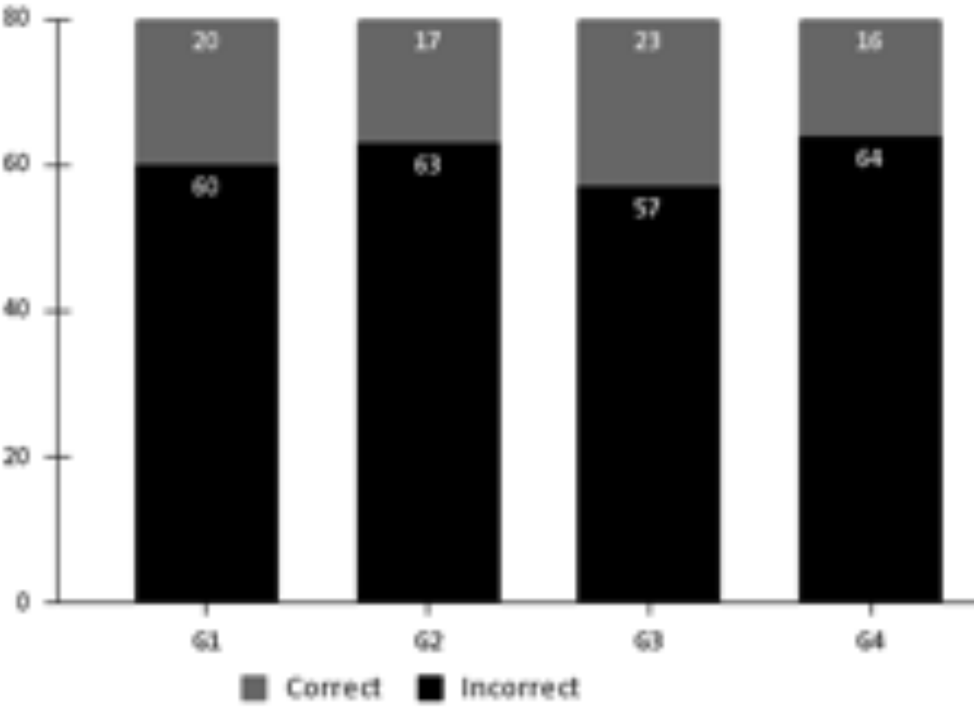
close (Figure 3). The chi-square test was applied between the number of correct and incorrect answers for the facial approximation and all groups of evaluators. The Chi-square (x^2) value was 2.070750 with 3 degrees of freedom, being the value *p=0.5578,* that is, there was no association between the groups and the recognitions. Still regarding correct answers, the average of each group ranged from 20% to 28.75%, being the lowest value observed in group 4 - composed of laymen; and group 3 with the highest average value, composed of undergraduate dentistry students.

After pondering on each evaluator, it was decided to verify the frequency of positive approximation, according to the number of evaluators. In other words, the frequency of how many approximations the five evaluators got right, and the frequency of how many approximations none of the evaluators got correct answers to, going from zero (no evaluator) to five (all evaluators), and this is described in Table 2. Figure 4 shows the number of negative and positive recognitions of each approximation.

Figure 3. Number of correct (gray) and incorrect (black) answers obtained by the evaluators in each group: Group 1 – forensic dentists; group 2 – criminal experts; group 3 – undergraduate students; group 4 - laymen



Number of evaluators	G1		G2		G3		G4		Total
	AF	RF(%)	AF	RF(%)	AF	RF(%)	AF	RF(%)	
0	6	37.50%	8	50.00%	6	37.50%	8	50.00%	28
1	4	25.00%	4	25.00%	2	12.50%	3	18.75%	13
2	4	25.00%	1	6.25%	5	31.25%	3	18.75%	13
3	0	0.00%	2	12.50%	2	12.50%	1	6.25%	5
4	2	12.50%	0	0.00%	0	0.00%	1	6.25%	3
5	0	0.00%	1	6.25%	1	6.25%	0	0.00%	2
Total	16	100%	16	100%	16	100%	16	100%	64

Table 2. Frequency of correct facial recognition according with the number of evaluators in each group.
AF – Absolute frequency; RF – Relative frequency.
G1 – forensic dentists; G2 – criminal experts; G3 – undergraduate students; G4 – laymen

Figure 4. Frequency of positive (grey) and negative (black) recognition of each facial approximation.

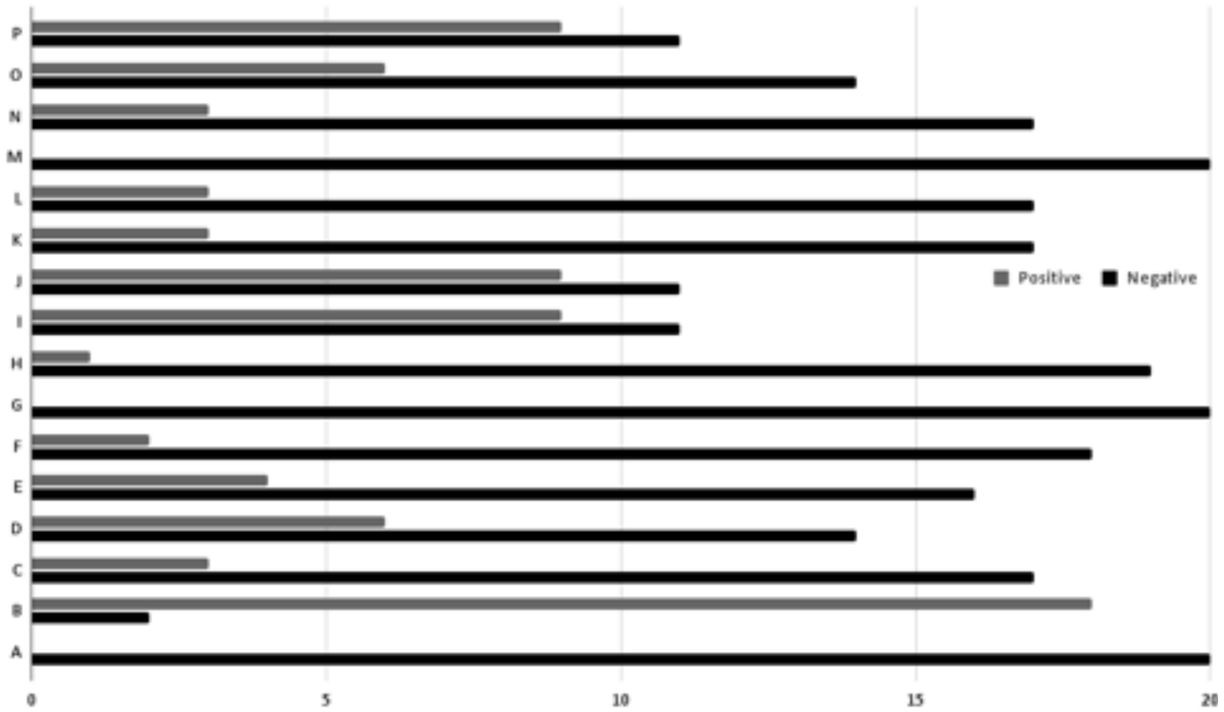


Table 3. Number of times that each photograph was chosen by evaluators in each approximation

	Position of the photographs						
Approximation	**1**	**2**	**3**	**4**	**5**	**6**	**0**
A	0	1	4	10*	0[a]	5	0
B	0	1	18*[a]	0	0	0	1
C	0	0	3	14*	0	0	3[a]
D	6a	1	1	0	0	7*	5
E	2	5*	4a	3	1	4	1
F	8*	1	3	0	3	2a	3
G	4	0	5	10*	0	1	0a
H	0	0	2	1a	12*	4	1
I	5	3	2	9*a	0	1	0
J	3	1	2	1	9a	2	2
K	2	0	2	8*	0	3a	5
L	3	3a	1	3	3	1	6*
M	14*	0a	0	1	1	3	1
N	2	3	3a	0	2	0	10*
O	2	0	5	1	4	6*a	2
P	9a	1	1	1	5	0	3

*Number of photograph chosen the most times for corresponding approximation.
[a] Number of photograph that corresponds correct to the approximation in question

Table 3 shows the data regarding the evaluators' choices concerning the position of the approximations and the photographs. Moreover, it is noted that in only five approximations (31.24%) the option of correct recognition of the individual was the one that obtained the largest number of selections. Another fact noted is that in six approximations, half or more of the evaluators mistakenly opted for the same photograph.

Of the 320 possibilities of correct answers, only 76 were correctly chosen. Of these, 51 (67.10%) were most often chosen by the highest number of evaluators, and concentrated on five approximations. In these approximations, 15.00% of the evaluators chose the correct photograph every single time.

