

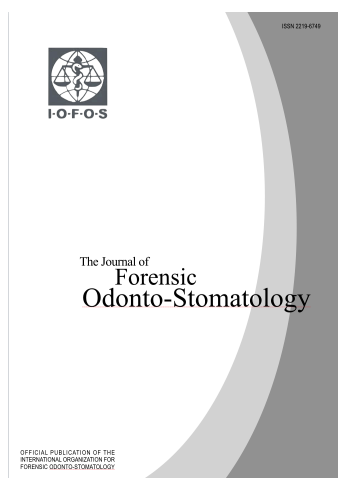


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Effects of long-term steroid therapy on the results of dental age estimation using pulp/tooth ratio

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KEYWORDS

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ABSTRACT

Forensic age estimation is performed by assessing pulp chamber constrictions due to physiological age-related changes on dental radiographs; however, the estimated ages occasionally deviate from the actual ages. In particular, long-term steroid users tended to demonstrate pulp chamber constrictions in all teeth. As this is uncommon among younger age groups, caution should be exercised when evaluating pulp chamber constriction. This study investigated the estimated ages of eight steroid users by applying the ratio of the pulp area to the total tooth area from canine radiographs. Patients in their 30–40s were examined at a dental outpatient clinic for the prevention or treatment of adverse events associated with the use of bisphosphonates to prevent steroidal osteoporosis and radiographs were obtained. The pulp and tooth areas were measured, and the estimated age was determined using regression formulas calculated from the canine teeth of the general Japanese study participants. The mean absolute error between the estimated and chronological ages of the patients was 19.24 years for the upper canines and 17.69 years for the lower. The root mean square error was 23.18 years for the upper canines and 20.00 years for the lower. The estimated ages were far from the actual ages of the steroid users. When estimating the age of an unidentified individual who has a pulp chamber constriction that is inconsistent with other forensic physical findings, this information may assist in predicting their medical background.

INTRODUCTION

Accurately estimating the age of unidentified bodies assists forensic investigations in identifying corpses, which is an important aspect of forensics. Human teeth exhibit prominent physiological age-related changes and are often examined for age estimation using forensic medical techniques. Since Gustafson proposed a method for age estimation in 1950,¹ many forensic scientists have conducted studies to accurately estimate an individual's age at death. However, in real-world forensic cases, various factors affect the accuracy of such estimates. Although an individual's age can be estimated using various methods, it is crucial to select one that has been scientifically proven to yield accurate results.

Cameriere et al. assessed pulp chamber constriction caused by the age-related physiological addition of secondary dentin and

estimating age using dental radiographs of the Italian population by means of a quantitative method.² This method has been applied by many researchers. Different methods for assessing pulp chamber constriction have been reported in various countries.³⁻⁷ However, the estimated ages occasionally deviate from the actual ages, especially in cases of prominent pulp chamber constriction throughout the jaw due to long-term steroid (LTS) use. As widespread pulp chamber constriction due to LTS use is inconsistent with the natural changes in the pulp area caused by aging, forensic scientists must exercise caution when estimating age using this aspect. Therefore, we verified the estimated age by applying the pulp/tooth ratio (PTR) of eight Japanese patients who had received LTS therapy to two regression formulae: the Japanese model that was derived from general Japanese subjects gathered in this study, and Cameriere's method.²

MATERIALS AND METHODS

Derivation of regression formulae for the Japanese population

We retrospectively examined 440 digital periapical radiographs of healthy Japanese canine teeth obtained for examination and treatment at the dental center of Iwate Medical University. The subjects consisted of 172 men and 268 women with an age range of 26–84 years (upper canines: men 68, women 122; lower canines: men 104, women 146) for the derivation of the regression formulae for the Japanese population (Table 1).

Table 1. Number of subjects distributed according to age group.

Part of teeth	Men (age)	Women (age)	Total (age)
Upper	68 (29–84 y)	122 (26–80 y)	190 (26–84 y)
Lower	104 (29–84 y)	146 (31–80 y)	250 (29–84 y)
Total	172 (29–84 y)	268 (26–80 y)	440 (26–84 y)

In the cases of patients in their fifties or older, it is difficult to determine whether the cause of the

pulp cavity constriction is due to LTS use or age-related changes. Therefore, relatively young patients, 30–40 years old, were selected for this study. The digital dental X-ray equipment used was ALULA-TM (Asahiroentgen Ind. Co., Ltd, Kyoto, Japan) and MAX-DC70 (J. MORITA MFG. Corp., Kyoto, Japan). Each image was converted to a JPEG and saved, following the method described by Cameriere et al.² The lasso tool in ImageJ (open source imaging processing program, National Institute of Mental Health, Bethesda, MD, USA) was used to measure the pulp areas and overall teeth in labial and buccal views of the upper and lower canines. All measurements were performed by the same observer. To test the intraobserver reproducibility, a random sample of 30 periapical radiographs was reexamined after an interval of two months. The intraobserver reproducibility of the measurements was assessed using the intraclass correlation coefficient (ICC).

Verification of the estimated age based on radiographs of the canines of Japanese patients who underwent long-term steroid therapy

Eight patients, all aged 30–40 years, had been administered a steroid, 1–75 mg converted to prednisolone (PSL), for more than four years. The LTS users were not included as general participants. All patients were examined at a dental outpatient clinic for the prevention or treatment of adverse events associated with the use of bisphosphonates to prevent steroidal osteoporosis (Table 2), and digital radiographs were obtained. All canines selected from the patients in this study were healthy and did not have any treatment marks. After measuring the canine pulp and tooth areas and calculating the PTR, we applied the PTR to the regression formulae (Japanese method) based on the Japanese population and Cameriere's method based on Italian subjects,² and compared the differences between their chronological and estimated ages in terms of the mean absolute error, root mean square error, and interquartile range.

The protocols for this study were approved by the Ethics Committee of the Iwate Medical University (approval no. 01352).

Table 2. List of Japanese patients on long-term steroid (LTS) therapy

Case No.	Age	Sex	Diagnosis	*Term / Quantity of PSL dosage
1	41	man	interstitial pneumonia with dermatomyositis	4 years / 15-75 mg
2	46	man	pancolitic ulcerative colitis	11 years / 5-50 mg
3	39	man	primary myelofibrosis	4 years / 30-50 mg
4	45	woman	scleroderma	6 years / 12.5 mg
5	46	woman	systemic lupus erythematosus	13 years / 5-50 mg
6	35	woman	systemic lupus erythematosus	15 years / 5-70 mg
7	47	woman	systemic myasthenia gravis	5 years / 5-20 mg
8	39	woman	systemic myasthenia gravis	18 years / 1-60 mg

PSL: Prednisolone.

* Medical agents administered as steroids were converted to PSL.

RESULTS

ICC analysis showed reliable results for intraobserver differences between the paired sets of measurements performed on the re-examined periapical radiographs [ICC (1,1) = 0.775].

The regression model for the upper and lower canines, based on the measurements of the Japanese population, yielded the following regression formula, which explained 83.8% of the total variance ($R^2 = 0.702$).

$$\text{Age} = -344.921 * \text{PTR upper} - 36.727 * \text{PTR lower} + 86.608$$

When only the upper canines were considered, the regression formula, which explained 92.0% of the total variance ($R^2 = 0.846$), was:

$$\text{Age} = 97.169 - 510.365 * \text{PTR upper}$$

When only the lower canines were considered, the regression formula, which explained 89.3% of the total variance ($R^2 = 0.798$), was:

$$\text{Age} = 88.535 - 429.939 * \text{PTR lower}$$

The accuracies are listed in Table 3. When Cameriere's method was applied to the Japanese subjects, the mean absolute errors between the estimated and chronological ages for the upper and lower canines, upper only, and lower only, were 5.87 years, 4.02 years, and 3.83 years, respectively; the root mean square errors were 7.19 years, 4.69 years, and 4.78 years, respectively; and the mean interquartile ranges were 7.75 years, 7.48 years, and 6.48 years, respectively. When the Japanese

regression formulae were applied to the Japanese subjects, the mean absolute errors between the estimated and chronological ages for the upper and lower canines, upper only, and lower only, were 4.32 years, 3.84 years, and 3.68 years, respectively; the root mean square errors were 5.46 years, 4.53 years, and 4.59 years, respectively; and the mean interquartile ranges were 5.33 years, 7.16 years, and 5.92 years, respectively. The regression formulae based on the Japanese population yielded more accurate estimates; however, it was proven that the regression formulae used in Cameriere's method also estimated the ages of the Japanese participants with reasonable accuracy.

The results of the verification of the accuracy of the estimated ages from the canines and chronological ages in LTS users were as follows: when using the regression formulae by Cameriere et al., the mean absolute error between the estimated and chronological ages of the eight patients was 20.45 years for the upper and lower canines, 20.52 years for the upper only, and 16.63 years for the lower only. Moreover, the root mean square error was 24.25 years for the upper and lower canines, 24.50 years for the upper only, and 19.06 for the lower only. When using the regression formulae from the Japanese subjects, the mean absolute error between the estimated and chronological ages of steroid users was 17.81 years for the upper and lower canines, 19.26 years for the upper only, and 17.69 years for the lower only. The root mean square error was 20.55 years for

the upper and lower canines, 23.18 years for the upper only, and 20.00 years for the lower only. The estimated ages of LTS users were far from

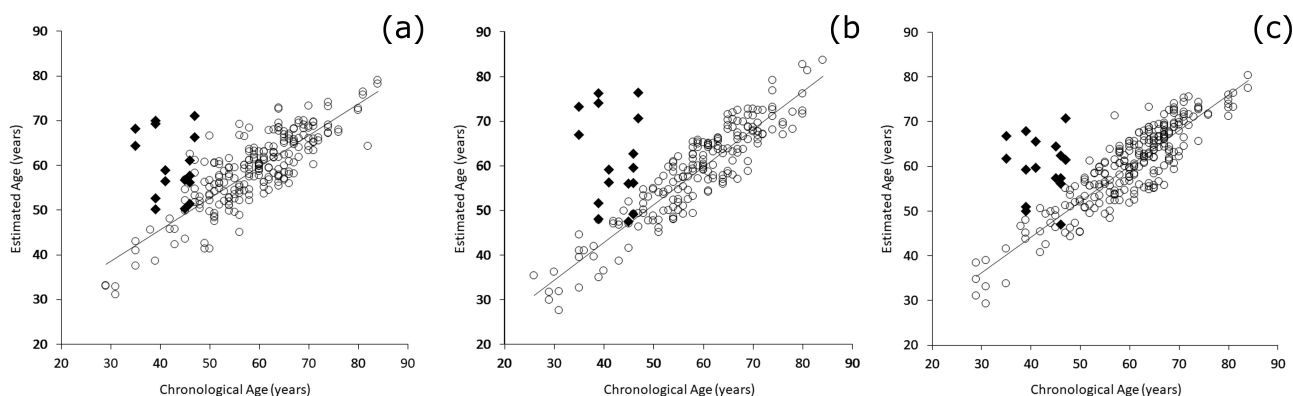
the regression line derived from the general study subjects as well as their chronological ages (Figure 1).

Table 3. List of Japanese patients on long-term steroid (LTS) therapy

Part of teeth	Subjects	MAE		RMSE		MIQR	
		CM	JM	CM	JM	CM	JM
Upper and Lower	General	5.87	4.32	7.19	5.46	7.75	5.33
	PSL user	20.45	17.81	24.25	20.55	9.63	5.74
Upper	General	4.02	3.84	4.69	4.53	7.48	7.16
	PSL user	20.52	19.26	24.50	23.18	8.55	8.18
Lower	General	3.83	3.68	4.78	4.59	6.48	5.92
	PSL user	16.63	17.69	19.06	20.00	4.14	3.85

MAE: mean absolute error
 RMSE: root mean square error
 MIQR: mean interquartile range
 CM: Cameriere’s method
 JM: Japanese method in the present study
 LTS user: long-term steroid user

Figure 1. Plots of data and regression line using a simple linear regression model of Japanese canines. Open circle: General subjects, Filled rhombus: LTS user. (a) upper and lower canines, (b) upper canines, and (c) lower canines.



DISCUSSION

Synthetic glucocorticoids, namely, steroids, are used for their strong anti-inflammatory and immune-boosting effects. Their main action is to suppress the production of prostaglandins by suppressing the expression of enzymes involved in arachidonic acid metabolism and to suppress the production of various inflammatory cytokines. Steroids are used to treat many diseases, including collagen diseases, respiratory diseases, allergies, kidney diseases, blood diseases, and post-transplant rejection, and it is commonly

used to treat autoimmune diseases.^{8,9} However, other effects may lead a variety of adverse reactions, such as neuropsychiatric symptoms, anemia, muscle weakness, and increased susceptibility to infection, diabetes, dyslipidemia, hypertension, and atherosclerosis.¹⁰ In particular, steroid-induced osteoporosis, which is caused by a decrease in bone strength, is more prone to fractures than primary osteoporosis even if bone density is maintained, and significantly reduces the quality of life of patients.¹¹ The mechanism of

onset is said to be inhibition of differentiation into osteoblasts¹², suppression of osteoclast apoptosis¹³, decreased calcium absorption and increased excretion^{14,15}, and the suppression of various hormone secretions by excess steroids, which promotes bone resorption.¹⁶

Bisphosphonates (BP) are administered as one of the medicines for treating steroid-induced osteoporosis, and many patients undergoing steroid therapy visit dental clinics for oral examinations and oral care to prevent the onset of osteonecrosis of the jaw, a side effect of BP. The causes of osteonecrosis of the jaw by BP include the fact that it is covered only by a thin oral mucosa, a large number of resident bacteria present in the oral cavity, and inflammation due to dental infections or invasive dental treatment that easily spread to the jawbone.¹⁷ However, within the scope of the authors' search, there have been no reports of BP having had an effect on the teeth.

All the patients in this report were undergoing LTS therapy to alleviate the symptoms of their primary disease, and dental X-rays were taken for oral management to prevent osteonecrosis of the jaw caused by BP, which confirmed pulp cavity constriction. However, the effect of steroids on teeth has not been clarified. The reported effects of LTS use on teeth include hyperesthesia-like symptoms^{18,19} and pulp chamber constriction.²⁰ The mechanism of the onset of pulp chamber constriction has not yet been fully determined, but it is thought to involve a phenomenon in which steroids enhance the expression of the

dentin sialophosphoprotein gene and type I collagen, as well as alkaline phosphatase activity.^{21,22} Moreover, steroids are fat-soluble and therefore distributed in a wide range of tissues; however, the proteins they bind to and their binding strength varies depending on the type of steroid.^{8,9}

Pulp chamber constriction due to steroids depends on when the steroid is used, how long it has been used, and the dosage.²⁰ Most patients in this study started using steroids when they were 20–30 years old. The estimated age of subjects with a history of high-dose steroid injections was extremely high. Among the subjects of this study, especially those in their twenties who had repeatedly increased and decreased their steroid dose for 18 years, we found pulp area stenosis that was inappropriate for their age (Figure 2). Furthermore, when we compared the previous and present results of patients who had radiographs taken twice, several years apart, we observed that the pulp area had clearly narrowed in a short period (Figure 3). In the upper canines, the estimated age did not match the actual age when the age estimation method derived in this study was applied.

Participants who showed a narrow pulp area included those with histories of mental illnesses such as depression, neurodevelopmental disorders, schizophrenia, and bruxism. There were also unknown causes besides LTS use; therefore, various aspects must be duly considered when performing forensic age estimation.

Figure 2. X-ray image findings of the patient (case No. 8), 39 years old, woman. Left: Upper left incisors and canine. Right: Lower left incisors and canine. The pulp area on each tooth is narrow, and the findings are not congruent for the age of the patient.

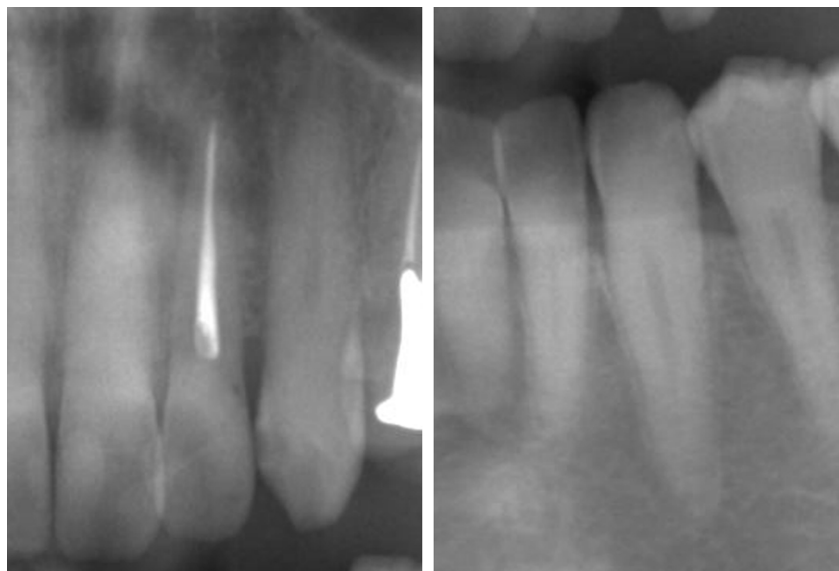


Figure 3. X-ray image findings of the lower left lateral incisor and canine in the same patient (case No. 5). Left: Two years ago, 44 years old. Right: Present findings, 46 years old. The pulp area of each tooth's crown section in particular has narrowed rapidly in just a few years.



CONCLUSION

The estimated ages of the Japanese LTS users were significantly different from their actual ages and from the regression line derived from the general study participants. For cases that require age estimation in the forensic field, the identity of the person is unknown and there is no way to

determine their medical history. In estimating age, if the findings of the relevant person's pulp area on the radiograph are inconsistent with other physical forensic findings, the information may assist in predicting their medical background.

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Third molar maturity index for discriminating between adults and minors: validation in an Iranian sample

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KEYWORDS

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ABSTRACT

The life-altering effects of criminal trials necessitate providing reliable methods to distinguish adults (≥ 18) from minors (< 18). The present study aims to evaluate the accuracy of the third molar maturity index (I₃M) introduced by Cameriere et al. (2008) in distinguishing adults from minors in the Iranian population. Panoramic radiographs of 800 Iranian individuals (400 males and 400 females) aged 14-23 were evaluated. The cut-off value of I₃M=0.08 was analysed to determine whether the individual is younger or older than 18. All male or female subjects with I₃M above 0.7 were below 18 years old. The cut-off value of 0.08 showed a sensitivity of 80.83% and 63.33% and a specificity of 88.12% and 93.12%, respectively, in males and females. The positive predictive values were 91.08% and 93.25%, and the negative predictive values were 75.40% and 62.87%, respectively, for males and females. The Bayes' post-test probability was 94% for females and 92% for males. 83.75% of males and 75.25% of females correctly classified as adults or minors. The most remarkable error rate occurred at 18 years old (67.5 % in females and 57.5% in males). By ROC curve analysis, the population-specific cut-off values of I₃M 0.097 for males, 0.116 for females, and 0.099 regardless of sex, were acquired, which improved the sensitivity of discrimination between adults and minors (86.3%, 73.8%, and 78.1 %, respectively). The results showed that I₃M is a reliable method for distinguishing between minors and adults in the Iranian population. This method provides a higher accuracy level in identifying individuals under 18 years old. Population-specific I₃M cut-off values increased accuracy, sensitivity, and NPV, especially for females. The I₃M method produced better results in diagnosing adult males than adult females and a higher accuracy level in identifying individuals under 18.

INTRODUCTION

Forensic age estimation has become a growing concern in criminal and civil cases involving young individuals, illegal immigration, and a variety of refugee status difficulties. It has been shown that half the refugees from Middle Eastern or African countries are children below 18 years of age. ¹ In many countries, this age represents a legal cut-off to distinguish minors from adults. ²⁻⁶ Also, the International Criminal Court, founded in 1998 to investigate and prosecute those suspected of committing an offence, considered 18 years old, the age of

the legal majority.⁷ The life-altering effects of criminal trials and the protection of minors' legal rights necessitate providing reliable, lawful, and non-invasive methods to distinguish adults from minors accurately.

Following the guidelines of the Study Group on Forensic Age Diagnostic (AGFAD), the age estimation of a living individual includes 1) Physical examination to record anthropometric data, sexual maturity signs, and age-appropriate developmental anomalies; 2) Radiography of the left hand or the clavicle (in case of completed wrist skeletal development); 3) X-ray analysis of the dentition to determine dental maturity.⁸ Based on AGFAD recommendation, combining three independent developmental systems enhances the diagnostic accuracy of age estimation.⁹

Skeletal techniques have some disadvantages due to the substantial heterogeneity of bone growth, which is impacted by environmental variables.¹⁰ However, dental development has less variability since it is under common control by genes rather than environmental factors.¹¹ It is indicated that socio-economic status is more pronounced in skeletal maturation than in dental development.¹² Cardoso¹² showed that age estimation in pre-adolescents using long bone development leads to biased, divergent results, especially in children with low socio-economic status. In contrast, dental age estimation is almost buffered against environmental factors. For dental age estimation of individuals between 14 and 23 years of age and to discriminate children from adults, analysis of third molar development is the most practical and appropriate indicator since these teeth are still developing at this age.^{13, 14} Mincer et al.¹⁵ pioneered applying Demirjian's staging system for age estimation based on third molar teeth. They showed that the majority of male and female subjects (90% and 92%, respectively) with fully developed third molars (those in stage H) were adults. However, this yields significant false negative results due to the high number of individuals over 18 years of age with unmaturing third molar teeth.¹⁶ More recently, Cameriere et al.¹⁶ proposed the third molar maturity index (I3M), the ratio between the width of open apices and the third molar tooth length, as a method of age estimation based on third molar development. They provided a cut-off value of 0.08 to discriminate between adults (≥ 18) and minors (< 18). This method was shown to have

higher sensitivity, specificity, and lower frequency of false negative results.

Population affinity has been shown as a potential factor influencing the accuracy of anthropological methods,^{5, 17} including age estimation derived from third molar development.¹⁸ In this regard, several studies have evaluated the accuracy of the Cameriere cut-off value in different populations worldwide; however, the accuracy of the Cameriere cut-off value for discrimination of minors from adults has yet to be carefully addressed in the Iranian population. So, the main aim of the present study was to evaluate the cut-off value of I3M 0.08 to discriminate minors from adults in the evaluated sample.

MATERIALS AND METHODS

Samples

The institutional human ethics committee approved the research protocol for this cross-sectional study (#IR.Sums.Dental.REC.1399.088).

