

Chronological age estimation based on third molar development in a Portuguese population

Inês Morais Caldas · Paula Júlio ·
Ricardo Jorge Simões · Eduarda Matos ·
Américo Afonso · Teresa Magalhães

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Abstract Third molar development was assessed using a sample of 1,131 orthopantomograms from a Portuguese population. The methodology applied was the eight stages (A–H) method described by Demirjian et al. The final sample was made of 739 orthopantomograms, 387 (52.5%) of which belonging to females; age ranged between 6.1 and 22.5 years old (mean age=14.49, S.D.=4.37). For each developmental stage, mean age, standard deviation, and minimal and maximal age was assessed; evaluation of the rate formation of each tooth, according to sex, was calculated and data distribution expressed in percentiles for each stage; the probability of an individual being 16 was

also evaluated. The relationship between tooth development and chronological age had a statistical significance for all teeth and both sexes ($p < 0.0001$). The data described may provide reference for forensic application and agree with the thesis that each population need specific data.

Keywords Forensic dentistry · Dental age · Third molar · Teeth mineralization · Age estimation

Introduction

Age estimation is an important area of forensic dentistry which has been gaining increasing importance in the past few years. In accordance with the recommendations for age estimation in criminal proceedings of the study group on forensic age diagnostics, for an age estimation, a physical examination, an X-ray examination of the hand, and a dental examination should be carried out; if the skeletal development of the hand is completed, an additional X-ray examination (or CT scan) of the clavicles (medial extremity) should be performed [1].

Developing teeth are a useful indicator of maturation (and hence of biological age) because they are less affected than other body tissues by diseases and environmental insults [2]. Dental development may be measured either by tooth eruption or tooth formation. While eruption is a discontinuous and variable measurement, affected by several factors like malnutrition, premature loss of primary teeth, crowding and dental decay, tooth formation is seen as a more robust measure with high heritability, low coefficient of variation and resistance to environmental effects [2]. Most authors have chosen the eighth-grade scheme of Demirjian for dental age estimation, for reasons of accuracy and practicability. Third molar development is particularly

I. M. Caldas (✉) · P. Júlio · R. J. Simões · A. Afonso
Dental Medicine Faculty of Porto University, Faculdade de
Medicina Dentária da Universidade do Porto,
Rua Dr. Manuel Pereira da Silva,
4200-393, Porto, Portugal
e-mail: icaldas@fmd.up.pt

I. M. Caldas · A. Afonso · T. Magalhães
Center of Forensic Sciences,
Largo da Sé Nova,
3000-213, Coimbra, Portugal

E. Matos · T. Magalhães
Biomedical Sciences Institute “Abel Salazar” of Porto University,
Largo Prof. Abel Salazar, 2,
4099-003, Porto, Portugal

T. Magalhães
National Institute of Legal Medicine–North Branch,
Jardim Carrilho Videira,
4050-167, Porto, Portugal

T. Magalhães
Faculty of Medicine of Porto University,
Al. Prof. Hernâni Monteiro,
4200-319, Porto, Portugal

Table 1 Orthopantomograms distribution according to age and sex ($n=737$)

Age, in years	Female	Male	Total
6	14	16	30
7	20	33	53
8	31	32	63
9	38	38	76
10	28	42	70
11	18	20	38
12	27	27	54
13	30	35	65
14	24	16	40
15	20	9	29
16	15	14	29
17	19	9	28
18	12	6	18
19	26	17	43
20	31	19	50
21	27	15	42
22	7	2	9
Total	387	350	737

important due to the fact of it being the only teeth still in development by age 16; however, previous findings showed that the mineralization of the wisdom teeth is a population-specific process and does not occur in every ethnic group at the same age [3–5]. According to Olze et al. [6] there are not enough data to decide how ethnic origin affects teeth mineralization, and the forensic value of age estimation depends on it. Therefore, it is crucial to use population-specific reference data in forensic age estimation of living people. Hence, the aim of this study is to obtain data regarding third molar development in a Portuguese population.

Material and methods

A total sample of 1,131 orthopantomogram collected from a Portuguese population aged between 6 and 22 years old was analyzed. The radiographs used were taken from patient files of the Faculty of Dentistry of Porto University, Portugal.

The exclusion criteria were the following:

- Other nationalities other than Portuguese;
- Absence of all third molars;
- Image deformity affecting third molars;
- Orthopantomogram exhibiting obvious pathology.