In every single photospread, there was at least one photograph that the approximation had not been performed. From the 18 photographs used, four of them (identified with the number 5, 11, 15, 16) had no facial approximation performed, with a total amount of 22.22% of the photographs used. These images appeared 21 times in the photospreads (Table 4), representing 21.87% of possible choices by evaluators. They were chosen as the correct option 41 times, thus corresponding to 12.81% of the evaluators' choices.

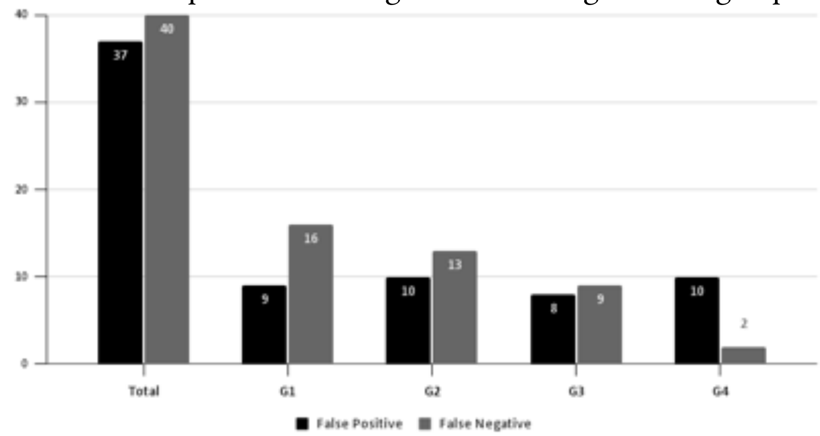
Table 4. Number of times that the four images which did not have a matching approximation appeared, along with the number of approximations in which these were chosen, and the number of evaluators who chose them as the correct option.

	Photographs without approximation *				**TOTAL**
	5	**11**	**15**	**16**	
Number of approximations they appeared in	9	3	3	6	21
Number of approximations in which they were chosen	7	2	2	2	13
How many times they were chosen as the correct option	15	5	15	6	41

The two facial approximations (C and G) that did not have images in the photospreads obtained just three correct answers out of 40, representing only 7.5% of the right answers. Thus, 37 evaluators identified those facial approximations, even when those did not have a photograph in the photospreads. It is worth mentioning that only the approximation C had correct answers.

Figure 5 brings the relationship between the false positives, when the evaluator chose some photograph when he should not have, meaning when the approximation did not have any correct alternative; and the false negatives, when the evaluator did not recognize the approximation in any photograph when it should have been recognized. It is possible to observe that the false positive and negative matched respectively 11.56% and 12.50% of the evaluators' choices, with nearly a quarter of their choices being the correct answers. It can be observed that the groups had very close amounts of false positive and negative, except for group 4 which only had two false negatives, that is, in only two answers out of 20, the evaluators chose to say that there would be no image related to the approximation when it existed.

Figure 5. Number of false positives and negatives according with the groups and the total of answers



DISCUSSION

Facial approximations can be presented to the public in a variety of ways, with the aim of encouraging recognition of the deceased individual by friends and family, and generate information about who the victim might be thus making it possible to apply the primary identification methods.[3,4,19] Posters with photos of missing children can be found on the streets and in different media, and the family of these children, along with the police, expect strangers to recognize the missing child through the poster. However, as previously mentioned, the recognition of people who have had prior contact is different to those who have not had, the former case subsequently making recognition easier than the later.[11]

Regarding the evaluators of the present study, the average of correct answers was 23.75%. However, it is important to emphasize that this study is not about familiar face recognition. The way people processed familiar faces is very different than they do unfamiliar ones,[12,25] and this could be a possible justification the low success rate in the recognition. However, Richard and Monson[19] found poor and similar performance recognition with people familiar and unfamiliar with the subject target.

Facts that can interfere in the facial recognition are lighting, positioning, quality of the image and the age difference between the suspect and the image to be recognized.[17,26,27] It was used in the present study photographs of real cases, and there were several of the factors mentioned above. However, it was decided not to change these aspects (except for the configuration in grayscale), as recommended in other studies [3,14] This characteristic is positive, once in real cases, people will provide any photograph of their parents such as selfies from smartphones, or photographs from old documents.[27] Therefore, this fact brought the study closer to day-to-day forensic practice, and not just a perfect laboratory pattern.

Henderson, Bruce and Burton[28] performed experiments using unknown people through surveillance camera images, and observed a high error of recognition (24%), even if the position and expression of the face were the same in both the surveillance image and the photograph used to perform the recognition. With this, it is observed that even having the so-called "gold standard", the recognition is not something simple and easy to be accomplished. Three facial approximation had no correct answers, in two of them is possible to suggest the cause of these results. In one of them, the photograph was about a man wearing beard, and in the other one, the person in the photograph was smiling. The facial approximation was performed without any characteristics, so they do not have smile or hair. Other studies[3,11,29,30] recommended that hair and clothes should be avoid, because they could complicate the recognition. However, one hypothesis for these two facial approximation were not recognized by anyone is that the evaluators could be expecting these characteristics in the facial approximation and in the photograph.

The defined groups in an attempt to cover various levels of knowledge, and the evaluators, whom had previous knowledge about the method, due to their professional or personal experiences. Caplova et al.[14] evaluated facial recognition of students and qualified professionals (forensic experts that deal with identification and facial recognition). The student average score was 78.1% of the correct answers and for the professionals 80%, with no statistical difference between the groups., concluding that facial recognition is an innate human ability. Countering the cited study[14] and the present study, Wilkinson and Evans[17] evaluated face recognition through photospreads, using groups composed of people from the general public and experts. Data showed that the general public recognized 46% correctly and experts 83%. For the authors, the results suggest that training and experience in facial analysis caused more reliable and accurate recognition. The present study found no significant differences between groups and no association between the level of knowledge and the groups themselves, however, the recognition rate was beneath 25%, well below the cited studies.[14,17]

Another study[31] conducted with passport officers' comparing photos with ID photos found a high frequency of mistakes even with people trained and with experience in this area. Burton et al.[32] concluded that even police officers with experience in forensic identification had a poor recognition rate just as people who did not know the subject targets. One of the possible reasons for the low right answer index is that the evaluators may be expecting to find facial approximations that would be practically photographs of the subjects, which is not possible, since the facial approximation only estimates and approximates a possible face.

In a criminal or identification context, it is necessary to be careful with false positives (when a different individual is mistakenly matched to the face reconstructed) and false negatives (when the identity of a person whose face appears in the image is dismissed as being correct). In the present study, false positives and negatives corresponded, respectively, to 11.56% and 12.50% of the evaluators' choices, being almost a quarter of their options for the correct answers.

Wilkinson and Evans[17] evaluated the recognition of people with two different experiments. In the first case, out of the ten photospreads, only six had the face to be recognized. The public had 10% false positives and 39% false negatives, and experts had 3% false positives and 8% false negatives. In the second case, the photospreads were composed of six sets containing the subject target and two without the face to be recognized. The public obtained 10% of false positives and 59% of false negatives. The experts, on the other hand, obtained 2% of false positives and 25% of false negatives. Likewise, in the present study there was a higher rate of false negatives than false positives, however, these values were very close to each other. Nevertheless, a discrepancy with the above study is that all groups analyzed in the present study (either with greater or lower knowledge) had similar false positive and negative rates, and the highest rate did not exceed 20% of the possibilities.

As presented, the methodology is limited and needs new studies to be more accurate. It is recommended additional investments in software, technology, high quality of vigilant images to improve this results. Moreover, this important technique is very viable in several aspects, and could be used in more cases than it already has. Finally, an increase in number of workshops will be helpful in training more forensic experts to put it in practice.

CONCLUSIONS

The methodology used increased in almost one quarter (23.75%) the chances of correct recognition, and with this, the possibility to find *ante mortem* data to compare the missing person with the body. In other words, this method helps to increase the identification of a missing person. It is important to highlight that this method is an auxiliary tool and cannot be used alone and exclusively to perform identifications.

The recognition process has no association between the level of understanding of the evaluator and the correct answer provided (p = 0.5578). It is noteworthy that in view of the important consequences in the lives of relatives of unidentified victims, in social and psychological spheres, as well as in relation to succession, economic and legal issues, the possibility of getting it right in almost ¼ of the possibilities makes the tool useful as an auxiliary method for investigation, allowing *ante mortem* data to be collected for the proper human identification process through primary methods.

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Forensic determination of dental age by cementum thickness of human teeth

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ABSTRACT

The purpose of this study was to assess the correlation between the known chronological age and the dental cementum thickness (DCT) in male and female subjects in different age groups. **Material and methods**: The study sample consisted of 57 donor teeth of both sexes. Teeth were classified by donors' sex and divided into three age groups: 10-19, 30-39 and 60-69 years. Tooth roots were cut with transverse ground sections in the apical, middle, and cervical thirds. DCT measurements were made on photomicrographs of light microscope. The correlation between DCT and the chronological age was calculated using the Spearman correlation coefficient. **Results:** A positive correlation was found ($r=0.47$, $p<0.001$) between DCT and age of the donor. DCT decreased from apical to cervical ground section (median [IQR] apical section 216.72 [128.25-375.00] µm, middle section 158.44 [87.66-284.90] µm; cervical section 96.60 [70.05-165.59] µm). DCT variability was influenced by sex, number of tooth roots and the condition of the tooth crown. The influence differed depending on the location of the section, being most prominent cervically. **Conclusion:** The present study showed correlation of DCT with age, with significant influence of sex, number of tooth roots, condition of the tooth crown and location of the root section.