Digital panoramic radiographs of 1296 Iranian living subjects aged 14-23 years were evaluated. The images were retrieved from the Oral and Maxillofacial Radiology Department of Shiraz Dental School archive. The radiographs were taken for specific clinical indications other than the present study. All the patients or their guardians had signed a written informed consent for the possible use of their radiographic data, preserving their anonymity in research or publications.

The images with low quality (N=122), any pathological lesion in the area of interest (N=38), multiple dental anomalies (N=3), hypodontia (N=1), or congenitally missing mandibular third molar teeth (N=46) were excluded. Mandibular third molar teeth with an extensive carious lesion (N=24), endodontic treatment (N=1), coronal restoration (N=11), or severe rotation (N=223) were also eliminated from the study. Moreover, radiographs from individuals with a history of systemic disease or developmental abnormality and dental / maxillofacial trauma (N=27) were also excluded from the study. Finally, the study sample comprised 800 panoramic radiographs (400 males and 400 females), then divided into ten one-year age groups. Each group included 80 subjects evenly distributed according to sex (40 male and 40 female subjects). (Table 1)

Table 1. Distribution of the subjects per age and sex groups (Female/Male). The numbers in the parenthesis show the subjects with completely developed left mandibular third molars ($I_3M=0.00$).

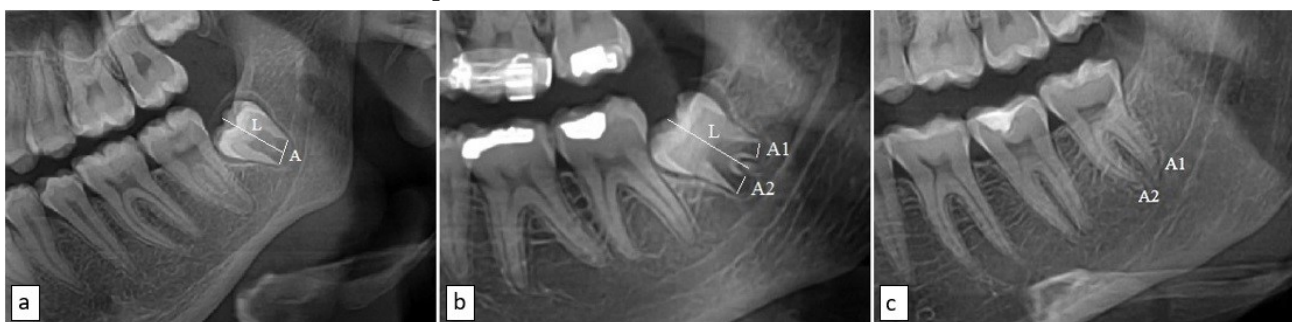
Age categories	Males	Females	Total
14-14.9	40(0)	40(0)	80(0)
15-15.9	40(0)	40(2)	80(2)
16-16.9	40(2)	40(0)	80(2)
17-17.9	40(3)	40(1)	80(4)
18-18.9	40(6)	40(3)	80(9)
19-19.9	40(11)	40(7)	80(18)
20-20.9	40(18)	40(9)	80(27)
21-21.9	40(22)	40(9)	80(31)
22-22.9	40(32)	40(19)	80(51)
23-23.9	40(32)	40(27)	80(59)
Total	400(126)	400(77)	800(203)

Age is presented in years.

All panoramic radiographs were taken by the Vatech PaX-I panoramic X-ray unit (Vatech Co., Seoul, Republic of Korea). According to the Cameriere et al.¹⁶ method, the I_3M of each subject was calculated as the ratio of the distance between the inner margins of the third molar's open apex to the total tooth

length to eliminate the effect of magnification differences in radiographs. In this regard, I_3M is measured as zero in completely developed roots, and in third molar teeth with two or more roots, it is defined as the sum of the width of open apices to the tooth length (Fig. 1).

Figure 1. Calculation of I_3M in third molar teeth with different numbers of roots and developmental stages. A1 and A2: width of the two open apices' inner margins; L: tooth length. a) A/L in a one-rooted third molar; b) $(A1+A2+...)/L$ in a third molar with two (or more) roots; c) I_3M is calculated as zero in a third molar tooth with closed root apices



The measurements were recorded on the left mandibular third molar teeth, either erupted or impacted, for standardisation. However, since a strong correlation has been shown between the development of the left and right third molar teeth,^{19, 20} the measurements were recorded on the right counterpart whenever

the left mandibular third molar did not meet the inclusion criteria. The radiographs were coded with an ID to obscure the subjects' demographic data and prevent observer bias. For calculating the chronological age of the subjects, the birth date of the individuals was subtracted from the date of radiographic

acquisition and then rounded to one decimal place.

Radiographic images were evaluated by a well-trained and calibrated last-semester dental student using EzDent-i software (Vatech Co., Ltd., Hwaseong, Korea) on a 14-inch monitor in deemed environmental lighting. The observer adjusted image contrast, brightness, and sharpness or used a zoom-in option to better visualise tooth anatomy. The same observer randomly selected 200 radiographs of the study sample and examined them again after two weeks to assess the observer-intra agreement.

Statistical analysis

To examine intra-observer reliability for the evaluated panoramic radiographs, the intra-class correlation coefficient (ICC) was calculated. The cut-off value of 0.08 was applied to distinguish adults from minors, as described by Cameriere.¹⁶ Subjects with an I3M less than 0.08 were considered adults (≥ 18), and subjects with an I3M greater than 0.08 were considered minors (<18).

The sensitivity (Se), the proportion of correctly determined adults out of all the subjects with the age of ≥ 18 , and the specificity (Sp), the ratio of precisely defined minors out of all the subjects with ages below 18 years old, as well as accuracy (Ac), a proportion of correctly classified subjects among all the adult subjects were calculated.

The positive and negative likelihood ratios (LR+ and LR-) and predictive values (PPV and NPV) were computed. The LR+ determines how much more likely the positive result ($I3M < 0.08$) is to occur in adults in comparison with minors, and the LR- determines how much less likely the negative result ($I3M \geq 0.08$) is to appear in adults compared to minors. LR+ values between 2-5 show a slight increase, and 5-10 and over 10, respectively, represent moderate and significant increases in the likelihood of being over 18 years old. LR- values of 0.5-0.2 demonstrate a small, 0.2-0.1 means a moderate decrease, while < 0.1 reflects a significant/almost definite reduction in the likelihood of being over 18 years old²¹. PPV represents a proportion of adults with positive

test results (true positives) in total subjects with a positive result ($I3M < 0.08$). In other words, PPV defines the probability of being an adult when I3M is < 0.08 . NPV represents a proportion of minors with a negative test result (true negative) in total subjects with negative test results ($I3M \geq 0.08$). NPV determines the probability of being under 18 years old when $I3M \geq 0.08$.²²

The post-test probability, which can help to differentiate adults from minors, was also calculated, which according to Bayes' theorem (Bayes PTP), is defined as:

$$P = \frac{Se P_0}{Se P_0 + (1 - Sp) (1 - P_0)}$$

P: the post-test probability, Se: Sensitivity, Sp: Specificity, P_0 : the probability that the individual is ≥ 18 within the target population aged 14-23 years. In this study, P_0 was calculated based on the Iranian Bureau of Statistics data and was considered 63% for females and 62% for males.

A receiver operating curve (ROC) analysis was conducted to determine whether there is an I3M value and a higher discriminant ability between adults and minors for the Iranian population according to the highest Youden index (J).

Data analysis was performed using PASW SPSS software for Windows version 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

ICC showed a perfect intra-observer agreement.

Its value was 0.96 (95% CI 0.92, 1.00).

Table 2 shows the mean chronological age of the female and male subjects in different I3M classes. The values varied between the two sexes. The differences were statistically significant in I3M 0.04-0.5 (P value < 0.05). The mean age was greater in female than male subjects in all I3M classes except for the I3M 0.5-0.7 and 0.7-0.9. The data reveals that the I3M value had a general reverse relation with the mean chronological age. Additionally, all male or female subjects with I3M above 0.7 were below 18 years old.

Table 2. Descriptive statistics of the chronological age of the subjects based on ranges of I3M and sex

I3M	Female								Males								P value*
	N	Mean	SD	Min	Q1	Med	Q3	Max	N	Mean	SD	Min	Q1	Med	Q3	Max	
[0.0,0.0]	122	20.95	1.89	15	20	21	23	23	164	20.96	1.73	16	20	21.0	22	23	0.976
[0.04,0.08]	41	19.76	2.22	14	18	20	21.5	23	49	18.80	1.83	15	17.5	19	20	23	0.027
[0.08,0.3]	150	17.88	2.28	14	16	18	20	23	110	17.08	1.88	14	16	17	18	23	0.003
[0.3,0.5]	55	16.01	1.97	14	15	15	17	23	42	15.09	1.28	14	14	15	16	18	0.010
[0.5,0.7]	11	15	1.22	14	14	15	16	18	14	16	2.21	14	14	15.5	16.5	21	0.236
[0.7,0.9]	16	14.37	0.71	14	14	14	14.75	16	10	14.5	0.52	14	14	14.5	15	15	0.693
≥0.9	5	15	1	14	14	15	16	16	11	14.54	0.93	14	14	14	15	17	0.392

I3M: third molar maturity index; N: number of individuals; SD: standard deviation; Min: minimum value; Q1: 1st quartile; Med: median; Q3: 3rd quartile; Max: maximum value;

Table 3 reveals the association between the subject's age (adult ≥18 and minor <18) and the positive or negative test results (I3M <0.08 and I3M ≥0.08, respectively), and Table 4 summarises the measures for Ac, Se, Sp, LR+, LR-, PPV, NPV, and Bayes PTP. The data shows that 335 of 400 male subjects were correctly diagnosed as adults or minors. So, the accuracy of the I3M of 0.08 in the male subjects has been 83.75% (95%CI, 79.76-87.23%). Moreover, 301 of 400 female subjects were correctly diagnosed, which resulted in an accuracy of 75.25% (95%CI, 70.72-79.40%). The data in Table 3 also shows that in male subjects, the proportion of correctly determined adults (194) out of all the adult male subjects (240), the sensitivity of the I3M 0.08 in males, has been 80.83% (95%CI, 75.28-85.61%). For female subjects, this proportion and sensitivity were 152/240 and 63.33% (95%CI, 56.89-69.44%). In other words, an I3M of 0.08

produced better results in diagnosing adult males than adult females. On the other hand, the specificity, the proportion of correctly diagnosed minors out of all the subjects below 18 years old, was better for females (93.12%; 95%CI, 88.03-96.52%; 149/160) compared to males (88.12%; 95%CI, 82.08-92.70%; 141/160). The LR+ was 7.69 (95%CI, 5.44-10.86), showing that I3M < 0.08 is 7.69 more likely to be found in adult individuals (≥18) rather than minors (<18). The LR- was 0.31 (95%CI, 0.27-0.36), showing that I3M ≥0.08 is 0.31 less likely to occur in adults (≥18) than minors (<18). In the current study, the PPV was 92.02% (93.25% for females and 91.08% for males), indicating a high probability of being an adult if I3M is < 0.08. Post-test probability was 94% (95% CI, 88.8-97.0%) in females and 92% (95% CI, 87.2-95.0%) in males, considered excellent in both sexes. (Table 4)

Table 3. Cross-tabulation reporting discriminant function of cut-off value of 0.08 for the male and female subjects (male and female)

Quantities	Females	Males	Total
AC	75.25% (95% CI, 70.72-79.40%)	83.75% (95% CI, 79.76-87.23%)	79.50% (95% CI, 76.53-82.25%)
Sensitivity	63.33% (95% CI, 56.89-69.44%)	80.83% (95% CI, 75.28-85.61%)	72.08% (95% CI, 67.84-76.05%)
Specificity	93.12% (95% CI, 88.03-96.52%)	88.12% (95% CI, 82.08-92.70%)	90.62% (95% CI, 86.89-93.59%)
LR+	9.21 (95% CI, 5.17-16.43)	6.81 (95% CI, 4.44-10.43)	7.69 (95% CI, 5.44-10.86)
LR-	0.39 (95% CI, 0.33-0.47)	0.22 (95% CI, 0.17-0.28)	0.31 (95% CI, 0.27-0.36)
PPV	93.25% (95% CI, 88.25-96.58%)	91.08% (95% CI, 86.42-94.54%)	92.02% (95% CI, 88.81-94.55%)
NPV	62.87% (95% CI, 56.38-59.04%)	75.40% (95% CI, 68.59-81.39%)	68.40% (95% CI, 63.74-72.80%)
Bayes PTP	94% (95% CI, 88.8-97.0%)	92% (95% CI, 87.2-95.0%)	93% (95% CI, 89.5-95.1%)

AC: accuracy; LR+: positive likelihood ratio; LR-: negative likelihood ratio; PPV: positive predictive value; NPV: negative predictive value; Bayes PTP: Bayes post-test probability.

Table 4. The quantities from the cross-tabulation based on I3M of 0.08 for discrimination between adults (≥18) and minors (<18) in the sample of Iranian

	Test	Age		Total
		Adult≥18	Minor<18	
Males and females	I3M <0.08	346	30	376
	I3M ≥0.08	134	290	424
	Total	480	320	
Males	I3M <0.08	194	19	213
	I3M ≥0.08	46	141	187
	Total	240	160	
Females	I3M <0.08	152	11	163
	I3M ≥0.08	88	149	237
	Total	240	160	

Table 5 classifies the correct discrimination as an adult or minor based on age groups in both sexes. The data shows that the most remarkable error rate occurred in individuals with 18 years old (67.5% in females and 57.5% in males), followed by females at 19 years old (47.5%).

Based on the ROC curve analysis, the population-specific cut-off value of I3M 0.099 was acquired. When the subjects' sex was considered, the cut-off value was defined as 0.097 for males and 0.116 for females. As shown in Table 6, the cut-off value of 0.099 increased the sensitivity of discriminating adults from minors

(78.1% vs. 72.08%). The increase in sensitivity was greater when the specific cut-off value for females was used (0.116), which improved the sensitivity from 63.33% to 73.8%. Specific cut-off value for males (0.097) also increased the sensitivity from 80.83% to 86.3%. The specific cut-off values similarly improved accuracy (81.9% vs. 79.50%) and NPV (72.7% vs. 68.40%). On the other hand, these specific cut-off values decreased the specificity, which was more significant in females (87.5% vs. 93.12%), LR+ (6.25 vs. 7.69), LR- (0.25 vs. 0.31), and PPV (90.4% vs. 92.02%). (Table 6)

Table 5. The number of correctly diagnosed subjects as adults or minors/total subjects according to age groups using I3M of 0.08. The percentage of correct diagnoses is presented in parentheses.

Age (years)	Females	Males
14	39/40 (97.5%)	40/40 (100%)
15	37/40 (92.5%)	39/40 (97.5%)
16	38/40 (95%)	33/40 (82.5%)
17	35/40 (87.5%)	29/40 (72.5%)
18	13/40 (32.5%)	17/40 (42.5%)
19	21/40 (52.5%)	29/40 (72.5%)
20	26/40 (65%)	37/40 (92.5%)
21	24/40 (60%)	34/40 (85%)
22	31/40 (77.5%)	38/40 (95%)
23	37/40 (92.5%)	39/40 (97.5%)

Table 6. The quantities are based on population-specific I3M cut-off values of 0.116 for females, 0.097 for males, and 0.099 regardless of sex. (Please compare the results with Table 4)

Quantities	Females' cut-off value*	Males' cut-off value	Total cut-off value
AC	79.3% (95% CI, 74.9-83.1%)	86.5% (95% CI, 82.8-89.7%)	81.9% (95% CI, 79.0-84.5%)
Sensitivity	73.8% (95% CI, 67.7-79.2%)	86.3% (95% CI, 81.2-90.3%)	78.1% (95% CI, 74.2-81.7%)
Specificity	87.5% (95% CI, 81.4-92.2%)	86.9% (95% CI, 80.6-91.7%)	87.5% (95% CI, 83.4-90.9%)
LR+	5.90 (95% CI, 3.89-8.95)	6.57 (95% CI, 4.40-9.82)	6.25 (95% CI, 4.66-8.38)
LR-	0.30 (95% CI, 0.24-0.37)	0.16 (95% CI, 0.11-0.22)	0.25 (95% CI, 0.21-0.30)
PPV	89.9% (95% CI, 85.4-93.1%)	90.8% (95% CI, 86.8-93.6%)	90.4% (95% CI, 87.5-92.6%)
NPV	69.0% (95% CI, 64.1-73.5%)	80.8% (95% CI, 75.3-85.3%)	72.7% (95% CI, 69.1-76.0%)

AC: accuracy; LR+: positive likelihood ratio; LR: negative likelihood ratio; PPV: positive predictive value; NPV: negative predictive value.

DISCUSSION

Forensic age estimation is highly valued because of the effects the results would have on the individual's life. Lately, determining age has been one of the most common requests made by law enforcement and legal authorities, ²³ mainly due to the increasing number of immigrants/refugees, unaccompanied minors, and individuals with missing or uncertain birth documents. Based on statistics, in 2015, over two hundred thousand Middle Eastern persons, most of whom do not have personal documents, traveled through Serbia, and this number is rising due to the challenging situations in developing countries. ²⁴ The importance of age estimation stands out, especially in determining adults from minors. A child considered as an adult is at greater risk of improper conduct, precarious work, forced into early marriage, or the armed forces. ⁹ So, it is crucial to apply a reliable method for distinguishing minors from adults. Forensic dentistry works well in this regard by analysing the third molar maturity index introduced by Cameriere since third molars are still developing at 18 years old. ^{3, 5, 6, 25}

There are discrepancies in the literature regarding the effect of population affinity on the accuracy of age estimation. Some studies showed heterogeneity in dental development between different populations, ²⁶ and reported more accurate results using population-specific references. ²⁷ However, Sgheiza and Liversidge ²⁸ explain these differences by discrepancies in methodology between the reference and follow-up studies. The effect of sample size ^{28, 29} and uneven age distribution ^{30, 31} on producing bias

and increasing the error rate in age estimation methods have been discussed in the literature. Sgheiza ³² relates the population effects to the location-based factors instead of ancestry. On the other hand, multiple studies reported dissimilarities in the development, formation, and eruption of third molars between various populations. ^{14, 18, 26} Lewis and Senn ³³ recommended that population-specific investigations are necessary for dental age estimation based on third molars due to variable developmental rates and sexual dimorphism of the third molar teeth between various population affinities. Based on such findings, some researchers also suggest validating Cameriere's method across different populations. ^{6, 17} So, this study verified the Cameriere third molar maturity index in an Iranian sample and provided population-specific cut-off values.

We evaluated the panoramic radiographs of individuals aged 14-23, during which the third molar mineralisation is in process in most healthy individuals. ^{13, 34} Extending the upper age limit to older ages, whose third molars have closed apices, influences the mean value of I3M and adversely affects the discriminant performance of the test. Previous studies on different populations also applied a similar upper age limit for assessing third molar development. ^{16, 26, 35-37} The complete development of mandibular third molars in the subjects of the current study started at age 15 for females and 16 for males. This finding suggests that individuals belonging to this particular demographic who exhibit signs of complete apex closure should be acknowledged as being at the

age of at least 15 for females or 16 for males. Some previous studies ³⁶⁻³⁸ also reported a comparable minimum age for apex closure of the mandibular third molar in males. However, the minimum age reported in these studies for females was higher: 16, ³⁸ 17.63, ³⁷ and 17.74. ³⁶ Other studies ^{39, 40} reported a minimum age of 18-21 years for third molar apex closure in males and females.

Data from the present study demonstrates statistically significant (P value < 0.05) disparities in the mean ages of males and females within the range of I3M values from 0.04 to 0.5. The higher mean ages in females, with or without statistically significant difference, were evident in almost all the I3M values except for I3M 0.5-0.9. These results closely match those obtained by Deitos et al. ²⁵ in the Brazilian population, which revealed a notable difference in sex and early development in males throughout most of the I3M ranges examined, except for the I3M range from 0.7 to 0.9. Galic' et al. ³⁷ found comparable results in the Croatian population. They showed higher mean ages in female subjects in all the I3M ranges with statistically significant differences in I3M values between 0.00 and 0.3. A similar pattern was also noticed in the Albanian sample for the I3M range between 0.04 and 0.08. ⁴¹ On the other hand, some studies found no statistically significant difference in the timing of mandibular third molar teeth development between males and females across various I3M ranges. ^{3, 16, 36, 39}

Our findings also indicated that all individuals with $I3M \geq 0.7$ were under 18 years old. This amount was different in other studies as it was ≥ 0.9 for Serbian ⁴² and Croatian ³⁷ samples, ≥ 0.3 for the Libyan sample, ⁴³ and ≥ 0.7 for South Indian females and ≥ 0.3 for South Indian males. ⁴⁰ However, these differences should be interpreted with caution since the classification of I3M did not follow the same pattern in different studies.

This study employed the cut-off value of 0.08 for I3M, proposed by Cameriere et al., ¹⁶ to assess the efficacy of this technique in the Iranian population and to make the results comparable with those of similar studies. The test's sensitivity was 80.83% for males and 63.33% for females. The sensitivity is lower in both sexes than in most previous studies. ^{3, 5, 6, 36, 39-41, 44, 45} However, it is superior to that reported in Saudi Arabia (52.3% for males and 51.3% for females). ⁴⁶ The current study also exhibited a higher sensitivity in males

than females, indicating that the test identifies adults more accurately for males. Santiago et al. reported a similar pattern in a meta-analysis of multiple studies. ⁴⁷

Our results showed a relatively high specificity (90.62%, 93.12% for females, and 88.12% for males), better than the specificity reported by the studies on Brazilian, ²⁵ Australian, ⁵ and Chilean ⁴⁸ samples. Almost similar specificity, especially for females, was reported by Cameriere et al. ⁴¹, De Luca et al. ³⁹, and Galic' et al. ³⁷, respectively, for the Albanian, Colombian, and Croatian populations. However, some previous studies reported higher specificities. ^{3, 6, 44, 46}

Based on the current study's findings, the specificity was higher than the sensitivity. Most previous studies also showed this pattern. ⁴⁹ Only a few studies reported an inverse relation. ^{5, 39, 41} The tendency for higher specificity indicates that the examined index exhibits a higher accuracy level in identifying individuals under 18 years old compared to its effectiveness in detecting adults. The importance of prioritising specificity over sensitivity in age estimate methods has been emphasised from a forensic standpoint and adheres to the principles of medical ethics. ¹⁶ It is ethically unacceptable if an individual under 18 is incorrectly identified as an adult since it would breach their rights. ⁹ So, it is of vital importance to eliminate the false positive results (increase specificity).