A >cross-sectional study was carried out to assess chronological age based on the stages of third molar

development. Dental development was assessed using the method developed by Demirjian et al. [7]. To assess reliability, 80 randomly selected radiographs were examined by the two first authors; 80 randomly selected orthopantomograms were assessed twice by the first author, with a 1 month interval between the two observations. Intra- and interobserver agreement was determined using the Wilcoxon signed ranks. Statistical analysis was performed using the SPSS 16.0 (SPSS Inc., Chicago, IL).

Results

Repeated scoring of 80 radiographs revealed no significant intra- or interobserver differences ($p>0.05$). Intraobserver agreement occurred in 95% and interobserver agreement occurred in 93%. The final sample was made of 739 orthopantomograms, 387 (52.5%) of which belonging to females; age ranged between 6.1 and 22.5 years old (mean age=14.49; S.D.=4.37). Orthopantomograms distribution, according with age and sex is shown in Table 1. Of the orthopantomograms, 17.1% were excluded since they present no third molars; 4.9% presented one third of a molar; 6.1%, two; 6.9% had three third molars; and 65.0% presented all four third molars.

Results were studied according to sex and age for every third molar. Table 2 shows the mean age, standard deviation, standard error of the mean, and minimal and maximal age (in years) for third molars, for stages A–G. For stage H, mean values were not calculated, and the probability of excluding age younger than 18 years old was calculated for every third molar, both for males and females (Table 3). Table 4 presents 50% probability values for stage H.

To allow better visualization of the rate formation of each tooth, according to sex, the data distribution is expressed in percentiles for each stage, in Table 5. The frequency distribution by stages and sex for each group age is shown in Table 6. Stage A was not observed beyond the age group 13–13.9 years, and stage B was rare in those age groups. From stage D onward, all group ages older than 9, for females, and 10, for males, were verified.

A correlation analysis with Spearman rank order correlation (ρ) was used to assess the strength and direction of the linear relationship between tooth development and chronological age. There was a strong, positive correlation between the two variables for every tooth in both genders ($p<0.005$). The Kruskal–Wallis test was used to check for differences in tooth mineralization according to sex. It was found that, overall, there were statistically significant differences for all third molar mineralization rate in males and females (tooth 18, $p=0.002$; tooth 28, $p<$

Table 2 Age distribution by sex, stage, and tooth

Stage, sex	Mean			Standard deviation			Standard error of mean			Maximum			Minimum			Number						
	18	28	38	48	18	28	38	48	18	28	38	48	18	28	38	48	18	28	38	48		
A Male	8.5	8.4	8.3	8.5	1.0	1.0	1.2	1.2	0.17	0.17	0.17	11.0	11.0	12.1	12.1	7.2	7.2	7.2	33	35	54	56
Female	8.6	8.6	8.4	8.6	1.2	1.1	1.6	1.4	0.25	0.27	0.27	10.5	10.5	13.0	13.0	7.8	7.8	6.1	21	17	37	34
B Male	9.8	9.4	9.6	9.6	1.3	1.2	1.2	1.3	0.23	0.20	0.22	13.5	12.7	13.5	13.4	7.2	7.2	7.2	33	32	32	31
Female	9.3	9.6	9.3	9.8	1.2	2.1	0.8	1.4	0.18	0.32	0.16	12.5	13.0	12.0	14.2	7.9	7.9	8.1	41	42	23	20
C Male	11.2	11.0	11.5	11.5	1.9	1.9	1.7	1.6	0.27	0.27	0.22	16.2	16.2	16.2	15.9	7.4	7.4	7.4	47	46	62	63
Female	11.8	12.0	11.5	11.2	1.9	2.2	2.2	1.9	0.30	0.32	0.29	16.8	15.4	20.0	15.4	9.9	9.9	8.9	38	46	60	53
D Male	12.9	12.8	13.1	13.2	1.4	1.4	1.2	1.1	0.22	0.22	0.22	16.8	16.8	16.8	16.2	10.0	10.0	11.3	42	41	28	25
Female	13.5	13.2	14.0	13.4	2.2	2.0	2.3	1.9	0.29	0.28	0.34	20.0	20.5	21.9	20.0	9.7	9.7	11.5	58	52	45	46
E Male	14.7	14.6	14.7	14.3	1.8	1.6	1.4	1.6	0.54	0.40	0.56	17.2	17.2	16.2	17.2	12.9	12.9	12.9	11	16	6	10
Female	16.0	16.2	16.3	15.9	2.7	2.8	3.0	2.8	0.50	0.47	0.55	20.8	21.7	21.7	21.7	12.3	11.5	12.3	29	34	29	34
F Male	16.4	16.8	16.6	15.8	1.8	1.7	1.9	1.3	0.58	0.48	0.54	20.7	20.7	20.7	18.5	15.0	15.0	15.0	10	13	12	13
Female	17.3	16.8	16.5	16.8	2.5	2.4	2.7	1.9	0.58	0.51	0.70	21.0	21.0	21.6	21.6	14.9	13.8	13.3	19	22	15	16
G Male	18.1	18.3	18.4	18.4	1.6	1.7	1.6	1.6	0.58	0.40	0.36	21.0	21.0	21.2	21.2	16.4	16.4	16.5	17	19	19	23
Female	18.4	18.4	18.1	18.3	2.2	2.0	2.2	2.5	0.41	0.36	0.38	21.9	21.9	21.3	22.2	14.9	14.9	14.9	28	31	34	35
H Male	-	-	-	-	-	-	-	-	-	-	-	22.1	22.1	21.9	21.9	11.3	11.3	11.3	43	40	39	34
Female	-	-	-	-	-	-	-	-	-	-	-	22.5	22.5	22.5	22.5	13.4	13.4	13.4	61	60	56	56