INTRODUCTION

Age estimation is one of the key factors in identifying a person in both forensic research and clinical, anthropological and archaeological research of people and human remains. Although influenced by various environmental factors after death, skeletal and dental remains are the best preserved tissues of the human body. Age estimation is important for medico-legal reasons; therefore the accuracy of estimation is of particular importance for the purposes of forensic analysis.[1]

Teeth, based on their life-changing morphology, can be a valid source of information about a person's age.[2-4]

So far, various methods have been used in humans, some of which are invasive and some non-invasive. Invasive methods result in tooth destruction, but enable greater precision because they provide detailed insight into individual parts of the structure and morphology of teeth and allow checking the accuracy and validity of non-invasive methods. One of the first methods for determining dental age in adults based on life changes on teeth dates to the 1950s, and is named as the Gustafson method, after its author.[6] It is based on the evaluation of six parameters in the ground section: attrition, loss of periodontal attachment, secondary dentine, cementum apposition at the root apex, root resorption at the apex and dentine translucency.

None of the dental age estimation methods in adults has withstood the test of time due to problems related to the methodology such as: insufficient sample size, narrow age range of donors whose teeth were considered, type and condition of teeth.

All methods involving the assessment of dental age, which include markers measured or assessed on the tooth crown, have the disadvantage that the tooth crown, due to exposure in the oral cavity, is the most damaged part of the tooth .[6]Age estimation by occlusal tooth wear may be inaccurate due to various factors such as: diet type, bruxism, occupation, lateralization, masticatory forces, socio-economic status, oral hygiene, etc. [7]

It seems that the analysis of dental cementum could provide an answer to the existing shortcomings or further direct new research.

During root development, acellular cementum is deposited on the dentine of the tooth root, and from the moment the tooth comes into function, i.e. in occlusal contact with the antagonist tooth, cellular cementum is deposited throughout lifetime. Its deposition is related to age and strength of masticatory forces.[2,3,8-15] Since it is evident that the thickness of dental cementum increases with age, dental cementum can be a valid indicator in the assessment of dental age.

It is known that the crown of the tooth wears out during lifetime. By recession of the epithelial attachment, a part of the root becomes a part of the physiological crown, while the deposition of cellular cementum compensates for the length of the root in the alveolus.

The purpose of this study was to assess the correlation of the dental cementum thickness with the chronological age of subjects, tooth donors of both sexes in different age groups.

MATERIAL AND METHODS

Sample

The sample of this pilot study consisted of 57 donor teeth of both sexes. Teeth extracted because of periodontal disease or orthodontic and prosthetic reasons were used in the research, with a strict indication from a specialist. The teeth were collected by colleagues from the clinics of the School of Dental Medicine and from several private practices. The age of the donors at the time of tooth extraction ranged from 10 to 69 years. The teeth were divided into three groups according to the chronological age of the donor: 10 - 19, 30 - 39 and 60 - 69 years, and they were classified according to sex. The exclusion criteria were teeth with root lesions.

Each tooth was embedded in a quick-setting autoacrylate (Presi, France) and the roots were cut with transverse ground sections on the ISOMET 1000 precision cutter in the apical, middle and cervical third, using a 7 cm diameter diamond circular saw. Six transverse ground sections of the root were made on each tooth: 2 sections on the cervical part of the tooth root, 2 sections in the middle third of the tooth root, and 2 sections on the apical part of the tooth root. The thickness of each section ranged from 0.3 to 0.5 mm. The total sample consisted of 342 ground sections.

Horizontal root sections were visualized and analyzed using the Olympus model CX43 / CX33 light microscope (Olympus Corp. Tokyo, Japan), and the cementum thickness was measured on photomicrographs using the Olympus EP50 digital camera, Version: V3_20190202 under 4x magnification (Olympus Corp. Tokyo, Japan).

The measurement was performed at 4 measuring points on each of the ground sections (points a, b, c, d), clockwise, Figures 1-4.

Statistical analysis

Normality of the distributions was tested using the Kolmogorov-Smirnov test. The differences between sub-groups were tested using the chi-square test or Fisher exact test for categorical variables, and for continuous variables using the Student t-test or the Mann-Whitney U test (when comparing two sub-groups), or using the analysis of variance (ANOVA) or the Kruskal-Wallis ANOVA (when comparing multiple sub-groups), depending on distributions. For comparison of paired samples, the Friedman test was used. The association between the thickness of the cementum and the chronological age of donors was calculated using the Spearman's correlation coefficient. Predictability was calculated using models in analysis of covariance (ANCOVA).

Statistical significance for all tests was set at $p<0.05$, with corrections for multiple comparisons. Statistical analyses were conducted using statistical software packages STATISTICA version 12 (StatSoft, Inc. (2013) and MedCalc® version 19.8 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021).

Figure 1 Section lines and measurement points scheme

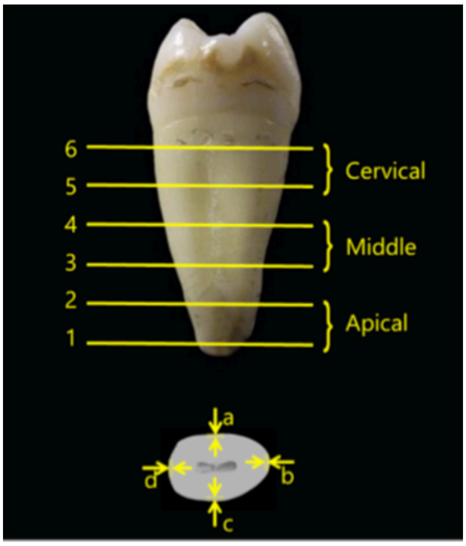


Figure 2 Cellular (C) and acellular (A) cementum, light microscope

Figure 3 Measurement of cementum thickness at 1st ground section

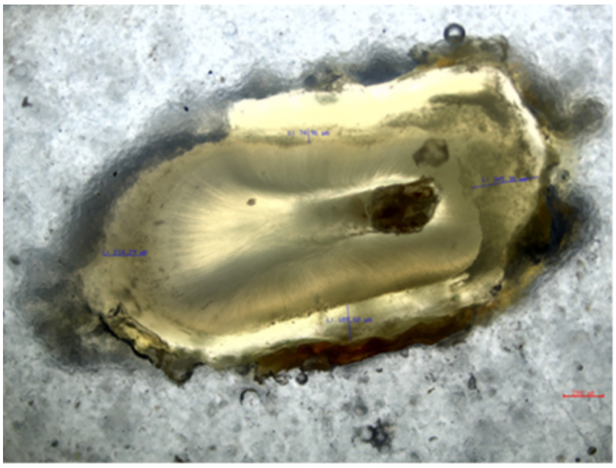
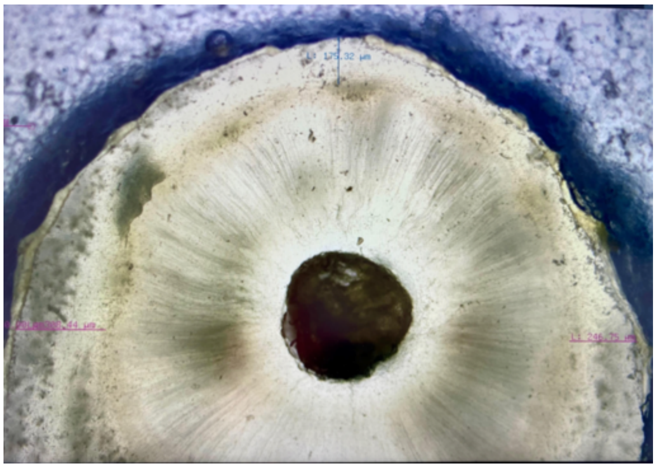


Figure 4 Measurement of cementum thickness at 3rd (middle) ground section



RESULTS

A total of 57 teeth from the same number of individual donors were analyzed, of which 39 (68.4%) teeth belonged to the female group. Donors ranged in age between 10 and 68 years, divided into 3 age groups (10-19 years, 30-39 years and 60-69 years). The characteristics of the donors and the teeth are shown in Table 1. The thickness of the cementum decreased from the apical to the cervical level (median [IQR] apical section 217 [128-375] µm; middle section 158 [88-285] µm; cervical section 97 [70-166] µm). Table 2 shows the average cementum thicknesses (median [IQR]) according to age groups, sex, number of tooth roots, and tooth crown integrity. The average (median [IQR]) cementum thicknesses were 95 [58-154] µm in the 10-19 age group, 232 [184-332] µm in the 30-39 age group, and 172 [136- 284] µm in the 60-69 age group, and they differed significantly in age groups with respect to the section site (apical, middle, cervical; p <0.001, the Friedman test). They also differed significantly by age for each section site (p<0.001, the Kruskal-Wallis test) (Table 2 and Figure 5 A-C). The cementum thickness has been found to be affected by sex (male teeth had a greater cementum thickness, median [IQR], 189 (104-297) µm vs. 157 [101-281] µm; Table 2), number of tooth roots (teeth with 2 or more roots had a greater cementum thickness, median [IQR], 186 [95-263] µm vs. 145 [100-262] µm; Table 2), and tooth crown condition (teeth with a destroyed crown had a two times greater cementum thickness compared to those with normal crown, median [IQR], 320 [117-468] µm vs. 160 [97-256] µm; Table 2).