In this survey, the PPV was 93.25% in females and 91.08% in males. PPV defines the probability of being an adult when the I3M is < 0.08 . This result shows that in the Iranian sample, when the I3M of an individual is < 0.08 , the probability of that person being an adult is 93.25% for females and 91.08% for males. PPV was reported 87% and 92% for Japanese female and male subjects. ⁴⁵ PPV was also reported in some previous studies as 94.6 to 98.1%. ^{3, 36, 42, 44} Previous studies also reported NPV between 87%-100%, ^{3, 36, 42, 44} while we found an NPV of 75.40% for males and 62.87% for females. The NPV defines the proportion of minors out of all the subjects with $I3M \geq 0.08$. In the present study, the higher specificity compared to sensitivity and greater PPV than NPV, especially in female subjects, suggest relatively high false negative results ($I3M \geq 0.08$ in adults) in females. In contrast, the false positive results ($I3M < 0.08$ in the subjects under 18 years old) are relatively low in our sample.

Most errors in classifying individuals as minors and adults by I3M 0.08 in the current survey occurred at 18 in both sexes (67.5 % incorrect classification in females and 57.5% in males). The greatest classification errors reported by the previous studies ^{42, 44, 50} were also at 18. The percentage of correctly classified subjects, *true* positive or negative, across all the adult subjects, or the general accuracy was around 80% (83.75% for males and 75.25% for females). Almost similar accuracy (83%) was reported by Cameriere et al. ¹⁶ These accuracies are higher than those reported by AlQahtani et al. ⁴⁶ in Saudi Arabia but are lower than other previous studies. ^{5, 25, 39, 45, 50-52} We should consider that the accuracy level for age estimation sufficient for criminal and civil cases is different. The threshold is usually considered higher in criminal cases compared to civil cases. However, accepted accuracies for criminal and civil cases vary among countries. ⁵³ For example, in Germany, high accuracy in age estimation is needed, even in civil cases. Still, in Italy and numerous countries, ³⁵ an accuracy rate of at least 51% might be considered adequate in civil cases. Additionally, the different types of age estimation errors are interpreted differently in the criminal and civil frameworks. For instance, in criminal cases, false adults (false positive results) are the least favorable errors, which were relatively low in the current investigation. This study provided specific I3M cut-off values (0.116 for females, 0.097 for males, and 0.099 regardless of gender). Applying these values increased accuracy, sensitivity, and NPV, especially for females. On the other hand, they reduced specificity and PPV to a smaller degree. These data may indicate a decrease in false

negative results (adults wrongly classified as minors) but, at the same time, an increase in false positive results (children wrongly classified as adults). So, although applying the population-specific cut-off values increased sensitivity due to the vital importance of the protection of the legal rights of minors, the authors suggest using Cameriere's I3M cut-off value of 0.08 in the Iranian population.

Age estimation using I3M exhibits limitations primarily associated with variability in third molars' position, morphology, and development. Considering these limitations in expert judgment is essential to prevent overestimation. ^{34, 54, 55} Moreover, this method is not applicable without a mandibular third molar tooth, which may be extracted due to malposition or other related complications.

In future studies, it is suggested that the I3M be accompanied by other age estimation methods to evaluate whether it would compensate for the lower sensitivity we found for the Iranian population.

CONCLUSION

The I3M of 0.08 is a suitable method for discriminating minors from adults in the Iranian population. This study also provided population-specific I3M cut-off values (0.116 for females, 0.097 for males, and 0.099 regardless of gender). These values increased accuracy, sensitivity, and NPV, especially for females. On the other hand, they reduced specificity and PPV to a smaller degree. Generally, the I3M method provides higher specificity than sensitivity, which implies a higher accuracy level in identifying individuals under 18 years old.

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Comparative analysis of estimated age by pulp-to-tooth area ratio using CBCT in three different teeth on a subset of the Hyderabad population: A preliminary study

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KEYWORDS

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ABSTRACT

Objectives: The study aims to evaluate the pulp-to-tooth area ratio in permanent maxillary central incisors, lateral incisors, and canines for age estimation using three-dimensional cone beam computed tomography images.

Methods: Hundred cone-beam computed tomography (CBCT) images of patients aged between 12-70 years were retrospectively studied using NNT Viewer software version 13. Pulpal and teeth area were evaluated with the “area tool” in the acquired images in all three planes, and the pulp-to-tooth area ratio (PTR) was calculated with the measurements obtained. The reproducibility of intra-observer bias using paired t-tests was calculated.

Results: The coefficient of correlation (r) values of maxillary central incisors with age are -0.54, -0.623, and -0.50 in the axial, sagittal, and coronal planes respectively. The r values of maxillary lateral incisors are -0.05, -0.52, and -0.25 in the axial, sagittal, and coronal planes. The coefficient of correlation values (r) of maxillary canine with the age is -0.53, -0.62, and -0.49 in the axial, sagittal, and coronal planes. A strong correlation between chronological age and PTR in multiple sections of the maxillary incisors and canines (p -value < 0.05) was observed.

Conclusion: The permanent maxillary central incisor and canine yielded promising results in predicting age. The predicted age derived by mid-sagittal sections was closer to the chronological age than the axial sections, which had the least predictability.

INTRODUCTION

The hardest material in the body is human teeth, which makes them incredibly durable and helpful in situations where the body's soft tissue structures are destroyed by fire or other natural disasters.¹ Post-mortem dental records are acquired using radiographs, visual examinations, and dental charting in combination. Forensic radiology is the practice, interpretation, and reporting of radiographic examinations and procedures necessary for law enforcement or legal processes. For identification, age determination, and diagnosing the cause of death, radiological techniques are commonly used.² Unlike dental charts or written records, which may contain genuine errors or falsifications, dental radiography offers an impartial anatomical overview. Forensic dental identification requires

radiology as a crucial component.³ The least invasive radiologic method for estimating age is using panoramic X-rays, which only need a single image to capture the entire dentition. In addition, bone structures including the jaw, nasal fossa, and vertebrae are also visible for further evaluation.⁴ When estimating an adult's age, several authors concentrated on changes associated with aging that can be seen by radiography. Three approaches can be recognized in this context.

A few researchers investigated estimating age using specific ratios or metrics. For instance, by evaluating the tooth pulp radiopacity, Kvaal et al. suggested an indirect method of measuring dentin apposition.⁵ Using linear ratios, investigators correlated the multiple linear measurements they took of the pulp and the tooth. Analogously, Cameriere et al. suggested area assessments in place of linear measurements.⁶ Another illustration of this type of measure is the Tooth Coronal Index (TCI), a height ratio at the crown level between the pulp cavity and the crown that was investigated by Ikeda et al.⁷ Two-dimensional imaging systems are inadequate due to their generation of dimensional fluctuations, mesiodistal dimension limitation, and analytical hindrance caused by superpositions and malpositions. For precise forensic identification, 3D images are therefore necessary. The use of 3D measuring techniques to infer age from secondary dentin deposition was pioneered by Vanderwoort et al.⁸ Using micro-CT, CT, or CBCT, subsequent studies have analyzed linear measurements, areas, and volumes.⁸

Dental growth, particularly in children, is a key factor in radiographic age estimation. In adults, however, a reduction in pulp area indicates the continuing deposition of secondary dentin throughout life.⁹ The interrelationship between pulp width and aging was established with a metric analysis of the pulpal dimensions introduced by Kvaal et al. in 1995, which is based on the deposition of secondary dentin.¹⁰ Cone-beam computed tomography (CBCT) has made it possible to view teeth in three dimensions (3D) and to take more precise measurements.¹¹ The present study aimed to analyze the pulp-to-tooth area ratio in three different teeth for age estimation using three-dimensional cone beam computed tomography images.

MATERIALS AND METHODS

This retrospective study included 3D CBCT scans of 100 patients with ages ranging from 12 to 70 years from the Department of Oral Medicine and Radiology at Army College of Dental Sciences (ACDS), Hyderabad, Telangana. Approval from the Institutional Ethics Committee (IEC) was sought for the same (ACDS/IEC/111/2022). The study included three-dimensional (3D) CBCT scans of the patients with fully formed roots of permanent maxillary central incisor, lateral incisor, and maxillary canine. CBCT scans were obtained using the NewTom GO Complete Vision machine (Cefla SC, Italy). NNT Viewer software version 13 (Cefla SC, Italy) was used to assess the pulp and tooth area of the maxillary central incisors, maxillary lateral incisors, and maxillary canines with fully formed roots using the area tool. The principal investigator (S B), an oral and maxillofacial radiologist with subject expertise used FOVs of 8X6 cm, 10X6 cm, and 10X10 cm to study the scans. The CBCT radiographic images used in this study were generated for diagnosis and standard dental care under the following exposure parameters: tube current 4.94 mA, tube voltage 90 kV, and exposure time 27.68 mAs. Patients with edentulous maxillary central incisors, lateral incisors, or canines, as well as those with root resorption (internal or external), teeth anomalies including but not limited to deformities, variations in tooth size or shape, or patients with any conditions affecting the pulp-to-tooth area, such as cysts or tumors in the region of interest, were excluded from the study. Additionally, radiographic images of patients with endodontic involvement in the selected teeth were excluded. Images for measurements were acquired using a multiplanar tool where the maxillary central incisors, lateral incisors, and canines were studied in all three planes, i.e., the axial at the level of CEJ, the mid-sagittal plane, and the mid-coronal plane (Figure 1). Pulpal and tooth areas were measured using the "area tool" in the acquired images in all three planes, and the pulp-to-tooth area ratio (PTR) was calculated from the measurements obtained (Figure 2). An image slice thickness of 0.15 mm was selected. A random sample of 70 radiographs was used for metric reiteration of the pulp-to-tooth area after two weeks by the same investigator S B, who was blinded to the chronological age of the subjects,

to test the reproducibility of intra-observer bias using a paired t-test. A descriptive analysis was performed to obtain the mean age of the study sample and the mean pulp-to-tooth area ratio of the maxillary central incisors, lateral incisors, and canines in multiplanar sections (viz., axial at the level of CEJ, mid sagittal, and mid coronal sections), as depicted in (Tables 1 and 2). Pearson

correlation coefficient between age and PTR based on tooth type, and the section type was calculated (Table 3). Linear regression analysis for age (dependent variable y) and the PTR of the three planes for the three tooth types (SEE= Std error of estimate) was calculated (Table 4). A multiple regression model with all the planes combined for each tooth was developed (Table 5).

Figure 1. Multiplanar selection of teeth to measure the pulp and tooth areas in axial, coronal, and sagittal sections.

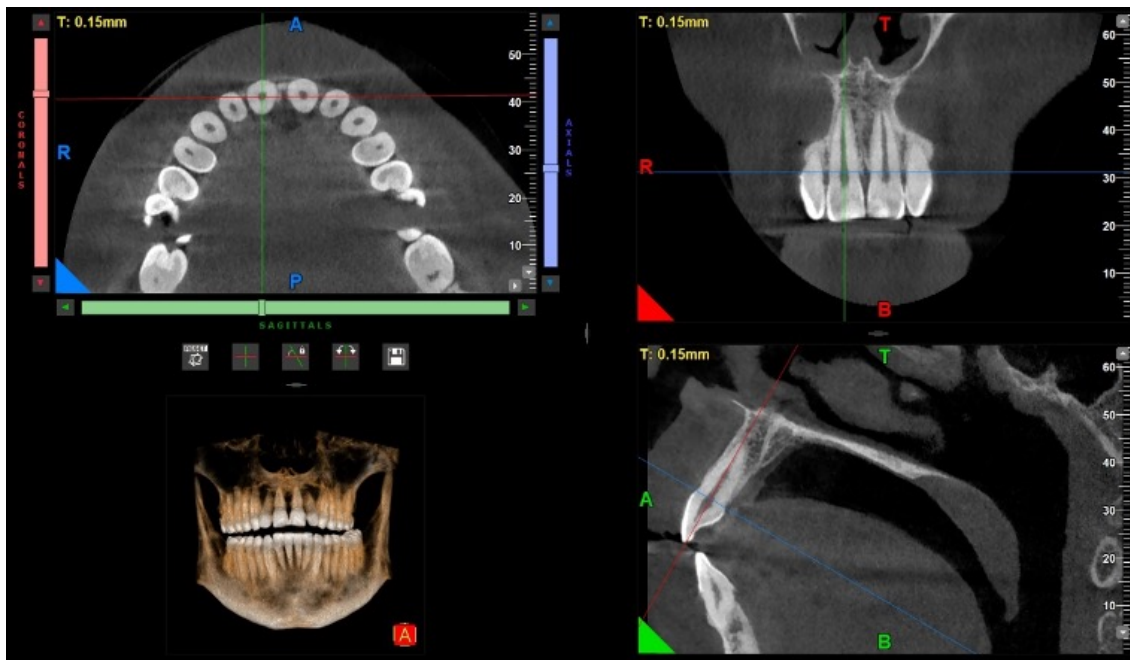
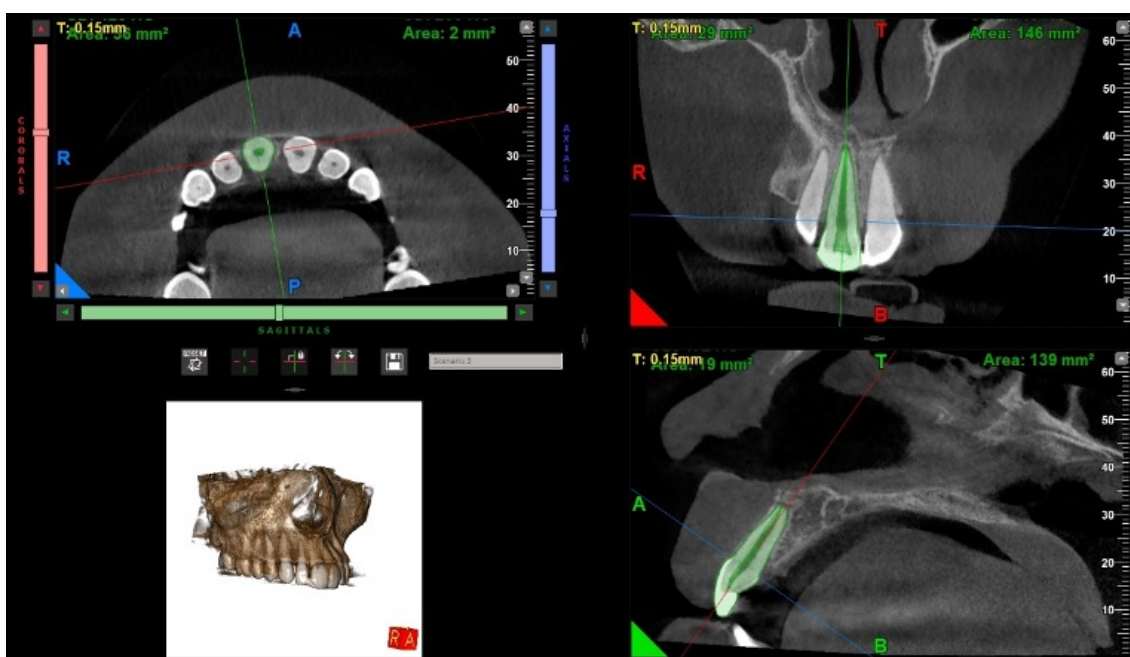


Figure 2. Tooth and pulp area measurements in the axial, coronal, and sagittal sections



Statistical analysis:

The obtained data were subjected to correlation and regression analysis using SPSS software version 25.0 (IBM Corp., USA). The standard error of estimate (SEE) was calculated to estimate the standard deviation of the estimated age using the PTR values. The coefficient of determination (R²) was calculated to measure the predictive power of PTR in different orthogonal planes for age estimation in the given sample.

RESULTS

The mean age of the study sample is 34.41 years, and the mean pulp-to-tooth area ratio of the

maxillary central incisors, lateral incisors, and canines in multiplanar sections was calculated and depicted in Tables 1 & 2.

The Pearson correlation coefficient between age and PTR based on tooth type and section type was calculated (Table 2).

Table 3 and 4 depicts the regression equation, standard estimate of error, and coefficient of determination of teeth of interest in all three sections (i.e., axial at the level of CEJ, mid-sagittal, and mid-coronal).

A paired sample t-test was used to analyze the intra-observer variation between the initial and subsequent measurements, and there was no statistically significant variation ($p > 0.05$).

Table 1. Sample size distribution.

Age range (years)	Sample size distribution	Mean age: 34.41 Median: 35
12-30	43	
31-50	42	
51-70	15	

Table 2. Mean values of continuous variables

Variable		Mean	Std. Deviation
Age (years)		34.41	13.479
Class of Tooth studied			
Maxillary central incisors	PTR axial	.0626	.02035
	PTR sagittal	.1523	.03875
	PTR coronal	.1482	.04959
Maxillary lateral incisors	PTR axial	.1269	.52383
	PTR sagittal	.1507	.04371
	PTR coronal	.1430	.04066
Maxillary canines	PTR axial	.0503	.01955
	PTR sagittal	.1924	.05117
	PTR coronal	.1261	.03715

Table 3. Age correlations with the type of tooth and the sections studied.

Type of tooth		Correlation coefficient (r)	p-value
Maxillary central incisor	Axial section at the level of CEJ	-.540	.000
	Mid sagittal	-.623	.000
	Mid coronal	-.500	.000
Maxillary lateral incisor	Axial section at the level of CEJ	.059	.541
	Mid sagittal	-.522	.000
	Mid coronal	-.255	.007
Maxillary canine	Axial section at the level of CEJ	-.530	.000
	Mid sagittal	-.625	.000
	Mid coronal	-.493	.000

Table 4. Regression equation to calculate the age.

Type of tooth		Regression equation (Y = age)	Standard estimate of error (SEE) in years
Maxillary central incisor	Axial section at the level of CEJ	$Y = 56.779 + (-357.485 * PTR \text{ axial})$	± 11.5
	Mid sagittal	$Y = 67.419 + (-216.77 * PTR \text{ sagittal})$	± 10.0
	Mid coronal	$Y = 54.567 + (-135.992 * PTR \text{ coronal})$	± 11.3
Maxillary lateral incisor	Axial section at the level of CEJ	$Y = 34.217 + (1.515 * PTR \text{ axial})$	± 14.3
	Mid sagittal	$Y = 58.676 + (-161.008 * PTR \text{ sagittal})$	± 11.7
	Mid coronal	$Y = 46.493 + (-84.483 * PTR \text{ coronal})$	± 14.0
Maxillary canine	Axial section at the level of CEJ	$Y = 52.786 + (-365.482 * PTR \text{ axial})$	± 10.4
	Mid sagittal	$Y = 66.103 + (-164.735 * PTR \text{ sagittal})$	± 08.0
	Mid coronal	$Y = 56.950 + (-178.729 * PTR \text{ coronal})$	± 12.2

Table 5. Regression analysis for all three planes combined for each tooth

Tooth	R	R ²	p-value for the model	Regression equation (Y = age in years)	Standard estimate of error (SEE) in years
Central	.696	.484	.000	$Y = 73.914 + (-179.85 * PTR \text{ axial}) + (-133.93 * PTR \text{ sagittal}) + (-52.99 * PTR \text{ coronal})$	9.817
Lateral	.531	.261	.000	$Y = 61.197 + (1.02 * PTR \text{ axial}) + (-152.31 * PTR \text{ sagittal}) + (-27.69 * PTR \text{ coronal})$	11.58
Canine	.698	.472	.000	$Y = 73.47 + (-149.62 * PTR \text{ axial}) + (-112.038 * PTR \text{ sagittal}) + (-82.533 * PTR \text{ coronal})$	9.79

DISCUSSION

Dental age estimation is a discipline of forensic dentistry that uses factors such as tooth development, biochemical alterations, and modifications following the completion of the teeth to determine dental age.¹² The present study calculated the pulp-to-tooth area ratio of the maxillary anterior teeth using 3D CBCT images. CBCT enables multiplanar cross-sectional images with 3D reconstruction in a single scan, in addition to nominal anatomic superimposition and geometric distortion.¹¹ Permanent maxillary anterior teeth are chosen considering the simplicity of the pulpal morphology as well as the fact that the incisors and canines are less likely to be affected by attrition and/or carious processes compared to the posterior teeth.¹³ In addition, the relatively longer roots with wider pulp areas offer ease in the metric analysis of the pulp-to-tooth area.¹³ Alkarni et al. proved that no significant statistical difference between the left and right sides of the jaw was noticed. Hence, maxillary anterior teeth on either side were chosen in this present study.¹³ The pulp-to-tooth area was measured as the method of calculating tooth and pulp area may be more indicative of changes within teeth than the one-dimensional measurements of length and width made by Kvaal et al. Additionally, calculating area is easier and requires less equipment than computing pulp and tooth volumes.^{11,13} Among the permanent maxillary anterior teeth studied, except the PTR of lateral

incisors in their axial sections all three planes for all tooth types were significantly (negatively) correlated to age (Table 3). The findings are consistent with Rai A et al. and Muralidhar et al.^{14,15} The linear regression equation to calculate the age in years depicted a lower Standard Error of Estimate [SEE] (± 08.0 yrs) in maxillary canines in their mid-sagittal sections (Table 4). An overall superior predictive power in age estimation was observed in the mid-sagittal plane compared with the other planes of study. One possible explanation for the superior accuracy of measurements at sagittal sections over axial ones could be the ratio being unaffected by image distortion from teeth that are misaligned.¹⁶ This is per the studies conducted by Lee et al. and Affiy MM et al., who used CBCT images in the buccolingual and axial dimensions of maxillary canines to measure PTR in Korean and Egyptian populations, respectively. The authors further proposed that the reliability of sagittal measurements in age estimation could be due to a larger PTR of the images in the sagittal section than in axial section images.^{16,17} A CBCT study by N. Jagannathan et al, using extracted human mandibular canines was used to estimate the age by modifying Yang's formulae and stated that the pulp-to-tooth volume ratio of mandibular canines is a useful predictor of age.¹⁸ The study results directed for a population-specific formula for age estimation. The regression equation in the current study was specific to each tooth type studied and the pulp-to-tooth area ratio served as

a useful predictor of age. In a study where maxillary and mandibular second molars were studied for age-related changes in the anatomical configuration of pulp volume, M A Helmy et al observed a statistically significant increase in the pulp chamber volume with an increase in age. This study also noticed significant differences in the pulp volume between males and females. The dependence and correlation of the pulp metrics with that of the chronological age are consistent with the findings in the present study. However, the regressive pulpal morphometrics were not studied among males and females independently in the present study.¹⁹ On the other hand, a study conducted by Sironi et al using the Bayesian method for evaluating age-related dental evidence found the influence of gender upon the narrowing of pulp was uneven.²⁰ The significance of PTR of the mandibular canines in age estimation was proven to be diverse based on a variety of studies.^{4,16,18,21,22} A C Pires et al in their CBCT study to estimate the age among the Portuguese population found that there are statistically significant differences between the chronological age and the dental age of individuals using pulp/tooth ratio, and concluded that Kvaal's formulae for age estimation are not reproducible in the Portuguese population when applied in CBCT which is in contrast with the present study.²³ In a pilot study, V Pinchi et al observed that the pulp chamber's narrowing is a dependable indicator of an adult's age, and CBCT is a simple, conservative method that provides precise tooth volume calculations. The suggested method is predicated on a geometric approximation of the upper central incisor volumes derived by free image processing software and assessed by CBCT.²⁴ Studies by Wu et al. and Haghanifar et al. are conclusive about the reliability of the maxillary central incisors in age estimation in comparison with that of the maxillary canines, which depicted a higher predictive power and lower SEE in maxillary central incisors.^{21,25} Studies by Biuki et al. and

Gulsahi et al. used teeth and pulpal volumes and found maxillary incisors as the better and superior means for age estimation.^{26,27} The multiple regression analysis with all the planes combined for each tooth type showed a SEE of ± 9.8 yrs and ± 9.79 yrs for maxillary central incisors and canines respectively, which reveals a comparable accuracy of these teeth types in age estimation using PTR (Table 5). Furthermore, the SEE values $< \pm 10$ years are of pertinent forensic value in age estimation studies. The limitation of the current study is that it excludes the possibility of inter-observer bias. Studying the pulp volume although sophisticated would give a deeper insight into the metric analysis however, the current study's primary objective included only linear measurements. Furthermore, the limited sample size in this study makes it difficult to apply the formula broadly.