Table 3 Probability of excluding age younger than 18 years if stage H is attained

Sex	Tooth			
	18	28	38	48
Male	97.3%	97.4%	97.5%	98.1%
Female	98.0%	98.1%	97.6%	98.0%

0.001; tooth 38, $p=0.005$; tooth 48, $p=0.002$); however, considering each stage separately, it was found that sexual dimorphism was not always the case, and significant differences concerned only stages F, G, and H for all four teeth. Differences in the mineralization rate between the upper and lower, left and right third molars were also evaluated (Table 7), and no significant differences were observed, except for females, between the upper third molars ($p=0.024$).

A logistic regression analysis was also performed for age 16 years old, with teeth staging (from D to F) as predictor variables. Analysis was done dividing the sample according to gender. For females, a total of 260 cases were analyzed and for males, 205 cases were analyzed (Table 8). Stage D was found to be a useful marker for diagnosing age equal or superior to 16 years, both in males and females, with 98.4% and 96.3% of correct predictions, respectively.

Criteria validity was also assessed, evaluating this method's specificity (the percentage of non-occurrences correctly predicted) and sensitivity (the percentage of occurrences correctly predicted) (Table 9). The results pointed out that excluding age 16 or over (true negatives) is a more secure prediction for every stage.

Discussion

Age estimation using dental development has been used for a long time since it is recognized that dental mineralization is an accurate measure of chronological age, not being particularly affected by other factors (as diseases, nutrition, among others); however, contradiction is reported on the usefulness of third molars for dental age estimation [8]. On one hand, there are various studies using distinct method-

ologies, for instance, Mesotten et al. [8] performed dental age estimation using a modification of the method of Gleiser and Hunt [9], wherein third molar development is assessed taking into consideration ten developmental stages. The same procedure was adopted by Gunst et al. [10] in a continuation and extension of the previous study.

According to De Salvia et al. [11], a method evaluation should be accurate, not invasive, reproducible and, if possible, easy exploitable. In this study, we chose to adopt the eighth-grade scheme of Demirjian et al. [7] because it obeys the characteristics; in addition, it does not require tooth measurements and is an easy, objective, and practical method requiring only experience in orthopantomogram interpretation. Besides, it is the preferred method by several authors [12–19], allowing us to compare our results with those from other studies. Olze et al. [6] also found the Demirjian method as the one more suitable for their investigation, claiming that other methods, such as the one used by Gleiser and Hunt [9] or the one described by Moorrees et al. [20] or yet, that applied by Kullman et al. [21], used many stages, making it harder to compare results and also use estimation values of the root future length, which results in a subjective approach to estimation. In another investigation, Olze et al. [22] referred to this method as the one revealing the highest inter- and intra-individual correspondence between evaluators and as the one with the highest correspondence between estimated age and chronological age as well.