The influence of these factors (sex, number of tooth roots, and condition of tooth crown) on cementum thickness depends on the site of the measurement (section site). Although it did not reach statistical significance, it was greatest in the cervical region, Table 2. These factors were not evenly distributed by age categories, Table 3.
A positive correlation was found between cementum thickness and known chronological age of the donor ($r = 0.41$, $p = 0.002$ for cervical cementum thickness; $r = 0.45$, $p < 0.001$ for middle and 0.47, $p < 0.001$ for apical and average cementum thickness).
The analysis of covariance, which included sex, number of tooth roots, condition of tooth crown and thickness of cementum, determined that the apical thickness of cementum ($F = 6.527$, $p = 0.014$) with the interaction of sex and number of tooth roots was the best determinant of donor age ($F = 5.376$, $p = 0.025$) with the coefficient of determination $R^2 = 0.321$ ($F = 4.626$, $p = 0.002$ for the model). The coefficient of determination for middle, cervical and average cementum thickness was 0.314, 0.267 and 0.317, respectively.

Table 1 Donors and teeth characteristics

Variable	
Subject age at extraction, median age (range)	33 (10-68) years
10-19 years (%)	22 (38.6)
30-39 years (%)	15 (26.3)
60-69 years (%)	20 (35.1)
Sex, female (%)	39 (68.4)
Number of roots	
1 root (%)	34 (59.6)
>1 roots (%)	23 (40.4)
Crown intact (%)	52 (91.2)
Apical cement thickness, median (IQR)	217 (128-381) μm
Middle cement thickness, median (IQR)	158 (88-286) μm
Cervical cement thickness, median (IQR)	97 (69-166) μm
Average cement thickness, median (IQR)	171 (100-281)

IQR – interquartile range

Figure 5 Apical (A), middle (B) and cervical (C) cementum thickness according to the age groups; median, 95% CI for the median, 25-75 percentiles, 10-90 percentiles, and range is presented

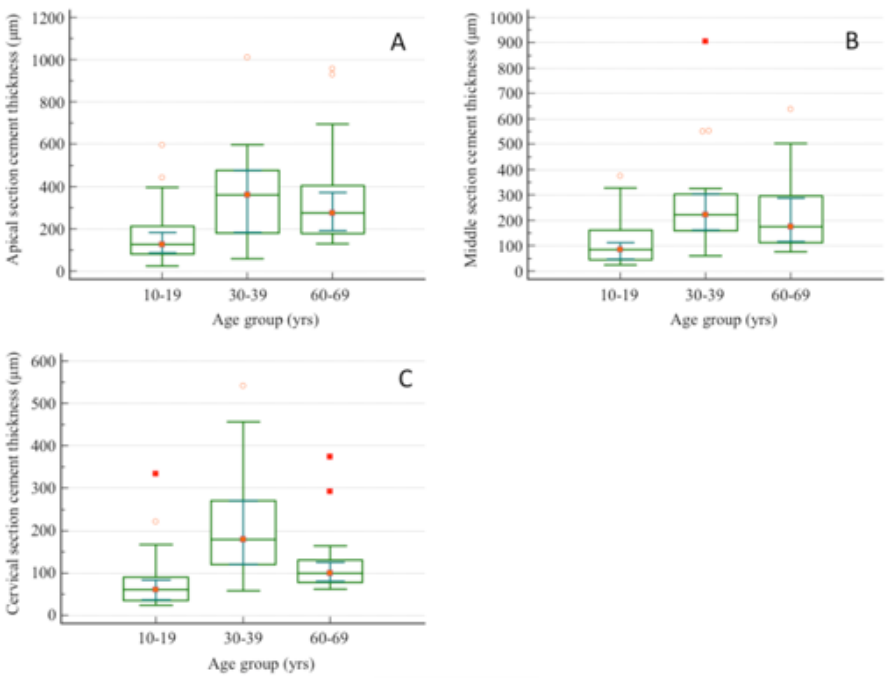


Table 2. Cementum thickness of different sections according to age groups, sex, root number and crown status

	Cement thickness, median (IQR)				
Categories	**Apical**	**Middle**	**Cervical**	**Average**	**p-value (Friedman test)**
Age					
10-19 years (%)	127 (81-214)	86 (45-161)	60 (36-90)	95 (58-154)	<0.001
30-39 years (%)	362 (181-478)	222 (161-304)	179 (120-271)	232 (193-326)	<0.001
60-69 years (%)	274 (179-405)	174 (112-296)	99 (78-131)	172 (136-284)	<0.001
p-value (Kruskal-Wallis test)	<0.001	<0.001	<0.001	<0.001	
Sex					
Female	196 (128-362)	155 (91-288)	90 (71-137)	157 (101-281)	<0.001
Male	251 (142-478)	163 (88-222)	136 (51-225)	189 (104-297)	<0.001
p-value (Mann-Whitney test)	0.286	0.813	0.343	0.520	
Number of roots					
1 root (%)	214 (128-361)	133 (88-287)	90 (65-124)	145 (100-262)	<0.001
>1 roots (%)	207 (122-366)	170 (85-239)	128 (71-226)	186 (95-263)	<0.001
p-value (Mann-Whitney test)	0.945	0.492	0.159	0.536	
Crown status					
Intact	203 (126-363)	155 (88-237)	91 (70-149)	160 (97-256)	<0.001
Destructed	411 (232-508)	274 (110-550)	223 (54-309)	320 (117-468)	<0.001
p-value (Mann-Whitney test)	0.082	0.153	0.185	0.096	

IQR – interquartile range

Table 3 Sex, root number and crown status distribution according to age groups

	Age group			
Variable	10-19 years	30-39 years	60-69 years	p-value (chi-square test)
Sex				
Female	14 (63.6%)	8 (53.3%)	17 (85.0%)	0.076
Male	8 (36.4%)	7 (46.7%)	3 (15.0%)	
Number of roots				
1 root (%)	14 (63.6%)	3 (20.0%)	18 (90.0%)	<0.001
>1 roots (%)	8 (36.4%)	12 (80.0%)	2 (10.0%)	
Crown status				
Intact	20 (90.9%)	11 (73.3%)	20 (100%)	0.049
Destructed	2 (9.1%)	4 (26.7%)	0 (0%)	

DISCUSSION

In this study, we wanted to assess the association between the known chronological age and DCT in male and female subjects in different age groups, regarding additional factors that could affect DCT. The results showed a moderate degree of correlation of the chronological age with cementum thickness. They also showed that there is a sex difference in cementum thickness. In the teeth of male donors, a greater thickness of cementum was measured in all age groups and at all root levels, however being the most pronounced in the cervical sections. We also found a significant difference in cementum thickness with respect to the distance from the tooth apex: cementum was thickest at the apex, and the thickness decreased toward the cervical portion of the tooth root. The number of tooth roots also influenced the thickness of the dental cementum.

It is interesting to note that the condition of the dental crown also affected the thickness of dental cementum. A significantly greater thickness of cementum was measured in teeth with a destroyed dental crown.

Previous research done by invasive methods was limited by sample size, selection of only one group of teeth, and the narrow age range. Therefore, positive correlations and statistical significance of the obtained data offer very limited practical application in the identification of human remains. Most of the research is based on the comparison of dentine and cementum as the preferred tissue for reliable data, the comparison of the measurement of the deposited cementum thickness and the number of incremental lines, as well as the comparison of vertical and horizontal sections in cement .[10-19]

In addition, most of the available scientific papers took into consideration exclusively intact teeth, mostly only one group. There were some exceptions that corroborate our data. For instance, the work of Pinchi et al.[20] found that upper teeth measurements offer more reliable data. However, the presented data for different groups of teeth (incisors, canines, etc.) offers small samples over a wide age range. On the other hand, they argue the advantage of using simpler analytical methods. Since in forensic and anthropological cases we cannot choose which teeth to have as material for identification and assessment of age, we conducted research on a sample of teeth of both sexes, all categories, without excluding teeth with partially destroyed tooth crown. Other authors, like Dias et al.[21] also explored the influence of dental pathology, specifically periodontal disease on the association between cementum thickness and age. They showed that this association is significantly disturbed by the presence of periodontal disease (leading to a prediction error of 22.6 years).