CONCLUSION

The present study analyzed the pulp-to-tooth area ratio in three different teeth for age estimation using three-dimensional cone beam computed tomography images, and the maxillary canines and central incisors have yielded acceptable results in predicting age. Mid-sagittal sections had the highest correlation to predict age, and the axial sections had the least correlation to predict age. Future directions: Extensive research to derive population-specific formulae is necessary. Future studies to include the impact of regressive alterations on the morphometrics of the teeth when studied radiographically.

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The manuscript has been read and approved by all the authors, the requirements for authorship have been met, and each author believes that the manuscript represents honest work, has not been published, accepted for publication, or is under editorial review for publication elsewhere and is free from conflict of interest.

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Age estimation at 18-year threshold: comparing Demirjian and Cameriere's methods for Thais

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KEYWORDS

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ABSTRACT

This study aims to compare the accuracy of Demirjian's and Cameriere's methods in determining adult age at the 18-year threshold using mandibular third molars in the Thai population. Panoramic radiograph images of 504 healthy subjects aged between 14 and 23 years were retrospectively collected. The developmental stages of mandibular third molars were evaluated using Demirjian's method, while the maturity index of mandibular third molars (I_{3M}) was assessed using Cameriere's method. Comparing the development of the left and right mandibular third molars, slight differences were observed: the left side developed 0.06 stages faster ($p = 0.01$) and had an I_{3M} 0.01 higher ($p < 0.01$). For the 18-year age threshold, Demirjian's Stage H demonstrated a specificity of 1.00 for both sexes on the left mandibular third molar, and 0.97 for males and 1.00 for females on the right. $I_{3M} < 0.08$ yielded specificities of 0.84 for males and 0.93 for females on the left, and 0.82 for males and 0.91 for females on the right. Reducing the cut-off to $I_{3M} < 0.02$ improved specificity to 1.00 for both sexes on the left side, and 0.96 for males and 1.00 for females on the right. Notably, using Demirjian's Stage H and $I_{3M} < 0.02$, the probability of being over 18 years was 1.00 for females on both sides, and 0.96 for males on the left and 1.00 on the right. Our study recommends using an I_{3M} cutoff of <0.02 for adult age assessment in the Thai population, as it achieves high specificity comparable to Demirjian's Stage H, with effective differentiation of adults.

INTRODUCTION

Accurate forensic age estimation for living individuals holds significant importance, both for the individuals undergoing examination and for society as a whole. It plays a crucial role in various contexts, including civil procedures, criminal investigations, and asylum cases.¹ Established protocols for age estimation in living adolescents and young adults are instrumental in ensuring justice, protecting individuals' rights, and facilitating informed decision-making in legal procedures.² In legal contexts involving young individuals, the age of 18 is widely recognised as a critical legal threshold. Internationally, the United Nations Convention on the Rights of the Child designates 18 as the age to categorise individuals as children or adults in legal contexts. In Thailand, this age holds significance in accordance with the Juvenile and Family Court and Its

Procedure Act, B.E. 2553. Therefore, this threshold impacts the legal procedure, potential sentencing and fundamental rights.

Dental age estimation is particularly useful because tooth development is influenced more by genetics than environmental factors and hormones, making teeth a reliable indicator of age.³ Dental age estimation methods, such as assessing dental developmental stages⁴ or measuring the open apices of developing teeth,⁵ can be applied in young individuals. However, beyond the age of 17, most teeth undergo complete development, except for the third molars.⁶ Hence, third molars are considered a key predictor for age estimation in teenagers and young adults. Current dental age estimation studies in the Thai population primarily rely on assessing tooth development stages of third molars.⁷⁻¹⁰ Notably, Duangto et al. (2016)⁷ utilised Demirjian's classification to estimate dental age from mandibular third molars, reporting that individuals with Stage H mandibular third molars were all confirmed to be over 18 years old. However, this method might suffer from limitations in sensitivity. Cameriere et al. (2008)¹¹ proposed the Third Molar Maturity Index (I_{3M}) as an alternative and compared this method against Demirjian's developmental stage-based method. I_{3M} achieved comparable specificity with Demirjian's method while exhibiting higher sensitivity, potentially minimising misclassifications. The proposed cut-off value of I_{3M} less than 0.08 allows for precise identification of individuals at the critical 18-year threshold.

Accurate dental age estimation relies on population-specific data, as methods effective in one group may not translate well to others due to genetic and environmental variations.¹² While the I_{3M} demonstrates promising potential exceeding Demirjian's method, its effectiveness remains untested in the Thai population. This critical lack of population-specific data hinders the confident application of I_{3M} for age estimation in Thai legal contexts. Therefore, this study aims to compare the applicability of Demirjian's and Cameriere's methods for identifying adults at the critical 18-year threshold within the Thai population. Identifying the most accurate approach can offer a valuable tool for legal proceedings, minimising the risk of misclassifying individuals and

contributing to fairer and more accurate legal outcomes.

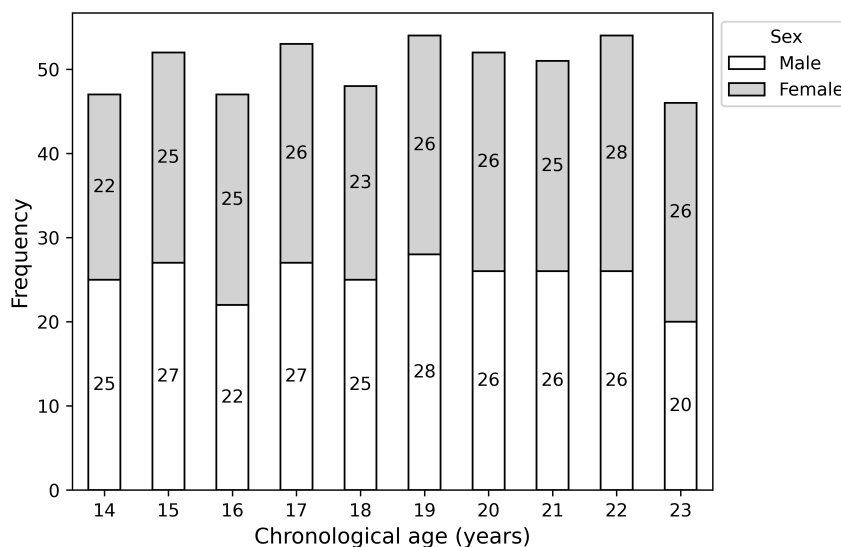
MATERIALS AND METHODS

Ethical approval and study sample

The research protocol received approval from the Human Research Ethics Committee (protocol EC6604-024) at the Faculty of Dentistry, Prince of Songkla University. This study retrospectively collected panoramic radiographs from the Radiology Clinic at the Faculty of Dentistry, Prince of Songkla University from 2018 to 2024. Two radiographic units were used to capture the radiographic images: GXDP-700 (Gendex Dental Systems, Hatfield, Pennsylvania, USA), and Orthopantomograph OP300 (Instrumentarium Dental, Charlotte, North Carolina, USA). These radiographs were exported from the system in JPEG file format. In addition, data including sex, date of birth, and radiographic were collected. Chronological age was calculated by subtracting the date of birth from the radiographic date. All collected data underwent anonymisation and were kept confidential.

Sample size determination was conducted using R Project for Statistical Computing,¹³ with epiR package.¹⁴ The calculation was based on the specificity of I_{3M} in age group assessment derived from Scendoni et al.'s study,¹⁵ with an estimated prevalence of 0.6, an epsilon value of 0.05, and a confidence level of 95%. The minimum required sample size was 490. Therefore, this study employed stratified random sampling to recruit participants. The radiographic prescription lists from the Radiology clinic served as the sampling frame. We stratified the sample by both age and sex. For age, the sample was divided into groups ranging from 14 to 23 years. Individuals were then randomly selected from each stratum defined by age and sex to ensure a representative sample across the targeted demographic. The exclusion criteria encompassed poor radiographic quality, absence of both left and right mandibular third molars, severe buccoversion or linguoversion, the presence of dental or bone pathology in the area of interest, and the existence of developmental or systemic diseases that could potentially impact tooth development. A total of 504 Thai healthy samples (252 males and 252 females) were included in this study (Fig. 1).

Figure 1. Distribution of the sample by age and sex

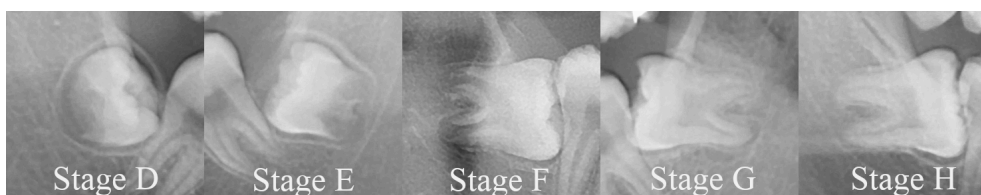


Radiographic assessment

Radiographic assessment involved two methods: Demirjian's and Cameriere's. For Demirjian's method, mandibular third molars were evaluated

based on developmental stages (Stages A to H). In this study, the collected samples only exhibited Stages D to H, corresponding to the age of the samples (Fig. 2).

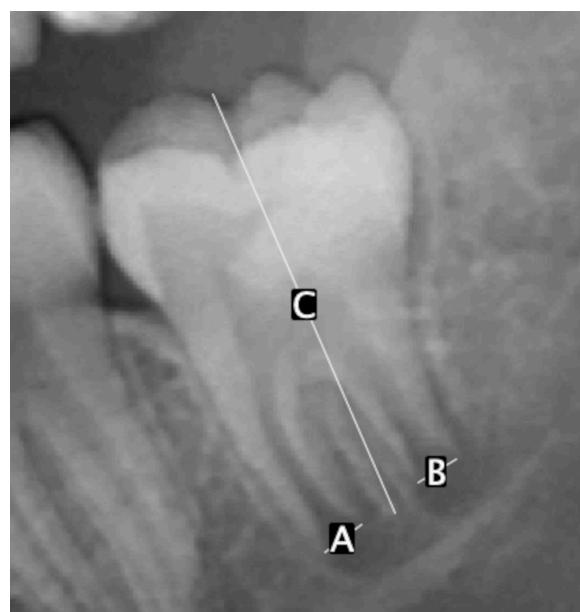
Figure 2. Examples of mandibular third molars Stages D to H according to Demirjian's classification



In Cameriere's method, determination of I_{3M} occurred in two instances. For complete root formation with a closed root apex, I_{3M} was designated as 0. In cases of incomplete root formation, I_{3M} was calculated by summing the distance from the inner to the inner surface of the root apex opening of each root (A+B) and dividing it by the tooth length (C) (Fig. 3). Measurements were conducted using ImageJ software.¹⁶

Two observers (KK and KC) evaluated tooth development using Demirjian's method, while measurement following Cameriere's method was performed by two different observers (AC and TP). All observers underwent calibration with an oral radiologist (WU), possessing five years of clinical experience, serving as the referee, before the commencement of the investigation. To assess inter-observer reliability, 50 radiographs were randomly selected. A month after the completion of the investigation, observers conducted a re-evaluation of the radiographs to determine intra-observer reliability.

Figure 3. An example of third molar maturity index measurements: A and B depict the distance of the open apices, while C illustrates tooth height



Statistical analysis

Cohen's kappa coefficient and intra-class correlation coefficient (ICC) were employed to assess the intra- and inter-observer reliability of Demirjian's and Cameriere's methods, respectively. The level of agreement was interpreted following Landis and Koch's guideline¹⁷ for Cohen's kappa coefficient and Koo and Li's guideline¹⁸ for ICC, respectively. Normality of the data was assessed using the Kolmogorov-Smirnov test for samples larger than 50 and the Shapiro-Wilk test for samples of 50 or fewer. Parametric statistics were employed for normally distributed data, while non-parametric statistics were used for data that did not follow a normal distribution.

Since the distributions of chronological age and I_{3M} for both the left and right mandibular third molars were non-normal ($p < 0.001$), and Demirjian's developmental stages are ordinal, Spearman's rank correlation was used to assess correlations between Demirjian's stages and chronological age, as well as between I_{3M} and chronological age. The independent t-test was applied for comparing age differences between males and females in groups with normal distribution, while the Mann-Whitney U test was used for non-normal distributions. For patients with both mandibular third molars, the Wilcoxon signed-rank test compared development between sides for both Demirjian's stages and I_{3M} , due to the non-normal distribution of I_{3M} data on both sides ($p < 0.001$).

The performance of Demirjian's developmental stage and I_{3M} in predicting age groups at the 18-year threshold was evaluated using sensitivity, specificity, accuracy, positive predictive value, and negative predictive value. Furthermore, Bayes post-test probability (p) was employed to determine the likelihood of individuals reaching the age of 18 years according to the two methods. This probability can be calculated as follows:

$$p = \frac{Se \times p_0}{Se \times p_0 + (1 - Sp)(1 - p_0)}$$

Where Se represents sensitivity and Sp represents specificity. Here, p_0 represents the probability of individuals being over 18 years old within the age range of 14 to 23 years. According to the Thai National Statistical Office, the values of p_0 for males and females are reported as 0.60 and 0.61, respectively. (<http://statbbi.nso.go.th/>).

The statistical analysis and data visualisation was performed within Jupyter Notebook, employing the NumPy, Pandas, SciPy, Matplotlib, and Seaborn libraries.

RESULTS

The inter-observer reliability of Demirjian's method was almost perfect, with a kappa value of 0.92. The kappa values for intra-observer reliability of the method were 0.93 (KC) and 0.92 (KK), indicating almost perfect agreement. For Cameriere's method, the inter-observer reliability yielded an ICC value of 0.99, interpreted as excellent agreement. The ICC values for intra-observer reliability were 0.99 (TP) and 1.00 (AC), indicating excellent agreement.

Table 1 and Fig. 4 illustrate the distribution of chronological age within each of Demirjian's developmental stages for the left and right mandibular third molars. The Spearman correlation coefficient between developmental stage and chronological age for the left mandibular third molar was 0.79 ($p < 0.01$) for males and 0.71 ($p < 0.01$) for females. These correlations for the right mandibular third molar were 0.82 ($p < 0.01$) for males and 0.78 ($p < 0.01$) for females, respectively. In the left mandibular third molar, there were no statistically significant differences in chronological age between males and females within each stage, except for Stage F ($p < 0.001$), where the median age of females was 1.67 years older than that of males. On the right side, differences in chronological age between sexes were observed at Stage G ($p = 0.017$), with females being 0.72 years older than males.

Table 1. Summary of descriptive statistics for chronological age (in years) and a comparison between males and females in each of Demirjian’s developmental stages for the left and right mandibular third molars (tooth 38 and 48)

Demirjian’s stage	Male				Female				p-value ^b
	N	Mean ± SD (years)	Median (years)	p-value ^a	N	Mean ± SD (years)	Median (years)	p-value ^a	
Tooth 38									
D	7	14.67 ± 0.50	14.49	0.201	16	15.12 ± 0.99	14.82	0.020 [†]	0.300
E	20	15.35 ± 0.82	15.35	0.735	37	16.17 ± 1.74	15.94	0.007 [†]	0.078
F	81	17.17 ± 1.82	16.85	<0.001 [†]	76	18.49 ± 2.34	18.52	<0.001 [†]	<0.001 [*]
G	70	20.09 ± 1.90	19.80	<0.001 [†]	61	20.49 ± 2.24	21.13	<0.001 [†]	0.183
H	54	21.53 ± 1.43	21.72	<0.001 [†]	35	21.71 ± 1.30	21.77	0.510	0.597
Tooth 48									
D	10	14.80 ± 0.66	14.66	0.145	13	15.23 ± 1.24	14.84	0.014 [†]	0.420
E	21	15.38 ± 0.83	15.14	0.117	34	15.86 ± 1.39	15.73	0.048 [†]	0.291
F	66	16.95 ± 1.68	16.80	<0.001 [†]	65	17.73 ± 2.09	17.60	<0.001 [†]	0.039
G	67	19.53 ± 1.90	19.55	<0.001 [†]	63	20.36 ± 2.03	20.27	<0.001 [†]	0.017 [*]
H	62	21.63 ± 1.51	22.01	<0.001 [†]	39	22.03 ± 1.48	22.14	0.020 [†]	0.186

N number of samples, SD standard deviation

^a p-value for normality test: Shapiro-Wilk test for N < 50; Kolmogorov-Smirnov test for N ≥ 50.

^b p-value for comparing mean age between males and females: use an independent t-test if normality is met; otherwise, use the Mann-Whitney U test.

[†] p-value < 0.05 indicates a significant deviation from normality, suggesting that the data is not normally distributed.

^{*} p-value < 0.05 indicates a statistically significant difference.

Figure 4. Boxplot illustrating the relationship between chronological age and Demirjian’s developmental stage of the left and right mandibular third molars, stratified by sex

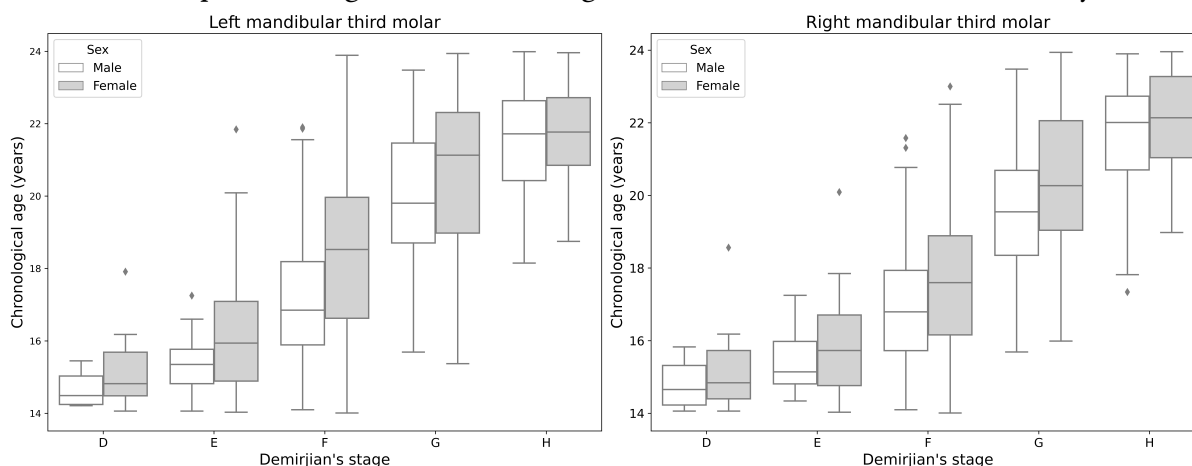


Table 2 and Fig. 5 illustrate the distribution of chronological age within each group of I_{3M}. The Spearman correlation coefficients between I_{3M} and chronological age were -0.81 (p < 0.01) for males and -0.79 (p < 0.01) for females on the left side. Correspondingly, for the right mandibular third molar, these coefficients were -0.83 (p < 0.01) for

males and -0.82 (p < 0.01) for females. In the analysis of age differences between males and females for the left mandibular third molar, statistically significant differences were found in the I_{3M} groups [0.08, 0.3) (p = 0.047), [0.04, 0.08) (p = 0.011), and [0.02, 0.04) (p = 0.026), where females had higher median ages than

males. For the right mandibular third molar, significant differences between males and females were observed in the I₃M groups [0.08, 0.3] ($p =$

0.021), [0.02, 0.04] ($p = 0.006$), and [0.00, 0.02] ($p = 0.012$), with females again showing higher median ages.