On the other hand, third molars are frequently absent, adopt aberrant positions, and vary in size, structure, formation, and eruption times [23–25]. Nevertheless, after a certain age, (approximately 15 years of age) third molars are the only teeth developing and therefore, the only useful age indicator [26], but because, root mineralization can be completed under age 18, other alternative methods that do not consider tooth mineralization as a factor are being studied. For instances, Olze et al. [27] described the radiographic visibility of the root pulp in third molars as a potential age estimation criterion, concluding that it is possible, “beyond reasonable doubt” state that a person is over 18 years old if a certain stage is attained. We chose to study the mineralization stages of third molars because in Portugal, the age with legal relevancy is 16 and not 18 years old.

Table 4 Fifty percent probability values for stage H, according to sex

Tooth	Males	Females
18	18.77	19.45
28	18.99	19.37
38	18.70	19.38
48	19.25	19.45

Table 5 Age distribution in percentiles by stage and sex

Stage		10					25					50					75					90							
		18	28	38	48	18	28	38	48	18	28	38	48	18	28	38	48	18	28	38	48	18	28	38	48				
A	Male	7.0	7.0	7.0	7.0	7.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	9.0	9.0	9.0	9.0	9.0	10.0	10.0	10.0	10.5		
	Female	7.0	7.0	7.0	6.0	7.0	8.0	8.0	7.0	7.8	9.0	9.0	8.0	8.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	10.0	10.0	10.2	10.5	
B	Male	8.0	7.3	8.3	8.3	8.2	9.0	9.0	9.0	9.0	9.0	10.0	10.0	9.0	9.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	11.6	10.7	11.0	
	Female	8.0	8.0	6.0	6.0	8.1	8.5	8.8	7.0	7.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	10.0	10.0	11.0	11.7	10.2	11.9	
C	Male	9.0	9.0	9.0	9.0	9.4	10.0	10.0	10.0	10.0	10.0	11.0	11.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	13.0	13.0	13.2	13.3	13.0	13.0	
	Female	9.9	10.0	9.0	9.0	9.0	10.8	10.8	10.0	10.0	10.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	11.0	11.0	13.0	13.0	13.0	13.0	12.0	14.0	14.3	14.0	13.6
D	Male	11.0	11.0	11.9	11.9	12.0	12.0	12.0	12.0	12.0	12.5	13.0	13.0	13.0	13.0	13.0	13.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.7	14.0	14.1	14.4
	Female	11.0	11.0	12.0	12.0	11.0	12.0	12.0	12.0	12.0	12.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	14.0	14.0	14.0	14.0	14.0	16.0	16.0	17.0	16.0
E	Male	12.2	12.7	12.0	12.0	12.0	13.0	13.0	14.3	14.3	12.8	15.0	15.0	15.0	15.0	15.0	15.0	15.0	14.5	14.5	16.0	16.0	16.0	15.0	15.3	17.8	17.3	16.0	16.9
	Female	13.0	12.5	12.0	12.0	12.5	14.0	14.0	14.5	14.0	14.0	15.0	16.0	16.0	16.0	16.0	16.0	16.0	15.0	15.0	17.5	18.3	18.3	19.5	18.3	20.0	20.5	21.0	20.5
F	Male	14.1	14.4	14.0	14.0	14.0	15.0	16.0	15.3	15.0	15.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	17.5	18.5	18.0	18.0	16.5	19.9	19.6	19.7	18.0
	Female	14.0	13.3	13.6	13.6	14.0	15.0	15.0	14.0	15.3	18.0	18.0	17.0	16.0	16.5	19.0	19.0	18.3	16.5	19.0	19.0	19.0	18.3	19.0	18.0	21.0	20.7	21.0	19.6
G	Male	16.0	16.0	16.0	14.0	16.0	16.5	16.0	15.3	17.0	17.0	18.0	19.0	19.0	19.0	19.0	19.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.2	20.0	19.7	20.0
	Female	15.0	15.0	14.0	14.0	14.6	17.0	17.0	16.0	16.0	16.0	19.0	19.0	18.0	19.0	19.0	19.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	21.0	21.0	20.4	21.0
H	Male	17.0	17.0	17.0	17.0	17.0	19.0	19.0	19.0	19.0	19.0	20.0	20.0	20.0	20.0	20.0	20.0	21.0	20.0	20.0	20.0	20.0	21.0	20.3	20.0	21.0	21.0	21.0	21.0
	Female	18.0	18.0	17.7	17.7	18.0	19.0	19.0	19.0	19.0	19.0	20.0	20.0	20.0	20.0	20.0	20.0	21.0	20.0	20.0	21.0	21.0	21.0	21.0	21.0	22.0	22.0	22.0	21.3