In this research, we decided to increase the accuracy of assessment through a previously unused method of analyzing the measurement of DCT on multiple horizontal sections, including all categories of teeth, both sexes, in a wide age range from 10 to 68 years. Horizontal sections were used to preserve the samples as based on the published experience of other authors. Amariti et al. state that longitudinal sections broke many teeth and introduced a lot of artefacts thus significantly affecting the results.[22]

By selecting the age groups for this study, we wanted to compare the teeth of the youngest group of donors (10-19 yrs.), in which the acellular cementum is dominant, middle age (30-39 yrs.), in which the deposit of cellular cementum is expected to be significant, as well as the influence of external factors on the tooth, and older age (60-69 yrs.), where the apical resorption of the cementum is expected to have already begun.

Shruuthi et al.[23] conducted a study to assess dental age in a population of South India, on a sample of 150 teeth, the age of the donors at the time of extraction 15-75 years, comparing two assessment methods: dentine translucency and deposited cementum thickness in single-rooted teeth (incisors and canines). The results showed that both methods were reliable in middle-aged subjects, while great deviations were obtained in younger and elderly people. Therefore, the authors suggest using the quantitative measurement of cementum deposition in younger specimens, and before the onset of the translucent dentine formation phase. The largest difference in reliability and accuracy was measured in subjects older than 60 years. Estimation of age by DCT, however, proved to be marginally better because dental cementum in the area of the tooth root was minimally affected by resorption processes.

Mohan at al.[24] examined age on a sample of 20 teeth using the number of incremental lines. They found a significant correlation between an individual's actual age and estimated age using

the dental cement annulations method, but in a narrow age range.
Mani-Caplazi et al.[25] investigated the causes of irregular incremental lines in cementum, which may be affected by pregnancy or some diseases, indicating a lack of reliability in the method of using incremental lines in age assessment.
Mallar et al.[26] compared longitudinal and transverse sections in a sample of 50 subjects of both sexes. Although the sample size of 25 male teeth and 25 female teeth is sufficient for such a study, the method of using only one (middle) section is not sufficiently reliable. The results showed greater reliability in longitudinal sections, but only in donor specimens younger than 30 years.
Swetha et al.[27] examined the correlation of known age and number of incremental lines in a sample of 80 single-rooted teeth, ranging in age from 22-60 years, to assess which tooth is the most reliable indicator of age. The study used longitudinal sections and the formula stating that the estimated age is equal to sum of the number of incremental lines and eruption age of the particular tooth. A positive correlation was obtained between known and estimated age, with a mean deviation of +/- 2 years. Lieberman [4] pointed out in his research that cementum is the best hard dental tissue for age assessment because it is continuously deposited, unlike other dental tissues, and due to no blood flow, it is less susceptible to remodelling and resorption processes.

CONCLUSIONS

There was a gradual increase in the thickness of the cementum commensurate with ageing, hence the amount of cementum can be used as an indicator of age in post-mortem forensic analyses. The thickness of the cementum is greater in males, particularly in the cervical third of the tooth root. The obtained quantitative values of dental cementum thickness yielded results that can be used in developing a new formula for dental age estimation in adults. More reliable results will be obtained by conducting a study with a larger sample, which is in progress.

Limitations of the study

Relatively small sample, uneven distribution of factors affecting the thickness of cementum by age groups, absence of comparing different methods of cutting or determining the thickness of cementum (different microscopes), absence of comparing different methods of fitting the samples into the cutting mixture, without the data on the method of extraction and storage of teeth after extraction and previous manipulations related to the root of the tooth are the main limitations of our study.

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Ethical codex: This research was approved by the Ethics Committee of the School of Dental Medicine, University of Zagreb at the 18th regular session held on 04.06.2020, decision number: 05-PA-30-XVIII-6/2020.

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Dental identification of unknown bodies through antemortem data taken by non-dental X-rays. Case reports

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KEYWORDS
Postmortem dental radiology, Body identification, Antemortem dental records, Dental x-rays matching

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ABSTRACT

The dental radiographic comparison is one of the most reliable and scientifically accepted methods for body identification (ID). The heterogeneity between AM (ante mortem) and PM (postmortem) x-rays images continues to stand as an issue for the forensic odontologist. Casual dental findings on X-rays for investigation of other structures than teeth or maxillaries, could eventually be a relevant source of dental data for the ID especially when AM dental files or X-rays are lacking. Two cases are reported in which the body ID was achieved through the comparison of PM dental X-rays with dental images obtained by radiographies of other structures (e.g. X-rays of the skull or cervical spine). These cases highlight that these occasional dental findings might provide sufficient evidence for a body identification. In the collection of AM data of missing people, the collection of all available records and radiographies of the head, neck and chest should be carefully reviewed by forensic odontologists, seeking for any available dental data.

INTRODUCTION

The usefulness of the dental structures for body identification (ID) purposes, and especially of dental treatments (e.g. conservative or prosthetic) or dental alterations for oral ritual mutilations [1] is well known. [2,3] The dental structures are rich of unique characteristics and are resilient to peri- and post-mortal agents [4] and they often therefore represent the main resources for identification either in cases of single corpses in which the body is extremely destroyed (e.g. skeletonized, charred, human remains) [5,6] or in the case of multiple bodies [7,8]. According to the literature, the x-rays comparison is the most reliable and scientifically accepted technique for the purposes of justice. [9-11] Post-mortem dental imaging usually implies the taking of intra-oral x-rays of the dental arches and, possibly, CT scans. [12,13] A panorex obtained from the CT images can anyway be used as an orthopantomogram (OPG), type of exam that cannot be usually taken from a dead body. [12] The lack of AM dental X-rays - one of the main issues which has hindered the radiological ID in the past - is nowadays largely bypassed by the digital X-rays and radiological archives which greatly improved the possibilities of storage and transmission of radiographs, thus facilitating the procedures in mass disasters and routinary identifications. [14-16]

Nonetheless, when AM (antemortem) radiographs are available, their heterogeneity towards the PM (postmortem) images is still an issue for the forensic odontologist who sometimes has nothing else to do than comparing PM intra-oral radiographies with AM extraoral x-rays or dental images occasionally taken from x-rays originally taken for investigation of different anatomical districts (skull, cervical spine or chest). [5, 17,18]

These radiological findings could indeed still represent a relevant source of dental data for body ID, when dental files or dental X-rays are not available after the AM data collection.

Two cases are reported here in which the body ID was achieved through the comparison of PM dental X-rays with very different dental images extracted by non-dental X-rays of the skull or cervical spine. The cases highlight therefore the importance of a complete collection of all the AM radiological data and of their careful review by a forensic odontologist to obtain dental images taken from radiological exams of the head, neck or chest.

CASE REPORTS

Case 1

One unidentified body in an advanced state of skeletonization was found in a wooded area. The identification team consisted of two forensic odontologists and a forensic pathologist.

The dental data were collected according to the INTERPOL DVI forms and a complete set of intraoral x-rays was taken.

Thirteen teeth were missing ante-mortem (already ossified alveolus), the remaining teeth presented multiple dental fillings, endodontic and prosthetic treatments and the upper left third molar semi-inclusion with a disto-vestibular tilting.

Some circumstantial data further oriented the search of the missing person but no direct relatives were found for DNA ID and no specific AM dental data or dental x-rays were available. Some AM clinical information and exams of the missing person were obtained by local hospitals. Two years old skull and chest CT scans, X-rays of the chest and cervical spine were collected. The skull CT scan showed an overall image of the maxillaries (scout view) and some upper molars (sagittal scans), whilst the transoral radiography originally taken to exclude fractures of the epistropheus and the lateral radiography of the cervical spine displayed some useful images of the teeth and dental treatments. With these AM radiographies the forensic odontologists were anyway able to fill the DVI Interpol AM dental form.

The comparison of the AM and PM charts revealed some consistencies (Fig. 1), but some AM evidence was missing and some others were regarded as possibly biased by the use of non-dental radiographies for AM dental charting and coding. Therefore, the forensic odontologists turned to a detailed comparison of the radiological images of the dental treatments retrievable by the AM and PM radiographies (Figg. 2-3). Relevant morphological similarities emerged for some dental traits and conservative and prosthetic treatments from the comparison of the AM non-dental radiographies and the PM intra-oral dental radiographies. The comparison of the images of the teeth 16, 26, 34 and 35 revealed the consistency of the type of treatments displayed by the AM and PM X-rays (fillings, crowns, etc) and a meaningful morphological similarity. The impacted upper left third molar was detected in both the AM and PM X-rays, with similarities for position and tilting of the tooth.