Table 2. Summary of descriptive statistics for chronological age (in years) and a comparison between males and females in each of third molar maturity index (I₃M) group for the left and right mandibular third molars (tooth 38 and 48)

I ₃ M	Male				Female				p-value ^b
	N	Mean ± SD (years)	Median (years)	p-value ^a	N	Mean ± SD (years)	Median (years)	p-value ^a	
Tooth 38									
{0.9,1.5}	3	14.64 ± 0.45	14.49	0.451	14	15.79 ± 1.44	15.72	0.066	0.200
{0.5,0.9}	16	14.86 ± 0.68	14.82	0.147	23	15.60 ± 1.28	15.37	0.025 [†]	0.074
{0.3,0.5}	17	15.46 ± 0.87	15.37	0.456	24	16.40 ± 2.13	15.92	<0.001 [†]	0.104
{0.08,0.3}	69	17.58 ± 1.92	17.23	<0.001 [†]	64	18.10 ± 1.83	18.20	<0.001 [†]	0.047*
{0.04,0.08}	39	19.33 ± 2.09	18.65	0.030 [†]	40	20.56 ± 2.19	20.80	0.117	0.011*
{0.02,0.04}	30	20.48 ± 1.60	20.66	0.846	21	21.38 ± 1.56	21.48	0.013 [†]	0.026*
{0.00,0.02}	58	21.44 ± 1.48	21.72	<0.001 [†]	39	21.85 ± 1.32	21.88	0.262	0.190
Tooth 48									
{0.9,1.5}	5	14.88 ± 0.84	14.49	0.143	9	15.46 ± 1.22	15.68	0.295	0.365
{0.5,0.9}	16	14.90 ± 0.46	14.92	0.561	26	15.28 ± 1.05	14.96	0.015 [†]	0.526
{0.3,0.5}	19	15.72 ± 1.12	15.75	0.575	18	16.47 ± 1.46	16.05	0.364	0.083
{0.08,0.3}	60	17.30 ± 1.86	16.99	<0.001 [†]	65	18.18 ± 2.26	17.95	<0.001 [†]	0.021*
{0.04,0.08}	30	19.42 ± 1.76	19.38	0.725	19	19.12 ± 1.57	19.09	0.389	0.546
{0.02,0.04}	25	19.33 ± 1.76	18.99	0.450	29	20.68 ± 2.08	20.88	0.012 [†]	0.006*
{0.00,0.02}	71	21.48 ± 1.63	21.92	<0.001 [†]	48	21.95 ± 1.46	22.18	0.012 [†]	0.120

I₃M third molar maturity index, N number of samples, SD standard deviation

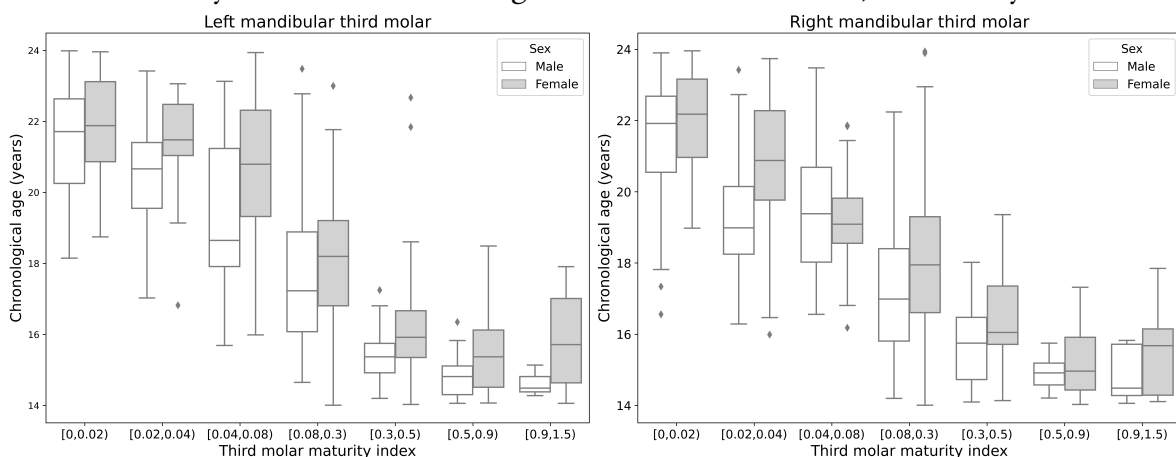
^a p-value for normality test: Shapiro-Wilk test for N < 50; Kolmogorov-Smirnov test for N ≥ 50.

^b p-value for comparing mean age between males and females: use an independent t-test if normality is met; otherwise, use the Mann-Whitney U test.

[†] p-value < 0.05 indicates a significant deviation from normality, suggesting that the data is not normally distributed.

* p-value < 0.05 indicates a statistically significant difference.

Figure 5. Boxplot illustrating the relationship between chronological age and Cameriere’s third molar maturity index of the left and right mandibular third molars, stratified by sex



In comparing the left and right mandibular third molars, Demirjian’s developmental stages were found to be statistically different between the two sides, with the left side developing faster than the right side by 0.06 stage ($p = 0.01$). Additionally, there was a statistically significant difference in I_{3M} between the left and right sides, with I_{3M} of the left side being 0.01 higher than that of the right side ($p < 0.01$). The performance of Demirjian’s stages and I_{3M} for age group classification at the 18-year threshold is shown in Tables 3 and 4. Concerning

accuracy, which reflects the overall correct classification, Demirjian Stage G and I_{3M} less than 0.08 appear to have the highest accuracy. However, in a legal context, a false positive is the worst-case scenario. Therefore, we aim to primarily focus on the specificity score. In all groups, Stage H and I_{3M} less than 0.02 achieve the highest specificity scores. The scores were 1.00, except for the right mandibular third molar of males, which demonstrates specificity scores of 0.97 and 0.96 for the prediction threshold at Stage H and I_{3M} less than 0.02, respectively.

Table 3. Performance of Demirjian’s stage and third molar maturity index (I_{3M}) in age group classification at 18-year threshold using left mandibular third molar

Sex	Thres hold	Accuracy (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)	<i>p</i> (95% CI)
Male	Stage E	0.62 (0.55, 0.68)	1.00 (1.00, 1.00)	0.07 (0.02, 0.12)	0.60 (0.54, 0.67)	1.00 (1.00, 1.00)	1.08 (1.02, 1.14)	0.00 (0.00, 0.82)	0.62 (0.56, 0.68)
	Stage F	0.70 (0.64, 0.76)	1.00 (1.00, 1.00)	0.28 (0.19, 0.37)	0.66 (0.6, 0.73)	1.00 (1.00, 1.00)	1.39 (1.23, 1.58)	0.00 (0.00, 0.21)	0.68 (0.62, 0.74)
	Stage G	0.86 (0.82, 0.91)	0.84 (0.78, 0.9)	0.90 (0.83, 0.96)	0.92 (0.87, 0.97)	0.80 (0.72, 0.87)	8.05 (4.46, 14.54)	0.18 (0.12, 0.27)	0.92 (0.89, 0.96)
	Stage H	0.65 (0.59, 0.71)	0.40 (0.31, 0.48)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	0.54 (0.47, 0.61)	NA	0.6 (0.53, 0.69)	1.00 (1.00, 1.00)
	$I_{3M} < 0.08$	0.83 (0.78, 0.88)	0.82 (0.76, 0.89)	0.84 (0.77, 0.92)	0.88 (0.83, 0.94)	0.77 (0.69, 0.85)	5.27 (3.29, 8.44)	0.21 (0.14, 0.30)	0.89 (0.85, 0.93)
	$I_{3M} < 0.04$	0.77 (0.71, 0.82)	0.62 (0.54, 0.71)	0.97 (0.93, 1.00)	0.97 (0.93, 1.00)	0.65 (0.57, 0.72)	20.00 (6.52, 61.38)	0.39 (0.31, 0.48)	0.97 (0.95, 0.99)
	$I_{3M} < 0.02$	0.66 (0.6, 0.72)	0.43 (0.34, 0.51)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	0.55 (0.48, 0.63)	NA	0.57 (0.50, 0.67)	1.00 (1.00, 1.00)
Female	Stage E	0.65 (0.59, 0.71)	1.00 (1.00, 1.00)	0.17 (0.09, 0.24)	0.62 (0.56, 0.69)	1.00 (1.00, 1.00)	1.20 (1.10, 1.32)	0.00 (0.00, 0.37)	0.65 (0.59, 0.72)
	Stage F	0.78 (0.72, 0.83)	0.97 (0.94, 1.0)	0.52 (0.42, 0.62)	0.73 (0.67, 0.80)	0.92 (0.85, 1.00)	2.00 (1.62, 2.47)	0.06 (0.02, 0.16)	0.76 (0.7, 0.81)
	Stage G	0.76 (0.70, 0.82)	0.66 (0.58, 0.74)	0.89 (0.83, 0.96)	0.90 (0.83, 0.96)	0.66 (0.58, 0.74)	6.28 (3.45, 11.44)	0.38 (0.29, 0.49)	0.91 (0.87, 0.95)
	Stage H	0.58 (0.51, 0.64)	0.27 (0.19, 0.35)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	0.50 (0.43, 0.57)	NA	0.73 (0.66, 0.81)	1.00 (1.00, 1.00)
	$I_{3M} < 0.08$	0.80 (0.75, 0.86)	0.72 (0.64, 0.79)	0.93 (0.87, 0.98)	0.93 (0.88, 0.98)	0.70 (0.62, 0.78)	9.71 (4.72, 19.97)	0.31 (0.23, 0.41)	0.94 (0.91, 0.97)
	$I_{3M} < 0.04$	0.68 (0.62, 0.74)	0.45 (0.37, 0.54)	0.99 (0.97, 1.00)	0.98 (0.95, 1.00)	0.57 (0.49, 0.65)	43.12 (6.08, 305.71)	0.55 (0.47, 0.65)	0.99 (0.97, 1.00)
	$I_{3M} < 0.02$	0.60 (0.53, 0.66)	0.30 (0.22, 0.38)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	0.51 (0.44, 0.58)	NA	0.70 (0.63, 0.78)	1.00 (1.00, 1.00)

PPV positive predictive value, NPV negative predictive value, LR+ positive likelihood ratio, LR- negative likelihood ratio, *p* Bayes post-test probability, CI confidence interval
 NA not applicable (LR+ was not applicable due to the calculation resulting in an infinite value)

Table 4. Performance of Demirjian’s stage and third molar maturity index (I_{3M}) in age group classification at 18-year threshold using right mandibular third molar

Sex	Thresh old	Accuracy (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	LR- (95% CI)	<i>p</i> (95% CI)
Male	Stage E	0.61 (0.55, 0.67)	1.00 (1.00, 1.00)	0.10 (0.04, 0.16)	0.59 (0.53, 0.66)	1.00 (1.00, 1.00)	1.11 (1.04, 1.19)	0.00 (0.00, 0.62)	0.63 (0.56, 0.69)
	Stage F	0.70 (0.64, 0.76)	1.00 (1.00, 1.00)	0.32 (0.22, 0.41)	0.66 (0.59, 0.72)	1.00 (1.00, 1.00)	1.46 (1.28, 1.67)	0.00 (0.00, 0.20)	0.69 (0.63, 0.75)
	Stage G	0.85 (0.81, 0.90)	0.88 (0.82, 0.93)	0.83 (0.75, 0.90)	0.87 (0.81, 0.93)	0.84 (0.76, 0.91)	5.04 (3.26, 7.81)	0.15 (0.09, 0.24)	0.88 (0.84, 0.92)
	Stage H	0.68 (0.62, 0.74)	0.46 (0.38, 0.55)	0.97 (0.94, 0.99)	0.95 (0.9, 1.00)	0.58 (0.50, 0.66)	15.00 (4.87, 47.00)	0.56 (0.47, 0.66)	0.96 (0.93, 0.98)
	I _{3M} < 0.08	0.83 (0.78, 0.88)	0.84 (0.78, 0.91)	0.82 (0.74, 0.89)	0.86 (0.80, 0.92)	0.80 (0.72, 0.88)	4.59 (3.01, 7.02)	0.19 (0.13, 0.29)	0.87 (0.83, 0.92)
	I _{3M} < 0.04	0.77 (0.72, 0.82)	0.67 (0.59, 0.75)	0.90 (0.84, 0.96)	0.90 (0.84, 0.96)	0.68 (0.60, 0.76)	6.58 (3.61, 12.00)	0.37 (0.28, 0.47)	0.91 (0.87, 0.95)
	I _{3M} < 0.02	0.71 (0.65, 0.77)	0.52 (0.44, 0.61)	0.96 (0.92, 1.00)	0.94 (0.89, 1.00)	0.61 (0.53, 0.68)	13.00 (4.84, 34.00)	0.50 (0.41, 0.60)	0.95 (0.92, 0.98)
Female	Stage E	0.63 (0.56, 0.69)	0.99 (0.98, 1.00)	0.13 (0.06, 0.20)	0.61 (0.54, 0.67)	0.92 (0.78, 1.00)	1.14 (1.05, 1.24)	0.06 (0.01, 0.47)	0.64 (0.58, 0.7)
	Stage F	0.78 (0.72, 0.83)	0.98 (0.96, 1.00)	0.50 (0.39, 0.60)	0.72 (0.66, 0.79)	0.96 (0.90, 0.99)	1.95 (1.59, 2.39)	0.03 (0.01, 0.13)	0.75 (0.70, 0.81)
	Stage G	0.82 (0.77, 0.87)	0.76 (0.68, 0.83)	0.90 (0.84, 0.96)	0.91 (0.86, 0.97)	0.73 (0.65, 0.81)	7.64 (4.08, 14.00)	0.27 (0.20, 0.37)	0.92 (0.89, 0.96)
	Stage H	0.61 (0.54, 0.67)	0.32 (0.24, 0.4)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	0.52 (0.45, 0.59)	NA	0.68 (0.61, 0.77)	1.00 (1.00, 1.00)
	I _{3M} < 0.08	0.80 (0.74, 0.85)	0.72 (0.64, 0.8)	0.91 (0.85, 0.97)	0.92 (0.86, 0.97)	0.70 (0.62, 0.79)	8.14 (4.16, 16.00)	0.31 (0.23, 0.42)	0.93 (0.89, 0.96)
	I _{3M} < 0.04	0.75 (0.69, 0.81)	0.59 (0.51, 0.68)	0.96 (0.91, 1.00)	0.95 (0.90, 0.99)	0.64 (0.55, 0.72)	14.00 (5.12, 36.00)	0.43 (0.34, 0.53)	0.96 (0.93, 0.98)
	I _{3M} < 0.02	0.65 (0.59, 0.71)	0.39 (0.30, 0.48)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	0.55 (0.47, 0.62)	NA	0.61 (0.53, 0.71)	1.00 (1.00, 1.00)

PPV positive predictive value, NPV negative predictive value, LR+ positive likelihood ratio, LR- negative likelihood ratio, *p* Bayes post-test probability, CI confidence interval

NA not applicable (LR+ was not applicable due to the calculation resulting in an infinite value)

DISCUSSION

This study employed both the Demirjian’s¹⁹ and Cameriere’s¹¹ methods for dental age estimation. The Demirjian method, commonly used among the Thai population,^{7, 10} assesses age by grading tooth development stages. However, its subjective interpretation can lead to errors, particularly with minor developmental variations. Alternatively, the Cameriere method¹¹ offers greater objectivity by quantifying the distance of open apices in radiographs but requiring meticulous measurements and specialised expertise.

Minimising false identification of minors as adults is critical in age estimation.²⁰ Therefore, the specificity score should be regarded as the most crucial factor. Previous studies conducted in the Thai population indicated that individuals reaching Stage H of mandibular third molar development were undoubtedly over 18 years old, with a specificity of 1.00, although a sensitivity is reduced.⁷ In our study, which is the first to use I_{3M} for adult age assessment among Thais, we found that an I_{3M} cutoff of less than 0.08 yielded lower specificity scores than Demirjian’s Stage H.

We then proposed a cutoff at I_{3M} less than 0.02, which achieved specificity scores comparable to Demirjian's Stage H but with higher sensitivity. These results align with previous comparative study, which revealed that I_{3M} criteria achieve comparable specificity scores to Demirjian's developmental stage while providing higher sensitivity scores.¹¹ In addition, comparing age estimation methods across different populations, this study noted that the development of third molars in the Thai population differed from that of other ethnic groups, highlighting the influence of ethnicity on tooth development. Therefore, employing population-specific reference data is essential for accurate age estimation in various population groups.

Sex dimorphism in the timing of mandibular third molar development was observed in our study. Using Demirjian's method, we found earlier dental development in males at specific stages. These results partially align with previous studies in Brazilians,²¹ Turks,²² and Thais,⁷ which found statistically significant differences in some developmental stages between sexes. However, they contradict studies in Southern Italian²³ and Peruvian populations,²⁴ which revealed no difference in developmental stages between the sexes. Notably, the latter two studies had smaller sample sizes (460 and 208 individuals, respectively) compared to the other studies, which employed samples ranging from 500 to 1,867 individuals. Considering investigation of I_{3M} , unlike studies on Croatian,²⁵ Serbian,²⁶ Turkish,²² and other multi-ethnic populations (Europe, Africa, Asia, and America)²⁷ that reported earlier development in males, we found no significant sex differences in mandibular third molar development using the I_{3M} method. This aligns with research from Southern Italy,²⁸ Botswana,²⁹ Peru,²⁴ and Northern China.³⁰ Ethnicity might explain this inconsistency. Additionally, variations in how studies group I_{3M} values could be a factor. Our study, specifically focused on evaluating cut-off points below 0.08, further divided I_{3M} values into groups like [0,0.02) and [0.02,0.04). These groupings were lower than those used in other studies.

Our study compared the development of the left and right mandibular third molars. Using Demirjian's method, while the right side showed a slightly faster development rate (mean difference of 0.06 stages), this difference was not clinically significant, and both sides can be used

interchangeably for age estimation when necessary. This finding aligns with previous research by Duangto et al.⁷ and Caggiano et al.,²³ which state that there were no differences in developmental stage among the left and right mandibular third molars. However, the maturity index analysis revealed a slight asymmetry, with the left side having a higher mean value (0.01), indicating a wider or slower developing root apex compared to the right. This finding is consistent with the multi-ethnic analysis of Angelaokopoulos et al.,²⁷ who reported similar asymmetry in Asian populations but not in Europe, Africa, and America. Regarding the final results of adult age assessment, we suggest that, in females, both left and right mandibular third molars can be used, as both of them achieve a high Bayes' post-test probability value. However, in males, the left side is preferred as it achieves a higher post-test probability value.

While valuable for age estimation of adolescents and young adults using third molars, Demirjian's and Cameriere's methods have limitations. These limitations include the presence of teeth that are absent (agenesis), misaligned, or obscured by pathology. For instance, studies have reported that bilateral mandibular third molar agenesis (missing wisdom teeth) occurs in 4.1% of females and 5.4% of males, highlighting a significant portion of the population where these methods would not be applicable.³¹ This highlights the need for alternative age estimation approaches in these cases. Other methods, such as assessing the visibility of root pulp in mandibular molars³² or secondary dentine formation in mandibular premolars,³³ can be helpful, as these teeth can serve as substitutes for age estimation when mandibular third molars are absent. Besides, estimating age from clavicle bone development using computed tomography could be one alternative,³⁴ but it is important to note that this technique carries a much higher radiation dose compared to the panoramic radiograph typically used for dental age estimation.

This study has limitations inherent to its cross-sectional design. While it offers valuable findings in dental development at a single point in time, it cannot establish causal relationships between development stages and age. Ideally, a prospective observational study following individuals over time would provide a more accurate understanding of third molar development and allow for refinement of the I_{3M} cutoff point.

However, ethically obtaining repeated dental radiographs in healthy individuals is not feasible. Additionally, the sample representativeness is limited. Although the study aims to propose a population-specific reference for Thais, data collection occurred at a single centre in the southern region. Future studies should incorporate a more diverse sample population to strengthen the generality of the findings. Furthermore, artificial intelligence (AI) for age estimation from maxillofacial radiographs shows promise, offering performance comparable to human examiners. AI could be especially useful in cases like mass victim identification, decomposed bodies, and criminal investigations.³⁵ Further research applying AI to this method could provide significant benefits.

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CONCLUSION

In conclusion, this study found that an I3M cutoff of <0.02 achieved high specificity and better sensitivity than Demirjian's Stage H method for adult age estimation in Thais. Slight asymmetries in third molar development were observed, with the left side developing faster and having higher I3M values. Both mandibular third molars performed similarly for females, while the left was preferred for males due to its higher post-test probability.

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The feasibility of the adult age estimation 3D-CBCT method on ancient human remains

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ABSTRACT

The age estimation of skeletal remains still represents a central issue for the reconstruction of the so-called "biological profile," but mostly for reconstructing investigation. This research aims to verify the feasibility of the adult age estimation method developed on living people by Pinchi et al. (2015 and 2018), for estimating the age at the death of 37 subjects from ancient populations found in two different Italian necropoleis of archaeological interest (Mont'e Prama and Florence, X-IX century B.C and V-VI century A.D respectively). The method is conservative and based on a geometrical approximation of dental volumes of the upper central left incisors on CBCT scans. The statistical distribution of the age and errors followed the Bayesian approach proposed by Sironi et al. (2018) applying the "a priori" values according to the estimates/classification obtained with anthropological methods (morphological). Results show higher accuracy for Mont'e Prama remains than for the Florentine sample due to the different characteristics of the two ancient populations (estimates varying from 18.4 up to 28.7 years with a maximum error of 6,14 years for Mont'e Prama, and from 15.88 up to 43.37 years with a minimum error of 1 year up to a maximum error of 7,85 years for Florence). The method proposed and validated on modern living people can represent a reliable tool for estimating the age of ancient human remains with a significant palaeodemographic value for archaeologists/anthropologists. Mont'e Prama sample could be defined as a homogenous group of males aged around 20-30 years, probably warriors, soldiers, or athletes; the Florentine sample as an inhomogeneous group of males and females from different families buried all together in a small area out of the city due to the probable occurrence of a special healthy emergency in the city.