Table 6 Frequency distribution, by sex, according with stage of mineralization

Age	Stage (tooth 18, 28, 38, 48)																														
	A			B			C			D			E			F			G			H									
	18	28	38	48	18	28	38	48	18	28	38	48	18	28	38	48	18	28	38	48	18	28	38	48	18	28	38	48			
6-6.9	Female	7.1	0	28.6	14.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
	Male	0	0	12.5	12.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
7-7.9	Female	15.0	16.7	35.0	30.0	5.0	5.6	0	0	5.0	5.6	5.0	5.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
	Male	18.2	24.2	40.6	30.3	3.0	9.1	3.1	6.1	3.0	3.0	3.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
8-8.9	Female	12.9	17.9	29.0	25.8	29.0	32.1	6.5	6.5	0	0	9.7	6.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
	Male	34.4	35.5	56.2	56.2	9.4	9.7	6.2	3.1	3.1	3.2	6.2	6.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
9-9.9	Female	26.3	15.8	28.9	31.6	36.8	42.1	34.2	23.7	5.3	5.3	5.8	8.4	5.3	5.3	0	0	0	0	0	0	0	0	0	0	0	0	0			
	Male	28.9	31.6	34.2	42.1	26.3	23.7	36.8	34.2	15.8	18.4	10.5	10.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
10-10.9	Female	7.1	7.1	10.7	10.7	39.3	35.7	21.4	17.9	21.4	28.6	35.7	39.3	7.1	7.1	0	3.6	0	0	0	0	0	0	0	0	0	0	0	0		
	Male	9.5	7.1	16.7	16.7	28.6	33.3	23.8	23.8	26.2	31.0	28.6	31.0	7.1	7.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
11-11.9	Female	5.6	5.6	5.6	5.6	22.2	11.1	11.1	33.3	44.4	50.0	44.4	22.2	16.7	16.7	22.2	5.6	11.1	5.6	5.6	0	0	0	0	0	0	0	0	0		
	Male	5.0	5.3	5.0	10.0	20.0	10.5	15.0	10.0	40.0	42.1	50.0	55.0	15.0	21.1	10.0	5.0	0	0	0	0	0	0	0	0	0	0	0	0		
12-12.9	Female	0	0	3.8	7.4	7.4	3.7	0	3.7	37.0	40.7	50.0	44.4	40.7	40.7	34.6	33.3	3.7	7.7	7.4	0	0	0	0	0	0	0	0	0		
	Male	0	0	3.7	3.7	3.7	3.7	0	3.7	25.9	22.2	40.7	44.4	33.3	33.3	25.9	18.5	3.7	3.7	3.7	7.4	0	0	0	0	0	0	0	0		
13-13.9	Female	0	0	3.4	0	0	3.3	0	0	30.0	30.0	34.5	23.3	46.7	46.7	37.9	53.3	10.0	3.3	10.3	13.3	3.3	6.7	3.4	0	0	0	0	0		
	Male	0	0	0	0	5.7	0	5.7	0	25.7	17.6	48.6	48.6	40.0	41.2	22.9	25.7	5.7	11.8	0	2.9	0	0	0	0	0	0	0	0		
14-14.9	Female	0	0	0	0	0	4.2	0	4.2	8.3	12.5	20.8	12.5	45.8	45.8	37.5	33.3	16.7	25.0	4.2	16.7	4.2	4.2	12.5	8.3	4.2	4.2	8.3	0		
	Male	0	0	0	0	0	0	0	0	12.5	12.5	18.8	12.5	56.2	50.0	56.2	50.0	12.5	12.5	0	12.5	6.2	6.2	12.5	12.5	0	0	0	0	0	
15-15.9	Female	0	0	0	0	0	0	0	0	0	5.0	5.0	5.0	15.0	15.0	15.0	30.0	25.0	35.0	45.0	25.0	30.0	15.0	10.0	15.0	15.0	15.0	15.0	0		
	Male	0	0	0	0	0	0	0	0	11.1	11.1	11.1	11.1	22.2	11.1	11.1	11.1	33.3	66.7	44.4	11.1	22.2	11.1	11.1	33.3	0	0	0	0	0	
16-16.9	Female	0	0	0	0	0	0	0	0	6.7	6.7	0	0	46.7	26.7	20.0	20.0	13.3	33.3	20.0	13.3	6.7	6.7	20.0	26.7	13.3	13.3	13.3	6.7	6.7	
	Male	0	0	0	0	0	0	0	0	7.1	7.1	7.1	7.1	14.3	14.3	7.1	7.1	7.1	7.1	7.1	7.1	28.6	28.6	35.7	28.6	35.7	21.4	28.6	0	0	
17-17.9	Female	0	0	0	0	0	0	0	0	5.3	5.3	5.3	5.3	5.3	0	21.1	5.3	26.3	26.3	15.8	15.8	5.3	15.8	5.3	15.8	26.3	26.3	15.8	21.1	10.5	
	Male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.1	11.1	0	11.1	11.1	22.2	11.1	11.1	33.3	22.2	44.4	44.4	44.4	
18-18.9	Female	0	0	0	0	0	0	0	0	0	0	0	0	8.3	0	0	0	8.3	8.3	8.3	8.3	25.0	33.3	0	16.7	0	8.3	50.0	16.7	50.0	
	Male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16.7	16.7	0	0	0	16.7	33.3	33.3	33.3	16.7	16.7	33.3	33.3	33.3
19-19.9	Female	0	0	0	0	0	0	0	0	0	0	0	0	3.8	3.8	7.7	3.8	7.7	3.8	7.7	3.8	7.7	11.5	7.7	3.8	7.7	19.2	34.6	23.1	19.2	57.7
	Male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.9	11.8	5.9	0	23.5	35.3	41.2	70.6	58.8	
20-20.9	Female	0	0	0	0	0	0	0	0	3.2	0	0	0	3.2	3.2	0	3.2	9.7	9.7	12.9	9.7	3.2	3.2	0	0	22.6	19.4	25.8	25.8	52.9	
	Male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.3	5.3	5.3	0	15.8	26.3	21.1	31.6	45.2	
21-21.9	Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.7	0	7.4	11.1	11.1	11.1	7.4	11.1	3.7	18.5	14.8	11.1	22.2	51.9	
	Male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.0	6.7	6.7	6.7	6.7	
22-22.9	Female	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.0	14.3	10.0	85.7	
	Male	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Table 7 Differences in tooth mineralization according to sex (Mann–Whitney *U* test)