Figure 1. AM Dental charting (left) and PM (right) according to the Interpol DVI forms and coding - FDI numbering.

A-M INTERPOL DVI Form - Missing Person 600's

ODONTOLOGY

630 Dental findings (for primary teeth change specific FDI code)

11	NON	NON	21
12	NON	NON	22
13	NON	NON	23
14	NON	NON	24
15	NON	NON	25
16	AMFO	AMFO	26
17	NON	AMFO	27
18	NON	IMX	28
48	NON	NON	38
47	NON	NON	37
46	NON	NON	36
45	NON	MCC	35
44	NON	RFX-MCC	34
43	NAD	NAD	33
42	NAD	NAD	32
41	NAD	NAD	31

P-M INTERPOL DVI Form - Unidentified Human Remains 600's

ODONTOLOGY

630 Dental findings (for primary teeth change specific FDI code)

11	MPM	NAD	21
12	TCFOD	MPM	22
13	NAD	MPM	23
14	MAM	MAM	24
15	MAM	MAM	25
16	AMFO	AMFO	26
17	MAM	MPM	27
18	MAM	IMX	28
48	MAM	MAM	38
47	MAM	MAM	37
46	MAM	MAM	36
45	NAD	MCC	35
44	NAD	RFX-MCC	34
43	ABR	ABR	33
42	ABR	ABR	32
41	MAM	ABR	31

Figure 2. AM radiological images useful for a comparison with PM dental X-Rays. a) lateral X-rays of the cervical spine, b). trans-oral X-rays of the cervical spine; c) scout view of the skull CT; d) sagittal CT view of the skull. The dental treatments of teeth no. 16, 26, 27, 34 and 35 are displayed. An impacted 28 is detected.

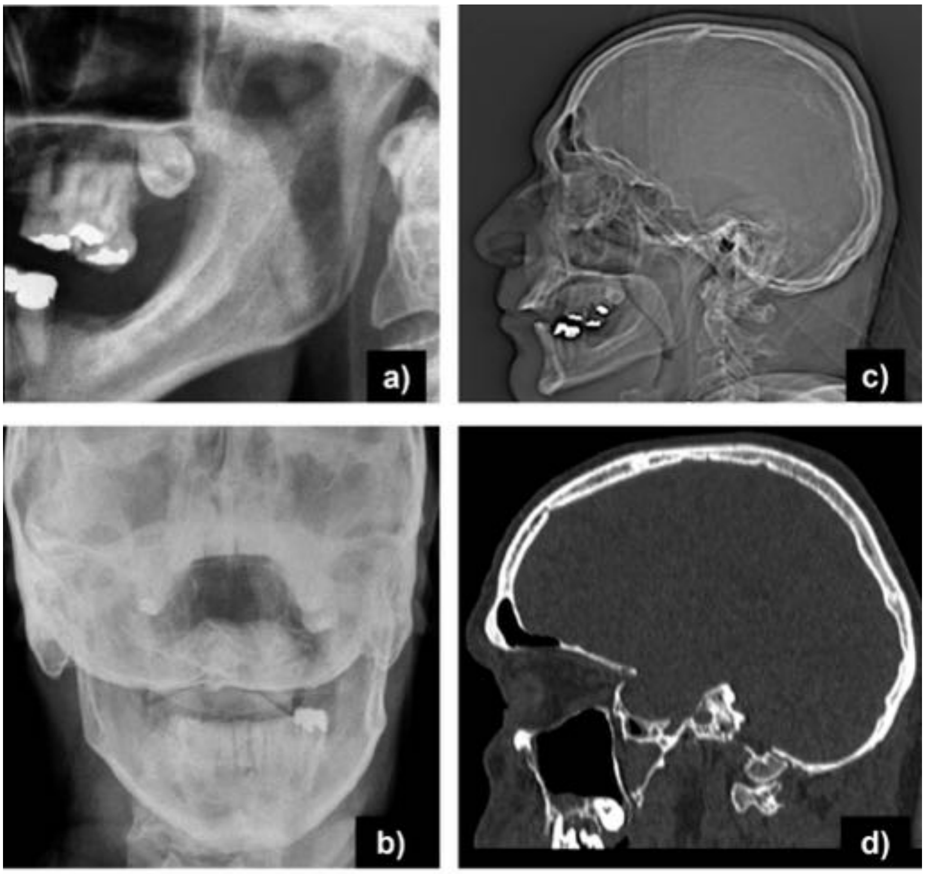
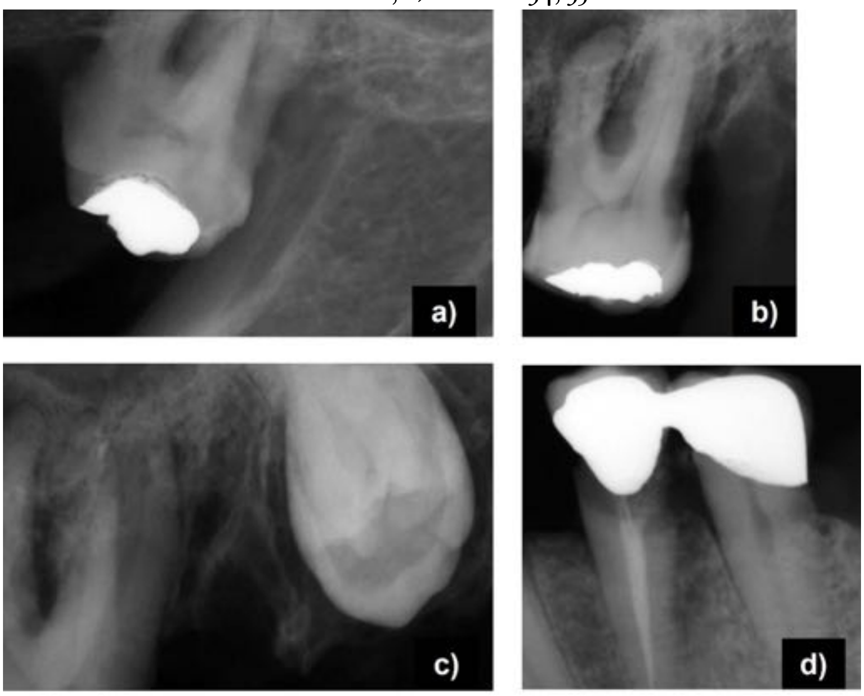


Figure 3. PM intraoral X-Rays used for comparison with AM images (Fig.2). a) tooth n. 16; b) tooth n. 26; c) tooth n. 28; d) teeth n. 34, 35.



Case 2

A deep charred corpse (Fig. 4) was found in a burned car. The body examination and ID procedures were performed according to the INTERPOL DVI protocol by two forensic odontologists and a forensic pathologist.

The post-mortem dental data were collected by a physical examination of the oral cavity, intraoral X-rays and a CT DentaScan. As usual, the carbonization far greater affected the facial tissues than the intra-oral ones. The tongue protruded between the arches and showed teeth marks. [19]

The oral autopsy and the dental X-rays revealed six ante mortem missing teeth with ossified alveoli, the presence of multiple dental fillings and endodontic treatments and the mesially impacted left mandibular third molar. The cross-sectional views of the DentaScan confirmed these findings, showing the vestibular-mesial impaction of the tooth n.38

The first hypothesis was that the dead man was the owner of the car. The AM data search revealed only one lateral radiography of the cervical spine, lateral and antero-posterior radiographies of the skull and a head CT, all dating back fourteen years.

No AM dental files or X-rays were retrieved.

First, the AM dental charting and coding resulted quite challenging and eventually deemed less reliable since the dental arches appeared partially or completely overlapped in the AM-X-rays originally taken for the detection of skull or cervical spine pathologies. Therefore, the comparison of the AM and PM dental charts, pursued with caution, revealed few consistencies (Fig. 5) and some discrepancies (teeth n. 34,35,36,47,15,26,27 e.g.). The latter were considered explainable given the long time (14 years) past from the day the AM radiographies were taken and the day of the death, meanwhile some dental treatments could have been changed or teeth extracted.

The limited evidentiary value of these findings demands that a detailed comparison of the AM and PM radiographic images of the teeth and dental treatments be made.

Despite the heterogeneity of the compared PM (Figg. 6-7) and AM (Fig. 8) imaging and the poor quality of the dental traits and treatments taken from the non-dental AM radiographies, some useful morphological similarities emerged from the matching.

Figure 4. Charred body, skull and intra-oral pictures. Tongue protrusion with teeth marks.

Figure 5. AM Dental charting (left) and PM (right) according to the Interpol DVI forms and coding - FDI numbering system.