INTRODUCTION

Age estimation is one of the main goals of the personal identification investigation, relevant for living subjects, single and multiple deaths,¹⁻¹⁴ but also for human remains of anthropological/archaeological interest.¹⁵⁻¹⁶

In the archaeological field, the age estimation of skeletal remains still represents a central issue not only for the reconstruction of the so-called 'biological profile,' but also for bioarchaeological investigations into the living conditions of ancient populations, the pathologies that may have led to

death, and any other useful data regarding the composition and characteristics of the studied population (palaeodemography).¹⁷⁻¹⁸

Furthermore, the age estimation procedure is complicated by adulthood due to the completion of skeletal and dental maturation and the need to apply the regressive and pathological skeletal/dental features affected by high variability. Teeth reveal a wide inter-individual variability due to different living conditions, the incidence of pathologies or para-functions, and personal habits which may have caused significant and specific changes in tooth morphology and anatomy over the years.¹⁹

On the other hand, ancient skeletal remains often offer limited available evidence, but represent archival interest for paleoanthropological nature museums. The museum value of ancient remains pursues the development and the application of conservative methods based on radiographic examination of teeth for estimating individual age at death.²⁰

The most applied dental techniques for age estimation are based on the direct evaluation of the macroscopic morphological changes in adult dental elements such as occlusal wear,²¹⁻²² usually highly variable since influenced by both exogenous and endogenous factors, or dentin translucency, root resorption, and periodontal attachment loss,²³⁻²⁷ physiological variations that occur in healthy teeth. These latter techniques are not suitable for archaeological finds due to their invasiveness, as they require the extraction or sectioning of dental elements. Other common methods rely on bi-dimensional radiographic measurements of dental pulp cavity reduction.²⁸⁻³⁰

However, when applied individually to estimate adulthood, none of these methods provide an accuracy better than 10-15 years. Therefore, it is often advisable to use a combination of different estimation methods to improve the confidence interval and increase the precision of adult age determination.^{18, 31-34}

Since the most reliable dental parameter for estimating adulthood appears to be the deposition of secondary dentin,³⁵ the introduction of the CBCT (Cone Beam Computed Tomography) in the common clinical practice has created new valid opportunities to obtain three-dimensional tooth radiographs to measure this phenomenon with absolutely non-invasive techniques.³⁶⁻³⁷

Some methods addressed to estimate the adult dental age are based on the ratio between 3D dental volumes using computed tomography: dental pulp chamber and the entire tooth volume (mineralized tissues) of various types of single-rooted teeth. Someda et al.³⁸ applied micro-CT scans to reconstruct three-dimensional structures of enamel, dentin, and pulp cavity of mandibular central incisors from subjects aged 12-79 years. They calculated the ratio between the pulp cavity and the whole tooth volumes, obtaining a high correlation between the pulp chamber narrowing and the increasing age in both sexes, but better accuracy in females. Similarly, Agematsu et al.³⁹ applied the micro-CT methodology and found a significant degree of pulp volume decrease between age-cohorts of the forties, fifties, and sixties years. Star et al.⁴⁰ developed a method based on CBCT scans of mono-rooted teeth (incisors, canines, and premolars) to calculate the ratio between the tooth volumes, requiring an automatic process of separation and segmentation of the tooth scans (applying a grey scale) to reconstruct and measure the entire 3D image. They found a difference between the real volume of the pulp and the volume measured with the software on the 3D structure of 21%, and 16% between the real tooth volume and the measured one. The entire procedure took on an average of 3 hours per tooth. Boedi et al.⁴¹ improved the segmentation method by focusing on the crown part of mono-rooted teeth measuring three parameters (enamel volume, dentine volume, and pulp chamber volume) and obtaining a pulp to dentine volume ratio and an enamel to dentine volume ratio. They concluded that the highest correlation can be found in the maxillary canines, but the 3D segmentation process, even if applied only for the crown scans, entails a prolonged time consumption.

Since most authors confirmed that central incisors show a stronger correlation between aging and pulp chamber volume decrease than premolars and canines,^{38-40,42} Pinchi et al. developed and validated a novel method for adult age estimation based on a geometric approximation system of dental volumes using CBCT scans.⁴³ Measurements were conducted on CBCT (Scanora 3D – Soredex) sagittal and axial scans of upper left central incisors. For the correlation between the volumes ratio and end age, a linear regression formula and a Bayesian statistical distribution method have been

developed.⁴³⁻⁴⁴ They concluded that the proposed approach remarkably reduced the operating time in comparison to the other more complex techniques based on the segmentation model.

The easy approach, the accuracy of the age predictions, and the evidence of no statistically significant differences between sexes in agreement with previous research,^{22, 39-40} led choosing for this study the method of adult dental age estimation developed by Pinchi et al.⁴³⁻⁴⁴ This method was applied to assess the age at death of subjects found in two different Italian necropolis (in Florence under the Uffizi Museum and in Sardinia, in Mont'e Prama) to investigate the reliability of the age estimates in ancient population of archeological interest.

In 2012, the forensic odontology group of the University of Florence was requested for a joint-

field research with the Authority for Archaeological Heritage of the Tuscany Region (Italy) to assess the age at death of human remains found in two different necropoleis, in Mont'e Prama (Sardinia) and then in Florence (Tuscany).

The two archeological areas of interest differ in several aspects: geographical site, historical era, graves setting, bones preservation. The necropolis of Mont'e Prama is located near Cabras (Sardinia) and the burial site consists of a total of 33 tombs cylindrical shaped dug in the ground and covered with chalky sandstone slabs. The skeletons of young adult males were buried in sitting or kneeling positions and the probable long time of exposure to the outside led to an extreme fragmentation of skeletal and skull bones (Fig. 1).

Figure 1. Human remains found inside a tomb of Mont'e Prama: Official images from "Mont'e Prama Foundation" website.



Fragments of stone statues, named "The Giants of Mont'e Prama", covered the graves and have been dated to the X-IX century B.C according to the radiocarbon analysis. Genetic analyses were conducted especially on available teeth. The historical period and the results obtained from the genetic and morphological analyses on remains suggest that the necropolis of Monte Prama might be the burial space reserved for a family group of young adults dominant in the Nuraghic society of the First Iron Age or a group of male warriors or athletes. Conversely, the necropolis of Florence is located near the Arno River. According to the radiocarbon dating of some groups of coins found alongside some skeletons, it probably dates from the V-VI century A.D. Seventy-five skeletons of adults and children (both males and females) were found concentrated in a small area, buried aligned in groups of three to eleven individuals in an

alternate head-to-toe manner with children's skeletons deposited in the free spaces between the adults' bones (Fig. 2). The small area of the necropolis settled along the riverside, the disorderly position of the skulls and upper limbs, and the absence of signs of fatal wounds or starvation, have been interpreted by the archeologists as the result of hasty burial due to a special emergency condition that led to the death of many people in a short time-lapse and in a critical condition of lack of space outside the town: a deadly epidemic plague in the town (Black Death) or an epidemic of salmonella during a very hot summer.

Fragments of stone statues representing 25 young male warriors and 13 models of "nuraghes" covered the graves. The statues were named "The Giants of Mont'e Prama" and have been dated to the X-IX century B.C according to the radiocarbon

analysis. Genetics analyses were conducted especially on available teeth.

The historical period and the results obtained from the genetic and morphological analyses on remains suggest that the necropolis of

Monte Prama might be the burial space reserved for a family group of young adults dominant in the Nuraghic society of the First Iron Age or a group of male warriors or athletes.

Figure 2. Human remains found inside a tomb of Florence lying in a disorderly position of the skulls and upper limbs head-to-toe: image from “Toscanaoggi” website.



MATERIALS AND METHODS

The about 108 skeletal remains recovered from Mont'e Prama and Florence underwent anthropological and dental age estimation analyses.

No inclusion criteria were applied for the anthropological age estimation sample collection. The age estimation methods were selected according to the available remains.

Inclusion criteria for the dental age estimation sample:

- The availability of intact and sound left central incisors;

Exclusion criteria for the dental age estimation sample:

- All the skeletal remains missing left central incisors were excluded from the analyses.

For the anthropological age estimation, morphological methods based on the assessment of the ecto/endo cranial suture closure, parietal thinning, pubic symphysis face and auricular surface of the ilium metamorphosis, development of the sternal rib ends, osteoarthritis, radiographic evidence of the

bone loss of clavicle and proximal femur, tooth wear, and bone histology features,^{22,45-51} were applied according to the available remains obtaining wide classification ranges from *sub-adults* (younger than 18 year old), *young adults* (from 18 to 20-25 years old), and *adults* (older than 30 years). In particular, the Lovejoy et al. method based on tooth wear²² has been applied when jaws were available. Tooth wear is classified into 9 stages for all maxillary dental elements and 10 for mandibular teeth (including third molars). Estimate inaccuracies result in line with those from other anthropological methods based on pubis, ilium, femur, and sutures (varying from 7,4 up to 12,2 years), thus suggesting the applicability in case of teeth availability but providing small ranges of age estimate (4-10 years) never overlapped (i.e., 25-29, 30-34, 30-40 etc.).²²

For the dental age estimation, the Pinchi et al. method of geometrical approximation of dental volumes⁴³ and the Bayesian statistical distribution⁴⁴ were applied to the available left upper central incisors.

A CBCT of the entire skulls, jaws or single teeth (Fig. 3) was taken and two experienced forensic odontologists performed the high and area measurements on sagittal and axial CBCT scans to calculate dental volumes according to Pinchi et al. methods ⁴³⁻⁴⁴.

This method calculates the different dental volumes (dental hard tissues, total volume of the tooth, and pulp volume) throughout a geometrical approximation of the different parts of the tooth (crown, roots, and pulp chamber).

The root and the pulp chamber are assimilated into an elliptical based cone; whilst the crown to an elliptical based truncated cone (Fig. 4).

The ratio between the pulp volume and the hard tissues (dental) volume is then measured according to the following equation: $\frac{V_{pulp}}{V_{ht}}$ [ratio between the pulp volume and the hard tissues (dental) volume] = $\frac{V_{pulp}}{V_{tot} - V_{pulp}}$ [volume of dental hard tissues] = $\frac{V_{pulp}}{V_{tot}}$ [total volume of the tooth] - V_{pulp} [volume of the pulp].

Figure 3. CBCT sagittal and axial scans and 3D reconstructions of skulls, jaws, and teeth from CBCT acquisitions.

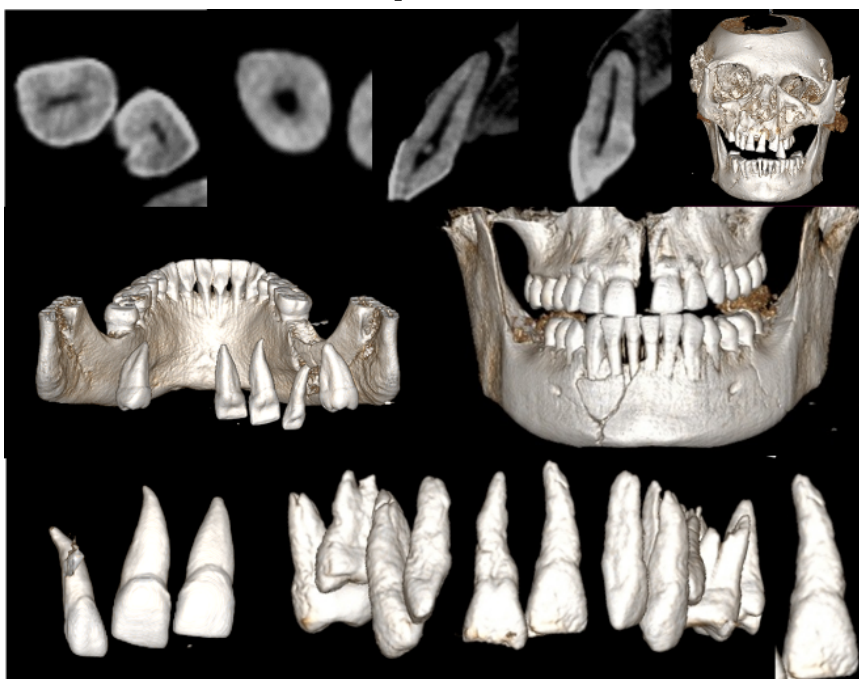
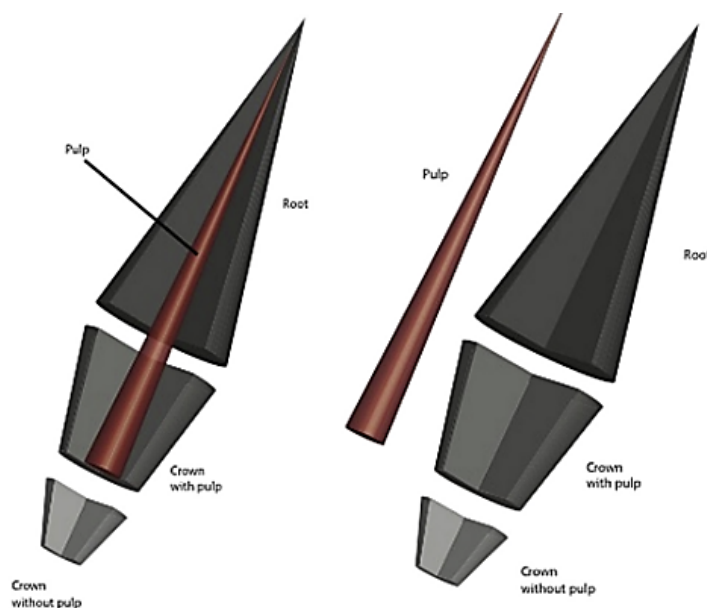


Figure 4. Geometrical approximation of the tooth. The root and the pulp have been assimilated into elliptical-based cones and the crown into an elliptical-based truncated cone ⁴³



Statistical analysis

The statistical distribution of age and errors followed the Bayesian approach proposed by Sironi et al.⁴⁴ The estimate of the age of each subject was obtained through the application of the following Bayes' theorem:

$$p(a|e, \theta) = \frac{p(e|a, \theta) \times p(a)}{\int p(e|a, \theta) \times p(a) da}$$

where e is the natural logarithm of the PHr; $p(a|e, \theta)$ is the linear relationship between the evidence and the age of the subjects with an uncertainty Normally distributed; θ represents the parameters of the Bayesian model estimated on a dataset of contemporary subjects collected from two private radiological studies, in Northern and Central Italy; $p(a)$ is the "a priori" probability of the age of each subject individually attributed in a range of a *maximum* and a *minimum* value on the basis of available information. A Standard Deviation (SD) for each evaluation was calculated as the Mean Squared Error of the discrepancy between the age estimated with the Pinchi et al. method⁴³ and the age assessment obtained with the anthropological methods, according to the Bayesian model proposed by Sironi et al.⁴⁴

The *maximum* and *minimum* setting of the "a priori" ranges was attributed differently for the two necropoleis groups, depending on the anthropological evaluations and historical information available. For the sample of Mont'e Prama, a *uniform priors* model was assessed considering the group homogeneity, thus the same *prior ranges* were imposed for all the subjects. The minimum age was set according to the previous anthropological estimates and complete dentition where possible; the maximum age was defined according to the average surviving age at that time. Due to the heterogeneity of the Florentine sample, a double validation of the age assessment was conducted, applying *uniform priors* in the first evaluation and *informed priors* for a second evaluation (intended as personalized prior ranges according to the specific morphological or physical characteristics of the remains). The *uniform priors* were

established according to the presence of permanent dentition, since the jaws were complete and available for all samples, and the maximum surviving age at the time. The *informed priors* were obtained for each subject according to personal dental data (tooth maturation and wear assessment, presence and formation of wisdom teeth) and on the lifestyle of the Florentine civilization in the respective historical period defined by the archaeologists.

The results obtained from the two prior models on the Florentine sample were then compared to appreciate if the informed prior could significantly improve the Bayesian evaluation in heterogeneous ancient populations.

RESULTS

Only 15 skeletons from Mont'e Prama Necropolis and 22 from the Uffizi Necropolis met the inclusion criteria for the dental age estimation.

The complete dental procedure, including the CBCT exposure, the image reconstruction, the pre-processing analyses (drawing the boundary of the images manually), and the post-measurements, required less than 15 minutes for each tooth.

For the sample of Mont'e Prama, the "a priori" minimum age was set at 12 years old, according to the previous anthropological estimates which defined all the subjects as *young adults* all in permanent dentition (including second molars). The maximum age was set at 35 and 40 years old, respectively, since the average surviving age at that time [Late Bronze Age to the Early Iron Age] was about 35/40 years old.

Table 1 shows the results of the Mont'e Prama sample obtained with the Pinchi et al. method⁴³ and the estimates obtained with the morphological methods.⁴⁵⁻⁵¹ The SD (standard deviation) was calculated between the Pinchi et al. and anthropological assessments. The age estimation with the "a priori" setting up to a maximum of 35 year varies from 18.4 up to 28.7 years with a minimum error between dental and anthropological analyses of 5,15, and a maximum error of 6,14 years. The age estimation with the "a priori" setting up to a maximum of 40 year varies from 18.71 up to 31,41 years with a maximum error of 7,14 years.

Table 1. Results of the sample from Mont'e Prama Necropolis with the "a priori" settings between the age range 12-35 and 12-40 years.

Sample ID	Dental Volumes Ratio	Prior U (12; 35)		Prior U (12;40)		Anthropological estimates
		Estimated dental age	SD	Estimated dental age	SD	
1	0,056	23,63	6,11	25,03	7,13	Young adult (20 years)
2	0,075	19,57	5,54	19,98	6,09	Young adult (20 years)
3	0,082	18,68	5,24	18,96	5,66	Young adult (20 years)
4	0,069	20,56	5,80	21,15	6,48	Young adult (20 years)
5	0,045	25,57	6,14	27,75	7,12	Young adult (20 years)
6	0,084	18,46	5,15	18,71	5,54	Young adult (20 years)
7	0,053	24,69	6,06	26,47	7,14	Young adult (20 years)
8	0,041	28,07	5,32	31,41	6,29	Young adult (20 years)
9	0,061	22,21	6,05	23,19	6,93	Young adult (20 years)
10	0,062	22,02	6,03	22,95	6,89	Young adult (20 years)
11	0,077	19,23	5,43	19,60	5,94	Young adult (20 years)
12	0,057	23,49	6,11	24,84	7,11	Young adult (20 years)
13	0,048	26,02	5,87	28,34	6,99	Young adult (20 years)
14	0,056	23,56	6,11	24,95	7,13	Young adult (20 years)
15	0,082	18,71	5,25	18,99	5,67	Young adult (20 years)

According to the information from the life conditions in the town of Florence in the Roman Era [V-VI century A.D.], for the "a priori" settings a minimum age of 12 years was established since all the subjects were in permanent dentition, and a maximum age of 70 years, since the unknown variability of the population examined (*uniform priors*). The anthropological estimates varied from sub-adults up to adults, and for all the sample the Lovejoy et al. method based on tooth wear²² could be applied, due to the presence of complete jaws. The SD (standard deviation) was calculated between the Pinchi et al. and the tooth wear Lovejoy et al. assessments.

Table 2 shows the results of the Florentine sample. The dental age estimation varies from 12.6 up to 49.31 years with a minimum error of 0.99 and a maximum of 8,8 years characterizing a heterogeneous sample.

A second phase of analysis was conducted in accordance with further information acquired from the available dental data, tooth wear assessment,²² and on the lifestyle of the

Florentine civilization in that specific historical period defined by the archaeologists. It was possible to reduce the range of "a priori" variability of the age of the sample, setting the minimum limit at 15, 18, and 23 years respectively depending on the characteristics of the individual subject (complete permanent dentition, complete second molars, or advanced formation of the root of wisdom teeth), and the maximum limit at 50 years (*informed priors*). All subjects with wisdom teeth with complete apex were evaluated with a *minimum prior* greater than 23 years, while those with wisdom teeth still in formation were considered a minimum of 15 or 18 years. The maximum age was placed at 50 years considering the more specific average survival information.

In Table 3, the results obtained in the Florentine sample with the *informed priors* set according to the individual characteristics at 15, 18, and 23 years up to 50 years. The age estimation varies from 15.88 up to 43.37 years with a minimum error of 1 year up to a maximum error of 7,85 years confirming the heterogeneity of the sample of Florence.

Table 2. Results of the sample from the Uffizi Necropolis with the “*a priori*” settings between the age range 12-70 years.

Sample ID	Dental Volumes Ratio	Estimated dental age	SD	Lovejoy estimates (dental wear)	Anthropological estimates
1	0,037	44,48	7,62	40-50	Adult
2	0,052	27,71	8,8	24-30	Young adult
3	0,094	12,60	5,41	18-24	Young adult
4	0,069	14,89	7,29	16-20	Young adult
5	0,078	12,89	6,45	16-20	NA*
6	0,088	12,62	5,74	16-20	Young adult
7	0,087	12,61	5,81	16-20	Sub-adult
8	0,028	49,31	5,78	20-24	Young adult
9	0,099	24,89	4,52	18-22	Young adult
10	0,103	22,94	4,48	20-24	Adult
11	0,060	21,72	8,27	16-20	Young adult
12	0,053	27,79	8,79	24-30	NA*
13	0,068	15,50	7,4	24-30	Adult
14	0,063	18,20	7,91	16-20	Young adult
15	0,046	34,45	8,76	20-24	Young adult
16	0,087	23,75	5,11	20-24	Young adult
17	0,043	37,21	8,55	24-30	Young adult
18	0,094	15,87	0,99	12-18	Sub-adult
19	0,057	23,99	8,55	24-30	Adult
20	0,074	13,06	6,82	NA*	Adult
21	0,076	12,94	6,59	12-18	Sub-adult
22	0,056	24,31	8,56	24-30	Adult

*NA: not available

Table 3. Results of the sample from the Uffizi Necropolis with the “*informed priors*” settings between the age range 15-50 years.

Sample ID	Dental Volumes Ratio	Prior I minimum	Prior I maximum	Estimated dental age (SD)	CI 95%	Lovejoy estimates (dental wear)	Anthropological estimates
1	0.037	23	50	39.74 (6.65)	25.51 - 49.48	40-50	Adult
2	0.052	23	50	33.22 (6.66)	23.51 - 47.5	24-30	Young adult
3	0.094	18	50	23.32 (4.85)	18.15 - 36.11	18-24	Young adult
4	0.069	18	40	25.22 (5.33)	18.27 - 37.51	16-20	Young adult
5	0.078	18	40	24.20 (4.96)	18.2 - 36.46	16-20	NA*

6	0.088	18	40	23.46 (4.63)	18.16 - 35.37	16-20	Young adult
7	0.087	15	30	20.10 (3.83)	15.17 - 28.71	16-20	Sub-adult
8	0.028	23	50	43.37 (5.45)	29.54 - 49.78	20-24	Young adult
9	0.099	20	50	24.90 (4.53)	20.14 - 36.93	18-22	Young adult
10	0.103	18	50	22.94 (4.58)	18.14 - 35.07	20-24	Adult
11	0.060	18	40	26.89 (5.73)	18.42 - 38.56	16-20	Young adult
12	0.053	23	50	33.04 (6.62)	23.49 - 47.28	24-30	NA*
13	0.068	23	50	29.74 (5.52)	23.22 - 43.71	24-30	Adult
14	0.063	15	30	21.99 (4.17)	15.36 - 29.46	16-20	Young adult
15	0.046	18	40	30.61 (5.79)	19.26 - 39.51	20-24	Young adult
16	0.087	18	50	23.76 (5.13)	18.17 - 37.2	20-24	Young adult
17	0.043	18	50	35.66 (7.85)	20.26 - 48.88	24-30	Young adult
18	0.094	15	50	15.88 (1.00)	13.92 - 17.83	12-18	Sub-adult
19	0.057	23	50	31.88 (6.33)	23.37 - 46.45	24-30	Adult
20	0.074	23	50	29.08 (5.19)	23.19 - 42.54	24-30	Adult
21	0.076	15	30	20.74 (4.00)	15.22 - 29.06	12-18	Sub-adult
22	0.056	23	50	31.96 (6.36)	23.38 - 46.56	24-30	Adult

*NA: not available

DISCUSSION

Age estimation is a critical aspect of analyzing human skeletal remains, both in the context of forensic identification (whether involving individual or mass deaths) and in anthropological and archaeological research, particularly for paleodemographic studies.¹⁻¹⁸ Given that ancient skeletal remains often provide limited evidence and must be preserved for archival purposes, it is essential to develop and apply conservative, reliable methods for age estimation, particularly those focusing on teeth.²⁰

This research aimed at verifying the feasibility and repeatability of the adult age estimation method developed on living people by Pinchi et al.⁴³⁻⁴⁴ for estimating the age at the death of subjects from ancient populations found in two different Italian necropolis of archeological interest. The method is extremely conservative since it is based on CBCT scans obtainable from Post-mortem entire skulls, jaws, or teeth. Furthermore, the entire procedure requires about 15 minutes for each tooth analysed.