Tooth	Sex			
	18	28	38	48
<i>z</i>	-3.128	-3.745	-2.815	-3.103
<i>p</i>	0.02	<0.05	0.05	0.002

Grouping variable: sex

The results of this investigation point to an almost linear relationship between age and third molar mineralization regardless of the individual sex. It also shows that complete mineralization occurs a little later in females. This is consistent, in general lines, with the relevant literature; however, not all data are consistent, and a clear tendency of earlier mineralization chronology is evident in our sample. Nevertheless, it must be taken into account that the mean age is influenced by the maximum age of the sample.

The present investigation showed no intra- or interobserver significant differences, and this finding is consistent with many other studies in the area [5, 15, 17–19, 28–30]. It was also found that there is a strong correlation between age and third molar development, both for males and females ($p < 0.001$, for both sexes). The correlation coefficients were 0.772 and 0.705 (tooth 18), 0.853 and 0.728 (tooth 28), 0.848 and 0.771 (tooth 38), and 0.843 and 0.759 (tooth 48), for girls and boys, respectively. Similar results were presented by other investigators [15, 17, 29].

Trends for an earlier third molar formation in males were apparent in all teeth and in all stages. These data agree with some and disagree with others, those from other investigations. For instance, in an Austrian sample of 610 individuals aged between 12 and 24 years old [5], a tendency for earlier third molar mineralization in females was apparent at earlier crown formation stages; the authors claim then an inversion and a faster root formation in male individuals, with boys reaching stage E 2 years earlier than girls. In this Portuguese sample, as stated before (stage E), these were also first reached by boys (1.3 to 1.8 years earlier). On the other hand, Lee et al. [17] stated that, as in

this study, third molar formation happens earlier in males. The same results were stated by Rai et al. and Olze et al. [28, 31].