A-M INTERPOL DVI Form - Missing Person 600's

ODONTOLOGY

630	Dental findings (for primary teeth change specific FDI code)		
11	NAD	NAD	21
12	NAD	NAD	22
13	NAD	NAD	23
14	NON	NAD	24
15	RFX-AMF O	NAD	25
16	TCF M	TCF M	26
17	NAD	RRX-RFX	27
18	NON	NON	28
48	NON	IMX	38
47	AMF V	AMF O	37
46	AMF O	RFX-AMF	36
45	NAD	NAD	35
44	NAD	RFX-AMF D	34
43	NAD	NAD	33
42	NAD	NAD	32
41	NAD	NAD	31

P-M INTERPOL DVI Form - Unidentified Human Remains 600's

ODONTOLOGY

630	Dental findings (for primary teeth change specific FDI code)		
11	NAD	NAD	21
12	NAD	NAD	22
13	NAD	NAD	23
14	MAM	NAD	24
15	RFX-TCF MOD	NAD	25
16	TCF M	TCF OM-TCF D	26
17	NAD	MAM	27
18	MAM	MAM	28
48	MAM	IMX	38
47	RFX-TCF OD	AMF O	37
46	AMF O-CAR D	MAM	36
45	NAD	TCF M-CAR M	35
44	NAD	RFX-TCF OD	34
43	NAD	NAD	33
42	NAD	NAD	32
41	NAD	NAD	31

Fig. 6 PM intraoral X-rays taken from the unknown dead body.

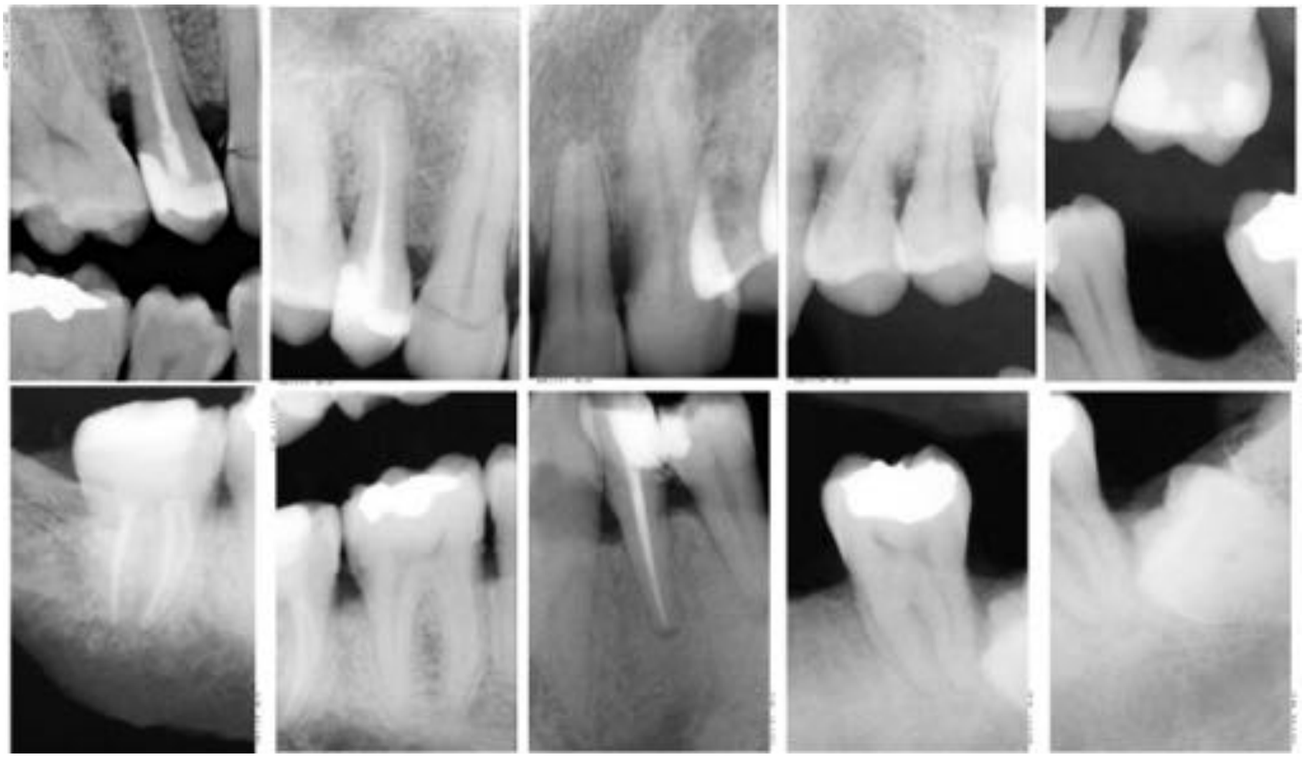


Figure 7. Some views taken from the PM DentaScan of the dead body. The tongue protruded between the incisors.

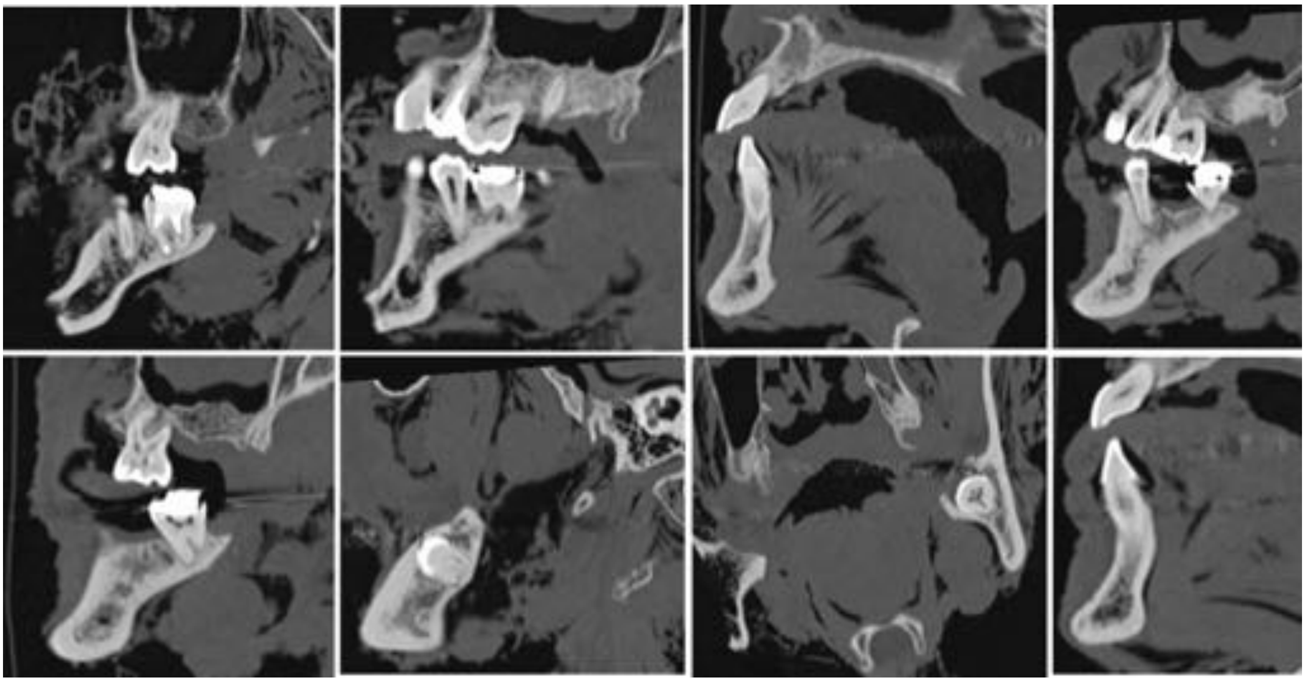
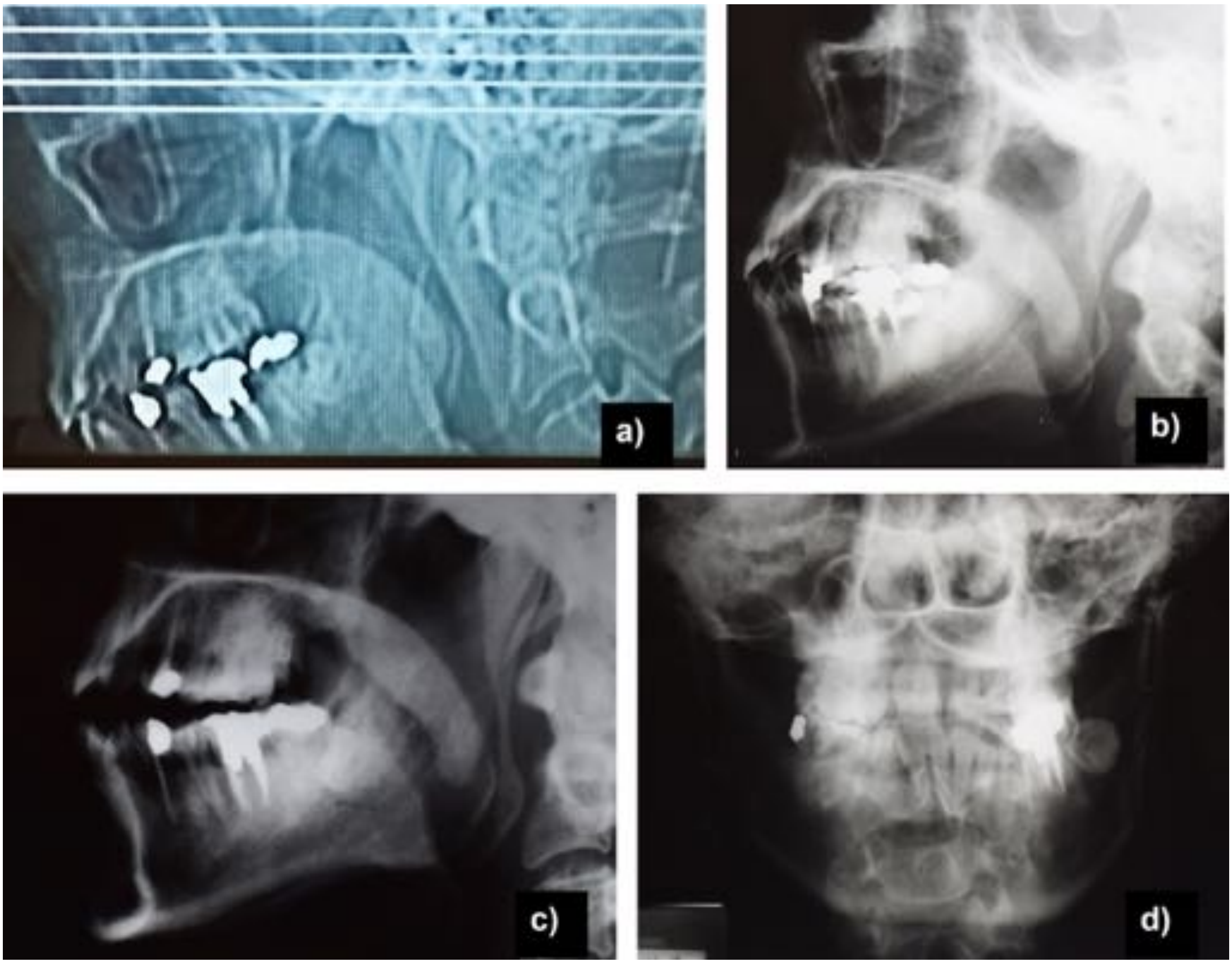