The study confirms the significant correlation between the reduction of the pulp chamber volume due to the progressive deposition of

secondary dentin during the life of an adult subject and the adult age even in ancient populations. The geometrical approximation of dental volumes measured on CBCT can be suggested as a reliable tool for estimating age in both modern and ancient populations, since it consists of a non-invasive/destructive three-dimensional radiographic technique and the correlation with the adult age is independent of sex.^{22, 39-40, 43-44} These features represent clear advantages in estimating the age of ancient populations or archaeological/anthropological interest since from human/skeletal remains it is often impossible to obtain all the information needed to construct the entire biological profile without using invasive methods (genetics) and therefore without sacrificing tissue.

A graduate and progressive scientific methodology combining the anthropological and dental age estimation was conducted to validate the Pinchi et al. methods⁴³⁻⁴⁴ in the ancient populations. The inaccuracy in the dental age estimation (SD compared to morphological evaluations^{22, 45-51}) varies from 5,15 up to 6,14 years in the sample of Mont'e Prama (Table 1), and from 1 up to 7,85 years in the sample of the

Necropolis of Florence (Table 3) with a mode of error of 4-5,5 in almost all age cohorts (from sub-adults 16-18 years old, up to adults older than 40 years). Some authors⁵² underlined that the Lovejoy methods^{22, 45-50} for age estimation, such as the other morphological assessments usually practiced by anthropologists⁵¹, consistently perform low accuracy for all age cohorts (from 21 up to 79 years old) providing small ranges of age estimate (5-10 years) never overlapped (i.e., 25-29, 30-34, etc.). This limit could lead to a higher risk of a systematic bias excluding the real age of the human remains. Otherwise, the prior selection of possible age ranges could strongly improve Bayesian methods to provide more accurate age estimates within the established age cohorts. The Pinchi et al. methods⁴³⁻⁴⁴ demonstrated to perform with high accuracy for all the age cohorts of ancient sub-adults and adults confirming the skeletal age estimation with a narrower inaccuracy range, without a systematic under or overestimation, and with higher reliability offering age estimates ranging between 10-12 years and CI 95% of error between 10 and 20 years (Table 3).

Furthermore, the analyses conducted with the Bayesian approach⁴⁴ indicate that outcomes in terms of accuracy might be considerably improved by integrating *prior* knowledge in the estimation procedure. For example, case 3 out of the Florentine sample gained an inaccurate estimate in Tab. 2 according to the tooth volume ratio evaluated within wider *prior* ranges, and an age younger than 18 years old was attributed (12,60 years old). After the application of informed priors, obtained from the estimates based on tooth wear and anthropological methods (Tab. 3, 18-24 years old), a minimum age of 18 years was set and the Pinchi et al. method evaluated 23.32 years old, in line with anthropological results. Comparing the results obtained on the Florentine sample in Tab. 2 (using a *uniform priors* with a wider range of variability) with the results obtained in the second analysis in Tab. 3 (using *informed priors* with narrower and personalized range obtained with the further archaeological/anthropological and dental knowledge) there is a significative reduction in the maximum average error (from 8.8 years in Tab.2 to 7.85 years in Tab. 3) which however is concentrated for the age cohorts between 30 and 50 years (from 5.79 up to 7.85 years). These findings are in line with Yang et al.⁵³

which obtained a square root of mean square error of 8.3 years applying CBCT measurements on the pulp chamber/tooth volume ratio on incisors and canines of a modern population mostly aged from 20 to 50 years. Similar results (mean absolute errors of 8.54 years) were gained by Jagannathan et al.⁵⁴ on a CBCT canines sample and by Ge et al.⁵⁵ demonstrated the highest variability of pulp chamber/tooth volume ratio between the age span from 25 to 40 years.

Whilst, the results of this study highlight an opposite trend compared to the Pinchi et al.⁴³ applied for the same age cohorts in living people, in which the highest accuracy in age prediction was obtained with residual errors of 0.71, 2.88, and 5.86 years. Similarly to Aboshi et al.,⁵⁶ their results are in line with what is known regarding the physiology of secondary dentin deposition, which presents a reduced speed in young adults (< 30 years) who have just completed the dental maturation, then increases considerably in the average adult up to a severe slowing down in adults >60 years.

These significant dissimilarities could be attributed to the different population analysed (modern and ancient) and the variability in the rate of tooth wear of the upper central incisors to which archaeologists report to be more exposed in correlation with lifestyles, habits, and with the physical properties of the consumed food and their preparation processes in ancient eras.⁵⁷ Therefore, the presence of major tooth wear could influence the dental age estimation based on the geometrical approximation of central incisor volumes resulting in an over-estimation in the adult age cohorts that has to be considered in studying archaeological populations, e.g. especially farmers. On the other hand, the estimates obtained using the *informed priors* of the Pinchi et al. method⁴³⁻⁴⁴ led to a reduction of the effects of dental wear on age assessment accuracy. Case 8 out of the Florentine sample, reveals a decrease in age evaluation in Tab. 3 which considers the results by Lovejoy et al.²² suggesting a minor tooth wear of the whole jaws than of the solely upper lateral incisor.

Since the high heterogeneity of the Florentine sample and the limited information available, our results can be considered in line with those obtained in previous studies, and even more accurate than those obtained by De Angelis et al.⁵⁸ on canines. They calculate a prediction interval

of around ± 30 years with 95 % confidence for both males and females and a prevision interval of ± 12 years in the female group and ± 14 years for males at 60 % confidence interval. Similarly, Marroquin Penaloza et al.⁵⁹ applied the Kvaal method on CBCTs of a modern population with a median age of 31 years (with different errors for diverse combinations of measurements from ± 10.58 years up to ± 12.84 years). According to Sironi et al.,⁴⁴ in the *informed Bayesian* approach the real risk is the expert's initial belief which could sensitively differ from the real age of the individual examined, leading to incorrect estimates. However, in the case of the Florentine population, a multi-trait approach was used in the *a priori* definition of each subject. The different information collected from the other dental elements present in the subjects' oral cavity, considering the complete permanent dentition, the complete formation of second molars, the advanced formation of the wisdom tooth's root until stage D of the Demirjian method,⁶⁰ and the maximum survival age of the historical period. The reduction of the standard error in almost all the estimates from Table 2 (*uniform priors*) to Table 3 (*informed priors*) confirms the better reliability of the results obtainable with narrower *a priori* age ranges. These results demonstrate the variability of the age sample suggesting to the archeologists that they were different family groups buried all together in a small area near to the center of the town.

On the contrary, the Mont'e Prama group demonstrates excellent accuracy of the measurement method applied with uniform Bayesian statistics (Tab. 1). The sample resulted extremely homogeneous so the error distribution was similar for all age cohorts and the accuracy obtained was even higher than Florentine group. According to the results obtained with the *prior* interval of 12-35 years, it seems to better identify the real age of the entire sample with reduced deviation from skeletal age estimation (Table 1). The analysis shows the absence of infants and elderly people, the presence of 5 subjects aged between 18-19 and the prevalence of "young adults" aged between 20-30 years, all of them males. The age and the sex of the buried bodies

led archeologists to define the deceased population of Mont'e Prama as a probable group of warriors or athletes.

The main limitation of the Pinchi et al. methods⁴³⁻⁴⁴ is the availability of the left upper central incisors. Of the entire sample discovered both in Florence and Sardinia, only 37 subjects presented the required characteristics (22 from the Uffizi Necropolis and 15 from the Mont'e Prama Necropolis) and then could be estimated. Despite the limited availability of information provided by the recovered remains, approximately 30% of the total cases (about 108) could be estimated from the only use of dental technique.

CONCLUSION

The pulp chamber narrowing, measured using tooth geometrical approximation on CBCT scans, demonstrates to be a reliable and conservative tool for estimating age in adult ancient populations of archaeological interest, offering an accuracy comparable to other methods that are invasive or destructive.

The *informed priors* model compared to the *uniform* one in a heterogeneous ancient population reveals a better accuracy in age estimation, but the *prior* setting needs reliable personalized information (e.g. dental maturity, etc).

The inaccuracy of dental age estimation, compared with skeletal age estimation, typically ranges from 1 to 8 years, with no systematic overestimation or underestimation, but in some cases could be influenced by significant tooth wear according to specific working or dietary habits of ancient populations.

Given the differing compositions of the two analyzed samples, the resulting age estimates are endowed with palaeodemographic value for archeologists and anthropologists. The Mont'e Prama sample was identified as a homogenous group of males aged around 20-30 years, suggesting they were warriors, soldiers, or athletes. In contrast, the population of Florence was more heterogeneous and probably composed of individuals from different families buried together in a small area due to a special social emergency, likely associated with a plague epidemic in the city.

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Professional liability and litigation in dental medicine: an analysis of the Portuguese context

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ABSTRACT

The activity of a dentist reveals itself in numerous aspects, and its regulation is determined by the Deontological Code of the Dental Association, which contains a set of rules that dentists are obliged to follow in the exercise of their profession. The regulation of this activity goes beyond following these precepts because, in the legal relationship that is established whenever an agreement is made with a patient to carry out the treatment deemed appropriate, a series of duties and obligations begin for each party, translated into a reciprocal contract, in which the non-compliance of one of them may result in a legal claim.

The objective of this study was to research most court decisions delivered in this century, in Portugal, regarding the activity of dentists when faced with patient claims and to outline a framework that better allows us to understand the regulation of this activity within the scope of the contracts established with them. This includes identifying the patients' sex, the judicial instance, the area of dentistry, the location of the injury, the type of liability, the characterization of the obligation, the basis of responsibility, the alteration of biological assets, and the outcome of the action while also not neglecting the identification of trends and jurisprudential evolutions, should they arise.

A survey of judgments from the Portuguese Superior Courts was carried out, which focused on decisions from the Supreme Court of Justice, Court of Appeal of Porto, Court of Appeal of Lisbon, Court of Appeal of Coimbra, Court of Appeal of Guimarães, and Court of Appeal of Évora.

To understand the judgments analyzed in this study, a brief approach to various legal concepts and institutions of interest was conducted. A summary characterization of the factual basis of the injured party's claim and the legal framework applied, as well as subsequent decisions by higher courts regarding the activity of dentists, was also elaborated.

It was impossible to identify any relationship between the field of dentistry and the characterization of the obligation and the basis of responsibility, nor between the outcomes of the actions and these two parameters mentioned. This is likely due to the small sample size, which, although limited, represented all available published superior court decisions in Portugal related to the proposed topic, and because there were different decisions for similar and identical factual situations due to the various scientific and social conceptions adopted by the courts being susceptible to other interpretations.

INTRODUCTION

For centuries, the medical profession was regarded as mythical-magical, making it unthinkable for doctors to be held accountable for their actions. The patient did not actively participate in his treatment; he was utterly dependent on the doctor and his conduct. Professionals made decisions exclusively, removing the patient's autonomy during his medical appointment.¹

If, once, medical activity was seen as superior, resulting in a significant lack of accountability for doctors, nowadays, due to the extraordinary scientific and social advancements, the standard that once existed is severely altered. The evolution of healthcare, which brought about new diagnosis techniques and advanced treatment methods, witnessed the patient's recent and active engagement. Since they were no longer stripped of their autonomy, patients could participate and critique the procedures adopted by their doctors. These different dynamics were translated into an altered relationship between patient and doctor, characterized by a bidirectional nature, allowing both parties to participate in medical consultations actively. As a result, clinical errors emerged, and doctors became susceptible to being held accountable for their acts and mistakes. This trend can be seen worldwide, with several countries' increasing medical liability concerning dentistry.²⁻⁷

Even though failures and errors in activities based on scientific criteria should be acknowledged because they are often based on probabilities rather than certainties, these are becoming increasingly less accepted.⁸

This reality has a strong impact in many healthcare areas, including dental medicine, because every activity performed by a human being, who is not perfect, has an inherent uncertain nature, carrying the potential for mistakes and errors and never being able to guarantee infallible results.⁹

Dental medicine professionals must adopt specific standards, respect various obligations, and fulfill different requirements to effectively practice and protect their profession. Above all else, dentists need to stay informed.

Dental medicine encompasses numerous aspects. Nonetheless, the correct conduct dentists must adopt is not limited to observing such principles. When a contract is established between the patient and the dentist, a legal relationship is also

fixed, and both parties need to respect a series of duties and obligations. Non-compliance or breach of this contract may result in legal claims. Here lies the core of the present article. Considering the factual and unequivocal truth that human activity is susceptible to errors and mistakes, dental medicine, even with the utmost diligence, can result in injuries due to unpredictable factors and unforeseen events. When one of these situations occurs, and the patient believes an error resulted in his harm, he may seek a court intervention^{10,11}. At this point, the courts may be called upon to analyze a specific situation and the conduct and medical activity practiced by a particular doctor to reach a decision ultimately.^{12,13}

MATERIALS AND METHODS

To evaluate how Portuguese courts see the activities performed by dentists, the research aimed to examine the treatment given by the superior courts to legal actions filed by patients against dental medicine professionals. It involved the search and analysis of all the published court decisions from the Portuguese Superior Courts, including the Supreme Court of Justice, Court of Appeal of Porto, Court of Appeal of Lisbon, Court of Appeal of Coimbra, Court of Appeal of Guimarães and Court of Appeal of Évora. The search for these court decisions was conducted using research tools such as the Supreme Court of Justice and the Court of Appeal of Porto databases and the database "jurisprudência.pt".

The inclusion criteria were:

- Court decisions issued after the year 2000 until 2023;
- The plaintiff is the injured patient;
- Indicating the specific area of dentistry that caused the situation;
- Court decisions from the civil or criminal field;
- Court decisions with proven facts.

As for the exclusion criteria, the following were considered:

- Court decisions before the year 2000;
- The plaintiff is a dentist or a clinic;
- Judgments not related to dental practice or where it is mentioned but not the focus;
- Writs of execution;
- Absence of proven facts.

The data were then statistically analyzed using IBM SPSS Statistics 27® (Statistics Package for

Social Science). The categorical variables were described using absolute and relative frequencies.

RESULTS

After the research and data collection, 30 court decisions were obtained, which could be included in the present study according to the inclusion and exclusion criteria (Table 1).

The Court of Appeal of Porto presented the highest number of cases (13; 43%), followed by the Court of Appeal of Lisbon (8; 27%). The

Court of Appeal of Guimarães had the lowest number of court decisions (1; 3%) in the databases used. The Court of Appeal of Coimbra was the only Superior Court with no results.

Female patients complained more frequently (22; 73.3%) than male patients (8; 26.7%). Prosthodontics (18; 60%) was the most frequent area involved, accounting for more than half of all cases, followed by Dental Surgery (9; 30%). Orthodontics also accounted for a tiny portion of the results (2; 6.7%). Only one case did not fit into a specific area (1; 3.3%) (Table 2).

Table 1. Court where the appeal was filed.

	Supreme Court of Justice	Court of Appeal of Porto	Court of Appeal of Lisbon	Court of Appeal of Guimarães	Court of Appeal of Évora	Court of Appeal of Coimbra
Cases (n)	5	13	8	1	3	0
Percentage (%)	17%	43%	27%	3%	10%	-

Table 2. Cases' distribution according to the dental medicine area

	Prosthodontics	Dental Surgery	Orthodontics	Others
Cases (n)	18	9	2	1
Percentage (%)	60%	30%	6.7%	3.3%

Regarding the location where the patient's lesion occurred, the areas were divided into the maxilla and mandible, and both concurrently. In most situations, the injury occurred in both locations (14; 46.7%), accounting for nearly half of all cases. The number of injuries that happened exclusively in the mandible (12; 40%) was significantly larger than those that occurred only in the maxilla (4; 13.3%) (Table 3).

Table 3. Local of the injury suffered by the patient.

	Maxilla	Mandible	Both
Cases (n)	4	12	14
Percentage (%)	13.3%	40%	46.7%

In most cases (26; 86.7%), permanent body injury occurred, while only a few did not show such alteration (4; 13.3%), which allowed the patient to return to their pre-injury state.

The outcome of the court decisions was classified as proven, partially proven, and

dismissed. The first outcome refers to situations where the amount claimed by the patient was equivalent to the amount awarded by the court (2; 6.67%). The second outcome relates to situations where, even though the dentist's responsibility was verified, the damage claimed by the patient was not fully proven, thus reducing the compensation amount (15; 50%). When the court decision was classified as dismissed, it referred to situations where the plaintiff failed in the lawsuit, and consequently, the dentist was acquitted (12; 40%).

Concerning the type of liability, it can be contractual when a contract is established between a dentist, clinic, or institution and a patient for dental services, and there is a breach of the obligations set in the agreement (22; 73.3%). It can also be non-contractual when an absolute right is violated, even though this was verified in one case (1; 3.3%).¹⁴ There were, however, situations where both types of liability were assessed simultaneously (3; 10%).

Regarding the traditional characterization of the type of obligation imposed on the dentist, it can

be an obligation of means or a result. The first one consists of cases where the dentist is not bound to produce any specific effect, only to practice his activities with the utmost care and diligence (9; 30%). In the second type of obligation, the dentist commits to guarantee the production of a particular result for the patient's benefit (9; 30%).¹⁵ Both kinds of obligation showed the same frequency.

From the analyses of the different court decisions, it was possible to observe a wide range of varying bases of liability used to hold the dentist accountable. In most cases, the basis of liability was the violation of *leges artis* (14; 47%). There were also an equal number of cases related to the defective performance of the contract (5; 17%) and violation of the duty to inform the patient (5; 17%) (Table 4).

Table 4. Basis of liability used to hold the dentist accountable (NA non-available data)

	Leges artis' violation	Defective performance of the contract	Defective performance of the contract and leges artis violation	Violation of the duty to inform	Error in consent	Physical harm	NA
Cases (n)	14	5	1	5	1	1	3
Percentage (%)	47%	17%	3.3%	17%	3.3%	3.3%	10%

NA: Non available data

DISCUSSION

The present study aimed to understand how dentists' activities are perceived within Portuguese jurisprudence.

Regarding the different areas of liability, implantology had the most considerable frequency within prosthodontics. Yet, compared to similar studies conducted in other countries, such as Brazil, implantology was only the fourth most frequent, representing 13% of all cases. At the same time, surgery was the most pervasive area, accounting for 33% of all instances.¹⁶ Similar research in Italy revealed that prosthodontics had the highest frequency there, accounting for about 70% of all the cases.¹⁷ Likewise, a jurisprudence analytic study conducted in Peru discovered prosthodontics was the field most closely connected with liability.¹⁸

The most frequent basis of liability in the current analysis was the *leges artis*' violation, which accounted for 47% of all cases. Comparatively, a recent study of the legal systems of another nation discovered a similar pattern in Chile, where the breach of *leges artis* was the most common basis of liability, accounting for 35% of all the cases examined.¹⁹

To determine whether the characterization of the obligation and the basis of liability were related to the varying degree of intrusiveness of different dental specialties, an analysis was conducted to

explore any statistically significant relationship between them. However, no significant relationship was found between these factors. We also attempted to identify any statistically significant relations between the outcome of the court decisions and the characterization of the obligation and basis of liability. However, it could not be done due to the small sample size or because identical situations saw different bases of liability attributed to them, different ways of evaluating the evidence, and above all, how the courts configured the legal issue and principles they relied upon to do so. This is troublesome as the same matters should be assessed similarly. Despite not being able to find statistical correlations, the in-depth analysis of the select court decisions made it possible to verify the existence of some jurisprudential trends:

- The predisposition to characterize actions based on aesthetic dentistry as obligations of result.
- The trend for the outcome of actions characterized as obligations of means to be dismissed since they are inherently more favorable to the doctor.
- The tendency for the outcome of actions characterized as obligations of means to be partially proven or partially proven since they are inherently more favorable to the patient.

- The transition from characterizing obligations as obligations of means or result to describing them as obligations with an indefinite and definite content, respectively.

The first mentioned trend was seen in 2013 when one of the court decisions concluded that “in the specific case of dental prostheses, we believe that not only their fabrication but also their application, representing different moments in dental practice, should be classified as obligations of results. As these procedures are commonplace in dental practice and the technique is highly advanced, the uncertainty regarding the desired outcome is negligible. Moreover, issues related to the acceptance or rejection of the prosthesis in the patient's mouth do not typically arise”. This understanding is then extended to other areas of dentistry when this court decision states that “dental interventions with predominantly aesthetic purposes, such as prosthetic placements, tooth restorations, and even dental implants, also fall under obligations of results”. It concludes that all these areas have negligible uncertainty in achieving the intended outcome. This trend is followed by six (of the nine) court decisions resulting from obligations.

In our modest understanding, we do not fully agree with this thinking. Despite the procedures being executed with highly advanced techniques, in constant evolution, and with great frequency, like many other areas of medicine, they are always dependent on the patient's body and associated variables, their behaviors and habits, and numerous factors that affect all procedures involved in patient treatment. Although highly

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sought-after and popularized nowadays, implants are not immune to failure, as they depend on a series of factors inherent to the dentist, the patient, and the implant itself.²⁰ Prosthodontics is also not exempt from uncertainty in its various forms, as it relies on different technical and biological factors.²¹⁻²³

While the level of uncertainty may be negligible since these procedures do not occur in an isolated system and are always dependent on multiple factors, considering that dentistry, like the human being, is not exempt from errors or mistakes, these procedures cannot be considered “certain”. The transition from characterizing obligations as obligations of means or result to describing them as obligations with an indefinite and definite content, respectively, arose from the fact that some court decisions believed that the distinction between the obligation of means and obligation of result led to inaccuracies. Specifically, the concept of an obligation of means creates an idea of diminished responsibility by the dentist.²⁴

CONCLUSION

While these jurisprudential trends were noticeable, meaning tendencies to decide similar issues in the same manner, this was not always followed, resulting in different rulings and understandings for identical factual situations, which can be related to the various scientific and social conceptions that the courts may adopt.