In this study, there were statistically significant differences for all third molar mineralization rate in males and females. Considering each stage separately, it was found that sexual dimorphism was not always the case, and significant differences concerned only stages F, G, and H, for all four teeth. These results partially agree with those from other studies; Meinel et al. [5] stated that significant sexual dimorphism was presented in stages E and F, Olze et al. [15] reported no significant differences between males and females, but no individual stage analysis was done. Prieto et al. [16] found that, as in our sample, there were no significant differences between genders, but when considering each stage isolated, significant sexual dimorphism was found in stages E, F, and G with males reaching maturity indicators earlier than females. Lee et al. [17] stated that significant sexual dimorphism as present in stages F and G. In a Chinese population, Bai et al. [18] also reported the presence of significant sexual dimorphism but on earlier and later stages (D, E, and H). These differences could be related with sample differences or with population-specific data.

No differences in the mineralization rate between the upper and lower, left and right third molars were observed, except for females, between the upper third molars. This difference was not observed in any of the investigations consulted [17, 19, 28].

Most authors chose to determine the likelihood that a subject is over 18 [11]; however, in Portugal, the age with legal relevancy is 16, therefore, 16 was the chosen age for this assessment. In our opinion, the diagnosis of being younger than 16 years old can be made with accuracy using stage D of the radiological development of the third molars. In fact, logistic regression pointed out that stage D can be used to predict age equal or superior to 16 with an accuracy of 96.3% for females and 98.4% for males. It should be stated that stage D is not the best stage to diagnose age over 16 since third molar mineralization increases with age. In this manner, stage E, F, G, and H are more suitable

Table 8 Binary logistic regression results, by sex, according with tooth staging

STAGE	Sex	Model significance			Variance explained (%)	Correct predictions (%)		
		Omnibus χ^2	DF	χ^2		<16	≥16	Total
D	Male	140.722	4	<0.001	49.7–69.8	82.3	98.4	87.3
	Female	128.619	4	<0.001	39.0–52.5	65.1	96.3	78.1
E	Male	189.894	4	<0.001	60.4–84.9	93.6	96.9	94.6
	Female	176.088	4	<0.001	49.2–64.8	84.9	90.7	87.3
F	Male	208.826	4	<0.001	3.9–89.8	97.6	93.8	96.6
	Female	170.519	4	<0.001	48.1–64.8	93.4	81.5	88.5

DF degrees of freedom

Table 9 Criterion validity (chronological age \geq 16) according to tooth staging for male ($n=350$) and female ($n=387$)

TP true positive, *TN* true negative, *FP* false positive, *FN* false negative

Stage	Sex	TP	TN	FP	FN	Sensitivity (CI 95%)	Specificity (CI 95%)
D	Male	66	240	28	16	0.19	0.69
	Female	112	194	56	25	0.29	0.50
E	Male	66	260	8	16	0.19	0.74
	Female	113	225	25	26	0.29	0.58
F	Male	74	263	5	8	0.21	0.75
	Female	101	236	14	36	0.26	0.61

indicators. Stage D is, perhaps, the earliest indicator of age over 16 years; however, dental age estimation is one more complementary method of age estimation, and other indicators should be used in order to improve specificity and sensitivity and diminish the occurrence of technically and ethically unacceptable errors. These type of errors were described by Garamendi et al. [32] who refer to the errors derived from a forensic age estimation indicating that a subject who is actually over 18 (in our case, 16) is a minor (technically unacceptable errors) and to the errors indicating that minors are over 18. The first type of error leads to a more benevolent criminal treatment and the second one leads to a violation of minors' rights. Taking this into account, additional age estimation methods should be used to reduce technically unacceptable to a minimum, and to erase ethically unacceptable.

Because in some few procedures, age 18 may be the age with legal relevancy (in determining illegal immigration, for instances), probability calculation of excluding age younger than 18 if stage H is attained was also calculated. As recommended by Knell et al. [33], mean values of age at stage H were not calculated, since they can lead to false conclusions.

Finally, we believe that, based on our results, and the differences between them and those of other populations, we do agree with Olze et al. [6] when they state that population-specific standards enhance the accuracy of forensic age estimation based on third molar mineralization in living subjects. Nevertheless, this remains to be proven, and some investigators do state that there is no indication at all that not using country-specific information influences the percentage of correctly identified subjects [30].

Conclusion

The present investigation provides important data on third molar mineralization in the Portuguese population. It can be concluded that stage D can be used to predict age equal or superior to 16 with an accuracy of 96.3% for females and 98.4% for males. It is also evident that different populations have different data; in this manner, these data may not be

valid to other populations. The data described may provide a reference for forensic application; however, additional studies with a larger population sample should be conducted to meet provisions of more accurate data concerning the Portuguese population.

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