Figure 8. Dental traits and treatments taken from the AM radiographies and used for the ID procedure. a) scout view of the skull CT; b) and d) x-ray lateral and anteroposterior view of the skull respectively; c) x-ray lateral view of the cervical spine.



The comparison of the AM and PM radiological images of the teeth n.15, 34, 37, 46 and 26 revealed the consistency of the type of treatments (fillings and endodontic treatments). The impacted lower left third molar showed relevant similarities in shape and position. The PM DentaScan provided more accurate information about the position and shape of the impacted third molar.

DISCUSSION

Few cases are reported In the Literature about body identification obtained through the casual retrieval of useful dental images in radiographies originally taken for investigation of the chest [17] or skull structures [5,18].Being such radiographies taken for investigation of structures other than the teeth or dental arches, the resulting "dental" images are far to be considered ideal and the matching with PM data is therefore challenging. Nonetheless, the radiopacity of the dental materials, the peculiar shape of some treatments or the occurrence of unusual dental traits can be detected and effectively used for ID purposes, despite the mis-projections, the overlapping and the poor quality of some AM images. [20,21]

The limits of the AM dental data proved to be important in both the reported cases and the comparison of the AM teeth images taken from the skull or spine X-rays even long before the death, represented the unique chance to identify the bodies. The lack of more reliable sources of dental data of missing people jeopardized the AM dental charting and coding, which could be performed merely looking at those non specific X-rays.

The anteroposterior views of the AM X-rays [Fig. 2 b); Fig. 8 d)] resulted actually useless in both the reported cases for the matching of the shape of

the fillings, but they anyway helped to solve some doubts about the distribution of the treatments per side that appeared overlapped in the lateral views [Fig. 2 a)- c; Fig. 7 a)-c)].
Franco et al [22] found the CBCT scans to be as effective as the OPGs to perform a proper dental charting and coding according to the Interpol DVI recommendations for ID procedures. The strongest associations in 2D and 3D-xrays were especially detected between the INTERPOL “non-erupted”, “partially erupted” and “impacted” third molar codes.
An antemortem CT scan was found in both the reported cases, but it was a skull CT. The head CTs resulted useless for dental charting and coding since the scans represented very few and incomplete dental images while only the scout images could be used for the dental ID,
Therefore, the AM dental charts obtained from antemortem 2D or 3D X-rays of the skull or cervical spine were regarded as probably biased in both cases, and consequently the comparison of the PM and AM odontograms devalued in their evidentiary role. This problem led the forensic odontologists to perform a radiological comparison of the dental traits and treatments of the non specific AM X-rays.
It is widely renown that a great reliability in the human identification procedures is attributed to some peculiar dental traits when compared on AM and PM dental X-rays, [23-24] and especially dental treatments such as root canal therapies [25] and amalgam [26] and/or composite fillings [27] are endowed with highly-specific morphological features. The peculiar shape of these treatments is reported to be highly recognizable on two-dimensional radiographies even if they are taken with a different angulation [28] and the matching of the dental radiographies is considered a more reliable item for identification than written dental records. [29] Forrest AS [30] reported that visual comparison or superimposition of "plain film radiographs" are the best methods for the recognition of similarities in the morphology of conservative treatments, and especially of the metallic ones. The maxillary CTs offer more 3D information about the extension of the dental treatments, but they inherently allow a scarce discrimination among the different dental materials. Moreover, the comparison of the 3D imaging of the teeth are more useful in the case of unrestored teeth.
In our Case 2, the findings detected by the intraoral X-rays compared with the AM data would have still been sufficient to identify the subject, but the Dentascan PM projections made it possible to add information about the position the position of the impacted third molar (Fig. 7).
In both cases, the lateral and antero-posterior x-rays of the cervical spine and skull allowed the detection of some treatments in the premolars and molars of the arches and some useful consistencies emerged from the comparison with the PM intraoral radiographies of the body. Relevant findings emerged about the type of the treatments (fillings, crowns, endodontic treatments), whilst shape similarities were detected only for some fillings and few dental traits. Only very few previous reports discussed about similar ID circumstances and provided variable conclusions. Minaguchi K et al [17] compared an AM chest x-ray which included only the frontal part of the two arches with the dental PM intraoral x-rays and found congruences between the conservative treatments detected. However, they concluded that similar findings are normally not sufficient to define a reliable dental ID and the body ID was obtained after additional genetic examinations.
On the contrary, Campobasso et al [5] identified a badly charred body from an anterior-posterior skull radiography taken 8 years earlier. The AM and PM dental data matching was limited to few dental treatments, being the other teeth in the PM data missing or with different treatments due to the long time past. Nevertheless the findings were sufficient for the establishment of a positive ID of the unknown body.
Pinchi et al [18] identified a case of a skeletonized body mainly thanks to the concordance of the position and morphology of an included upper third molar detected on a single scan of an AM skull CT.
As an interesting clue, in both cases an impacted third molar was found in the AM radiographies and in the unknown corpses. The peculiar type of impaction and some consistencies about the third molar traits retrieved in the AM X-rays of the skull and cervical spine and those found in the intra-oral radiographies of the bodies provided relevant evidence for the identification assessment.
Previous literature has shown that the position and shape of the impacted teeth, other than the impaction in itself, provide a strong evidence for the dental ID. [21] Pinchi et al [18] and Campobasso

et al [5] achieved a body ID by an effective comparison of radiographic images of impacted third molars found in aged unknown bodies and in AM radiographies of the skull of missing people respectively.

CONCLUSIONS

Dental data represents one of the primary identifiers according to DVI Interpol and the comparison of AM and PM dental records can effectively lead to a positive identification of an unknown or unidentifiable dead body. The antemortem dental radiographies of missing people have proven to be a reliable source of data when any other AM dental records lack. Two cases of body identification are presented in which no AM dental records or radiographies were available and a quite challenging dental charting was performed with dental traits or treatments captured just by AM radiographies of the skull or the cervical spine. The overlapping and misprojection of teeth represented in these non-dental AM radiographies and the relevant time past between AM and PM radiographies jeopardized the evidence obtained by the AM and PM odontograms matching. Despite the relevant diversity of the AM and PM radiographies, a comparison of dental traits and treatments images could be performed and provided an useful evidence in both cases. Occasional dental findings detectable in non-conventional radiographs may get sufficient evidence for a body identification, especially some dental treatments or impacted third molars.

The collection of Ante Mortem data of missing people should consider all the available records and radiographies of the head, neck and chest which should be carefully reviewed by the forensic odontologists in the search for AM radiographic dental data.

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