The unpredictability of court decisions appeared to be even more significant than the risks and uncertainties of a medical intervention.

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Professional liability in dentistry: structure and causes of judicial litigation

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KEYWORDS

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ABSTRACT

The great advances in diagnostic and therapeutic skills of most sectors of medicine and dentistry have led to an increasingly greater demand from patients for accuracy, attention and diligence by healthcare workers. Dentistry is one of the branches most frequently involved in claims for damages from malpractice, especially in those sectors that are particularly costly and of significant aesthetic value. Aim of the study was to compare data of malpractice claims with those of other Authors to identify similarities and/or differences in the results and to increase epidemiological knowledge in the area of dental malpractice. This work is a descriptive study performed analyzing medical malpractice claims in which one of the Authors was nominated as court professional expert advisor from 2018 to 2022 in two of the biggest courts in Campania: Naples and Santa Maria Capua Vetere. Findings of great interest were the greater involvement of the prosthodontic and implant-prosthetic sector, the significant recurrence of clinical records deficiency and the high prevalence of claims made by female subjects. Also, there was a greater incidence of emergent damage confronted to the non-pecuniary personal injury (biological damage).

INTRODUCTION

The great advances in diagnostic and therapeutic skills of most sectors of medicine and dentistry have led to an increasingly greater demand from patients for accuracy, attention and diligence by healthcare workers.

In the last twenty years there has been a profound transformation in the relationship between doctor and patient, with greater importance being placed on the decision-making role of the patient,¹ who must be provided with adequate and comprehensive information.²

Therefore, the accusations of presumed professional liability may arise not only for mistakes in the diagnostic (missed or wrong diagnosis) or therapeutic phase (absence or inadequate therapy or direct production of damage), but also for not providing the patient with enough information.³

Dentistry is one of the branches most frequently involved in claims for damages from malpractice, especially in those sectors that are particularly costly and of significant aesthetic value,⁴ such as prosthetics and implantology, followed by conservative/endodontic,⁵ oral surgery, orthodontics and periodontology.⁶

Therefore, the insurance market has evolved, adding different liability contracts for dentists, with coverage for damages deriving from inadequate diagnostic or therapeutic acts.⁷

In Italy, every year courts face more than 30.000 new medical malpractice claims (as shown by data from Osservatorio Sanità Ania in 2018⁸) and between 2017 and 2021 dental malpractice claims were at 4th place of the total medical malpractice claims (6,7%), with verdicts in favor of the patient in 74% of the cases (data from "Indagine Eurispes" 2023⁹).

Likewise, Manca et al.¹⁰ showed that litigations regarding dental malpractice from the Civil Forum of Rome (the biggest in numbers of dental malpractice litigations in Europe) represented about 10.4% of all health-related litigations and in 74% of cases the dentist was found guilty, with recognition of a permanent psycho-physical impairment in 62% of the cases.

These National data are aligned with several international studies on dental malpractice: for example, Calla and Muñoz¹¹ reported that dentists were found guilty in 84.8% of cases and Hashemipour et al.¹² in 56.7%. However, Thavarajah et al.¹³ reported a lower number of dentists pronounced guilty, with 39.63% of litigations decided in favor of patients.

In this context, the issue of identifying and delineating the boundaries of responsibility for the work of the single professionals involved arises, because of the possibility of carrying out dental work in an individual or associated form.

In fact, the expansion of scientific knowledge in the dental field has facilitated the fragmentation of the skills of the oral health professional, encouraging collaboration between specialists or professionals with specific knowledge (orthodontist, endodontist, periodontologist, oral surgeon, implantologist, prosthetist, pedodontist), sometimes in an equal position (horizontal team) and other times in a hierarchical organization (vertical team) in which non-medical health professionals also take part.

Moreover, the different health professionals can intervene in a synchronic or diachronic cooperation, with the former being a provision of different treatments practiced in contemporaneity and the latter being a provision of different treatments practiced at different times and stages.

In this scenario, the dentist can intervene in the treatment process with a counselling report, without there being any relationship of dependence between the owner of the dental practice and the consultant, or as an operator consulted (most often by the patient) to remedy a result deemed unsatisfactory.

Because of these peculiarities that characterize the practice of the dental profession, aim of this study was to compare data of malpractice claims with those of other Authors to identify similarities and/or differences in their results and to increase epidemiological knowledge in the area of dental malpractice.

MATERIALS AND METHODS

This work is a descriptive study performed analyzing medical malpractice claims in which one of the Authors (Di L. P.) was nominated as court professional expert advisor from 2018 to 2022 in two of the biggest courts in Campania: Naples and Santa Maria Capua Vetere.

Malpractice claims were divided by area (medical malpractice vs dental malpractice) and all information was divided based on multiple items: branch of dentistry involved, sex of the claiming party, type of defendant, defects in the clinical records available, the losing party, type of conduct defects claimed, temporary and permanent damage.

Data was then statistically described - and results compared.

RESULTS

A total of 161 medical litigations were retrieved in the indicated time frame.

Dental claim verdicts were firstly compared with the total number of medical litigation verdicts and the number of dental malpractices: out of 161 medical malpractice claims, 19 regarded dental malpractice, corresponding to 12% of the total medical liability claims (Figure 1).

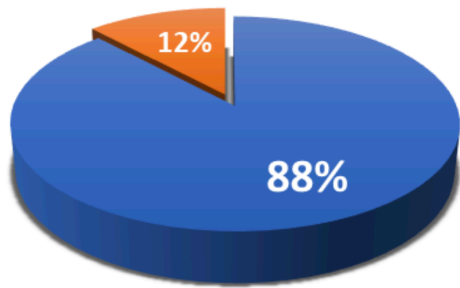
All dental malpractice litigations were in reference to conducts that occurred before 2017.

Analyzing dental malpractice claims by year of ruling (Figure 2) the number of cases has slightly decreased in recent years, with 5 claims in 2018, 4 case in 2019, 5 cases in 2020, 3 cases in 2021 and 2 cases in 2022.

In 15/19 (78.9%) of all cases the sex of the claiming party was female (Figure 3).

Figure 1. Number of dental malpractice claims vs total of medical litigations

Litigations distribution



■ Medical litigations ■ Dental malpractice

Figure 2. Dental malpractice claims distribution by year of ruling

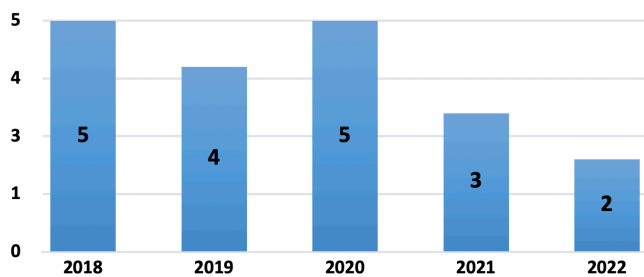
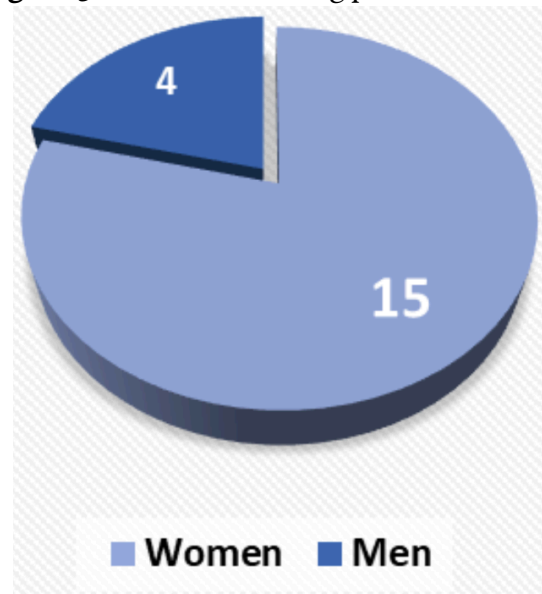
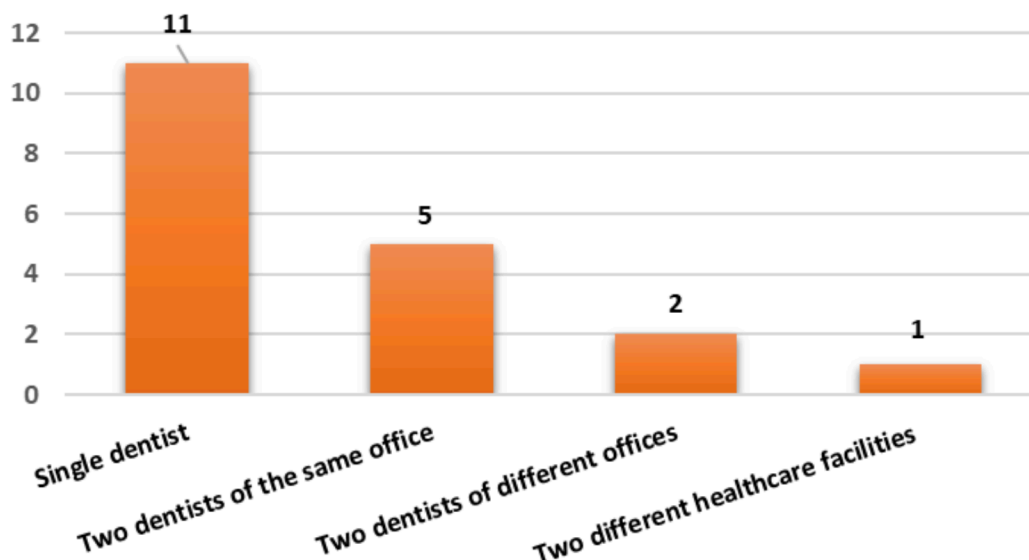


Figure 3. Sex of the claiming part



By analyzing the content of the verdicts, in 74% of cases (14/19) the dentist was found guilty. As shown in Figure 4, in 11 cases the defendant was a single dentist, while the defendants were two dentists of the same office in 5 cases, of different offices in 2 cases and in 1 case two different healthcare facilities were involved.

Figure 4. Type of defendants



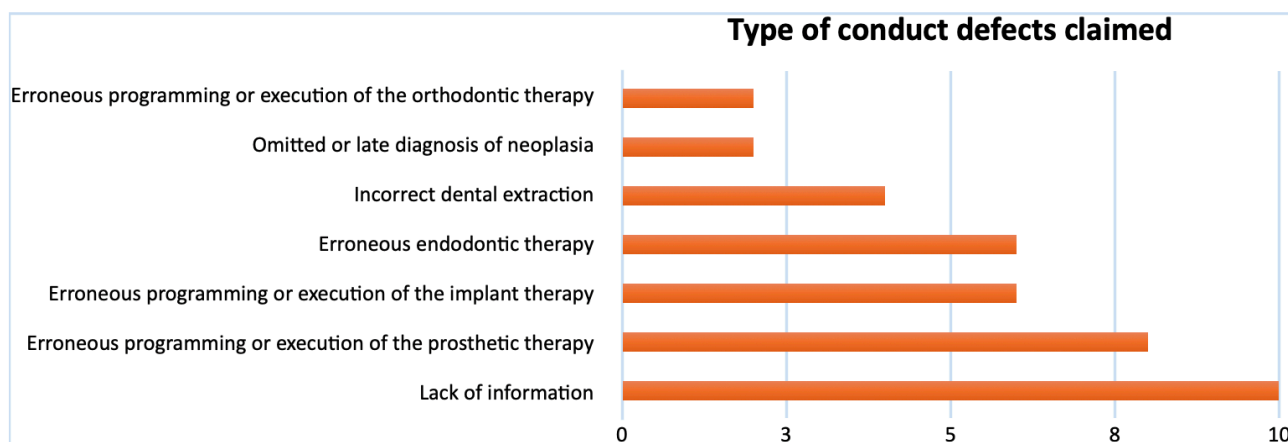
As for type of conduct defects claimed (Figure 5), keeping in mind that dentists are often simultaneously charged with different conduct defects, in 2 cases were of erroneous programming or execution of the orthodontic

therapy, in 2 cases were of omitted or late diagnosis of neoplasia, in 4 cases were of incorrect dental extraction, in 6 cases were of erroneous endodontic therapy, in 6 cases were of erroneous programming or execution of the

implant therapy, while in 8 cases were of erroneous programming or execution of the

prosthetic therapy. Finally, in 10 cases a defect in the information was claimed.

Figure 5. Type of conduct defects claimed



As for available clinical records (Figure 6), in 7/19 cases (37%) a lack in the clinical records was found and in 9/19 cases (47%) there was absence of informed consent.

Figure 6. Results of clinical records' analysis

ANALYSIS OF CLINICAL RECORDS

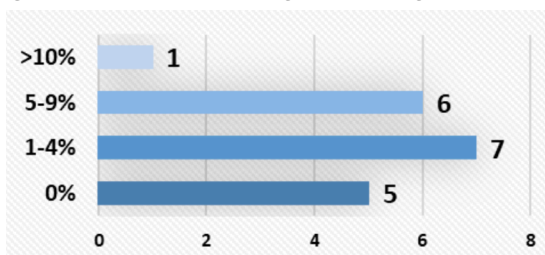
Lack in the clinical records	7/19 cases (37%)
Absence of informed consent	9/19 cases (47%)

This means that in 9 out of 10 cases of claims of lack of information this conduct defect was actually found.

Regarding the compensation voices valued by the Italian law, Biological Damage (BD), a quantification of psychological and physical permanent impairment percentage that goes from 0 (nothing) to 100 (the complete loss of physical validity), was recognized (figure 7) in 74% of cases, with DB of 1-4% in 7 cases, 5-9% in 6 cases and 1 case over 10%.

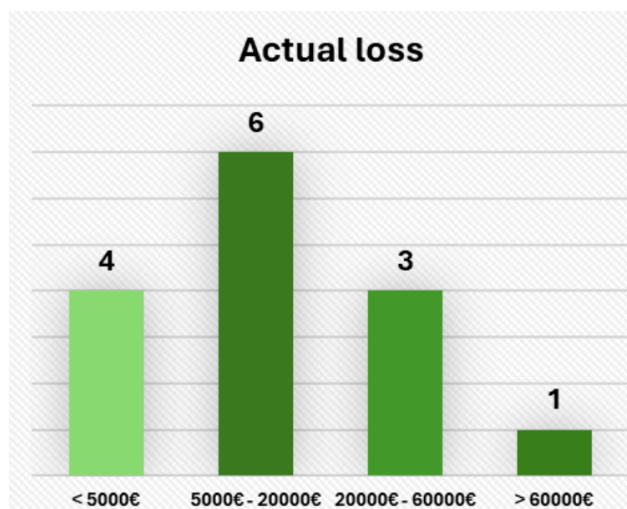
Moreover, a temporary damage was not always found. A partial inability was found in most of the cases, but with low incidence on validity of the patient.

Figure 7. Rates of biological damage found



Patrimonial damage intended as already sustained or future expenses (figure 8) was recognized in 14 cases, with 4 cases under 5.000 euros and a minimum of 500, 6 cases between 5.000 and 20.000, 3 cases between 20.000 and 60.000, and only 1 case over 60.000.

Figure 8. Actual loss compensation



DISCUSSION

Dentistry is an area characterized by high manual dexterity of the operator, collaboration among several specialist figures, use of sophisticated technical equipment and of constantly changing materials, aesthetic implications of dental treatments, high costs of most services which are frequently performed in election. These features, together with the predominantly commissive nature of dental damage and the fact that dental tissue easily shows signs of practiced therapy,

make dentistry a fertile field for malpractice claims.

The dentist has an obligation to the patient to use the most suitable materials to achieve the required aims, and is liable for the quality of materials chosen, for the aesthetic results of the treatment carried out and for optimistic unfulfilled promises.¹⁴

Failure to reach expectations results in a significant increase in judicial and extrajudicial litigation, in terms of professional liability. In fact, dental treatments are carried out as elective services, for which there are less justifications and excuses for errors.

Moreover, since dental treatment is carried out on hard tissues, which makes the damage produced more easily identifiable even after some time has passed, it is usually easier to demonstrate, a posteriori, the technical error at the litigation stage.

Possible productive misconduct involving the professional responsibility of the dentist can be identified in defects of information concerning privacy matters or diagnostic-therapeutic treatment, incorrect collection of anamnestic data, incorrect or incomplete clinical examination, inadequate evaluation and/or misinterpretation of instrumental and/or laboratory data and inadequate patient follow-up.

Therefore, the issue of dental malpractice is ubiquitous worldwide, contributing to the rise of the phenomenon of defensive medicine and dentistry. Moreover, numerous studies have shown how the dentist is frequently found guilty.

For example, Wu et al.¹⁵ reported that in a study carried out specifically on endodontic malpractice litigations in the United States from 2000 to 2021 the dentist was found liable in 43.3% of cases. Additionally, they showed that 45.3% of cases consisted of pre-procedural allegations (meaning issues in the diagnosis or failure to obtain informed consent) and 77.9% had intra-procedural allegations and that plaintiff won 75% of the cases attributed to post-procedural infections. Similarly, Al-Fraidi et al.¹⁶ have reported dentists to be found guilty in 47% of cases.

As for the identification of which branch of dentistry is more involved in dental malpractice claims, the results found in our study are similar to those presented by Kiani et al.¹⁷, Yu et al.¹⁸, Alsaeed et al.¹⁹ and Manca et al.¹⁰ who reported

that the majority of clinical complaints involved prosthodontics.

This information also aligns with those published by Nassani²⁰ who indicated that the available evidence suggest that prosthodontics may come at the top of dental specialties in terms of inciting patient complaints and filing of dental claims, while our results are in contrast with those of de Castro et al.²¹ and Fernandes and Junior²² who found endodontics and oral and maxillofacial surgery to be, respectively, the branches of dentistry most involved in malpractice litigations.

Other crucial findings were those of a significant absence of informed consent given to patients prior to procedures and lack in the clinical records, which were found in almost every case in which they were claimed. This data aligned with the findings of Hesham F. Marei,²³ Al-Fraidi et al.¹⁶, Kim²⁴, Corte-Real et al.²⁵ and Hamasaki and Hagihara²⁶, who showed how a vast number of intra-procedural mistakes are associated with the lack of adequate informed consent.

Such defects are of great importance and show how much work is still needed to ensure adequate information to all patients undergoing dental procedures, while also highlighting the ever-important need to produce retrievable clinical records, which are important to both patients (who may need them for future procedures) and health professionals (who may need them to adequately support their case in malpractice claims).

However, some of these deficiencies may find explanation in the fact that all of the claims were referred to procedures performed before 2017, the year Italian government passed the law that made informed consent mandatory for every medical treatment.¹⁸

In this perspective, adherence to these provisions of law will be of great interest in a potential future analysis, with a focus on claims related to procedures performed after 2017.

Another interesting finding in the dental litigations analyzed in this paper was that the sex of the claiming party was predominantly female. This discrepancy has been traced back by many Authors to the fact that women undergo, in general, more dental therapy than men²⁷ and are more concerned about oral health²⁸, while it also could be hypothesized that females give higher importance to the aesthetic aspects of treatment

because of the pressing beauty standards of modern Society.

Finally, biological damage was most often found between 1 and 9%, similarly to the mean of 4,31% found by Manca et al.¹⁰

With regard to malpractice claims and the role of different health care professionals, the peculiarities of both diachronic and synchronic cooperation make the dentist responsible not only for their own actions, but also for the errors of other team members if they could have been recognizable and avoidable.

In fact, based on traditional principles of warranty and fault, health professionals cannot avoid knowing and evaluating (to the extent that they can actually know and evaluate) the previous and contextual activity of another colleague by verifying its correctness and, if necessary, remedying the error of others.

Therefore, the dental practice owner may face two types of faults: in the first case, the dentist is culpable if they delegate a procedure to another person who is unable to properly perform it; in the second case, the owner is culpable if they do not adequately supervise, where necessary, the performance of certain interventions, or do not have organizational arrangements to prevent the occurrence of events harmful to the health of patients.

As a result, there are some obvious issues in the discrimination of who might actually be at fault in many dental malpractice claims. It is often difficult to demarcate the actions of the individual health care professionals involved and to distinguish between the responsibility of the individual, the responsibility of the team, and the responsibility of the dental practice owner.

In this context, we can highlight some precautions to be taken in the management of clinical risks in dentistry.

For example, as a lot of claims were related to erroneous programming or execution of prosthetic or implant therapy,¹⁹ this shows the primary importance of a careful assessment of the patient's medical condition and their suitability for dental treatment, together with constant diligence in diagnosis and treatment.

Moreover, use of the necessary precautions to minimize the risk of failure of dental treatment along with identification of factors that may hinder the success of treatment and information to the patient of how they may affect the outcome need to be of constant interest in dental

practice, as to not create unreasonable expectations and for the patient to understand that there is no guarantee of success. In this regard, written or implied warranties should be avoided.

In addition, it would be advisable to record pretreatment and treatment data and store them properly for a reasonable time, as well as to suggest a maintenance protocol designed to achieve long-term success and record patient cooperation data.

The roles of information and informed consent are to be considered of primary importance in dental treatment: the dentist must prospect, preferably in written form, the patient with prior comprehensive, detailed and comprehensible information about the health treatment and its foreseeable consequences, including the discomforts of the treatment (e.g., post-operative suffering) and the possibility of aggravation of health conditions as a result of the performance of the treatment itself, as well as about therapeutic alternatives. The need of a written consent is crucial to prove that they properly fulfilled the prior informational obligation.

Finally, the dentist must provide all documentary evidence as the person in charge of the formation of clinical records (such as, but not limited to, informed consent form, instrumental investigations, laboratory tests, medical certificates) and must show that they took all due precautions to avoid the occurrence of complications.

One limitation of this paper can be identified in the data collection methodology, which consisted of analyzing cases in which one of the authors personally worked as a court advisor, meaning that the sample size is relatively small and that the outcomes of the litigations were partly influenced by his personal medico-legal analysis of the cases.

CONCLUSION

The results of this study were aligned with those of other international authors.

Findings of great interest were the greater involvement of the prosthetic and implant-prosthetic sector, the significant recurrence of clinical records deficiency, the lack of consent recording and the high prevalence of claims made by female subjects. Also, there was a greater incidence of emergent damage confronted to the non-pecuniary personal injury (biological damage).

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