Age estimation using mandibular third molars in Chilean individuals

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SUMMARY

The aim of this study was to determine differences in maturation stages of the mandibular third molars (MTM) of Chilean individuals by age, sex, and side. The maturation stage of 930 lower third molar teeth were analysed, in patients aged between 9 and 24 years (432 males and 498 females), by panoramic radiography. The stage of maturity of the MTM was classified from A to H, following the method established by Demirjian. Multinomial logistic regression was performed for maturation stage by age.

The lowest age of complete maturity of the MTM was 16 years in both sexes (Stage H). The correlation between maturation stage and sex was significant, positive, and high for both sexes, in both tooth 48 and tooth 38, showing that increasing age and maturation stage are correlated. In both males and females, maturation Stage H of the MTM was associated with the age of 18 years. The fit of the model was significant, showing that it has a greater predictive power than the null model. Nagelkerke's pseudo R^2 explained 73.5% of the variations of the model. In the contemporary Chilean population, if the maturity of the MTM is at a stage prior to complete calcification of the crown (Stage D), it is very unlikely that the individual is aged 18 years or over. It is highly probable that a Chilean individual with complete root formation of the MTM is legally of age, regardless of side or sex.

Key words: Age estimation by teeth – Forensic dentistry – Panoramic radiography – Third molar

INTRODUCTION

Age estimation is an important factor for establishing the identity of an individual (Lewis et al., 2015) and represents a significant contribution to the identification of living or dead individuals by means of forensic odontology (Adserias-Garriga, 2019). Many methods have been reported in the

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literature to be good predictors for age estimation, such as radiographic methods, evaluation of joint surfaces (rib-ends, pubic symphysis, auricular surface, cranial sutures), dental histology, protein racemization, and DNA-based methods (mitochondrial DNA deletions, telomere shortening, DNA methylation and protein-based methods, etc.) (Hartomo et al., 2019; Larsen et al., 2023; López-Alcaraz et al., 2015).

The teeth are conserved for a long period (Lewis et al., 2015), and are frequently used in forensic medicine, as they are reliable indicators for age estimation (Kurita et al., 2007). This is because they are very resistant structures, even to environmental factors, and are therefore often found among human remains (Mesotten et al., 2002). The formation of the teeth and the changes associated with their development occur progressively with the morphological development of the crown, root and apex of the teeth and/or their opportune sequence of emergence and eruption (Lewis et al., 2015); they are closely related to the development of the individual.

Age estimation is also important for identifying whether an individual is legally of age, when his/her rights must be defined for the purposes of criminal proceedings, accusations of a crime or asylum processes (Santiago et al., 2018). In general, at 18 years most individuals already have fully formed third molars, a fact which can assist in establishing the chronological age of young individuals (Santiago et al., 2018). The teeth are fully developed by the age of around 14 years, with the exception of the third molars, which complete their development at around 18 years (Bhat and Kamath, 2007). Thus, analysis of the maturation of third molars can be a helpful tool for forensic scientists in estimating the age of an individual and identifying whether he/she has reached the age of 16 or 18 years (Kanchan et al., 2021). A combination of the estimation of dental age with other indicators for estimating skeletal age, together with more specific population studies, can increase the precision of age estimation and help in legal investigation (Kanchan et al., 2021).

Different methods exist for assessing the maturity of teeth; one of these is the method of Demirjian et al. (1973), which is based on qualitative analysis and classifies the maturity of the tooth into one of 8 stages. This method was developed in French Canadian children and is widely employed due to its precision and ease of use. Studies carried out in Brazilian and Indian populations have shown that Demirjian's method of age estimation is reliable. It presents great precision and can identify differences between sides through identification of maturation stages (de Oliveira et al., 2012; Kachan et al., 2021).

One important aspect that has been reported in the literature is that the development stage of third molars at a given age may differ between populations (Meinl et al., 2007); it is therefore important to obtain specific information for each population. So far as we know, there are no pre-existing studies on determining the maturation stage of third molars in the Chilean population. The object of this study was to determine differences in maturation stages of the mandibular third molars (MTM) of Chilean individuals by age, sex, and side.

MATERIALS AND METHODS

We carried out a descriptive inferential, cross-sectional study. The sample was non-probabilistic, by convenience. All the patients involved in this study signed an informed consent which stated their free right to participate in the study or not, with no influence on their dental treatment. This study was carried out following Chilean laws and in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants and/or their legal guardians. The study was approved by the Scientific Ethics Committee of La Frontera University, Folio Nº120_21.

Using panoramic radiography, the maturation stage of 930 lower third molar teeth were analysed in patients aged between 9 and 24 years (432 males and 498 females), who received attention at the radiological centre of the Dental Teaching Attention Clinic of Universidad de La Frontera, Temuco, Chile, between December 2018 and December 2021. The mean age of the patients was 16.7 years (±4.31) for males and 16.8 years (± 4.47) for females, and a median age of 17 years for both sexes. In total, 465 mandibular third molars were analysed on the right

side (teeth 48) and 460 on the left side (teeth 38). Table 1 summarizes the distribution of MTM analysed by age range, by side and in total, between sexes.

Table 1. Distribution of analysed third molars by age range, by side and in total, between sexes.

Sex	Age	Analysed mandibular third molars		
		Right side (48)	Left side (38)	Total
Males	9 – 12	46	45	91
	13 – 18	92	95	187
	19 – 24	81	73	154
	Total	**219**	**213**	**432**
Females	9 – 12	57	56	113
	13 – 18	91	94	185
	19 – 24	101	99	200
	Total	**249**	**249**	**498**
Total		**468**	**462**	**930**

The following were excluded from the study: PR with any kind of distortion, artefacts in the image, or anatomical alterations; MTM with caries evident in the PR; mandibular second molars with distal caries evident in the PR; patients who, at the date of the radiographic examination, exhibited orthodontic apparatus in one or both dental arches; PR in which any indicator of prior orthognatic surgery or surgical resolution of mandibular fracture could be observed, such as presence of osteosynthesis plates, or radiographic evidence of cyst-like or tumoral lesions around the MTM.

The maturation stage of the MTM was classified from A to H, following the method established by Demirjian et al. (1973) (Fig. 1): Stage A: Initial calcification of the cusps; Stage B: One third of the crown is calcified; Stage C: Two thirds of the crown are calcified; Stage D: Complete calcification of the crown; Stage E: One third of the root is formed; Stage F: Two thirds of the root are formed; Stage G: Three thirds of the root are formed, the apex remains open; Stage H: Apical closure is complete. Before taking the analysis, the investigators were calibrated by an experienced radiologist.

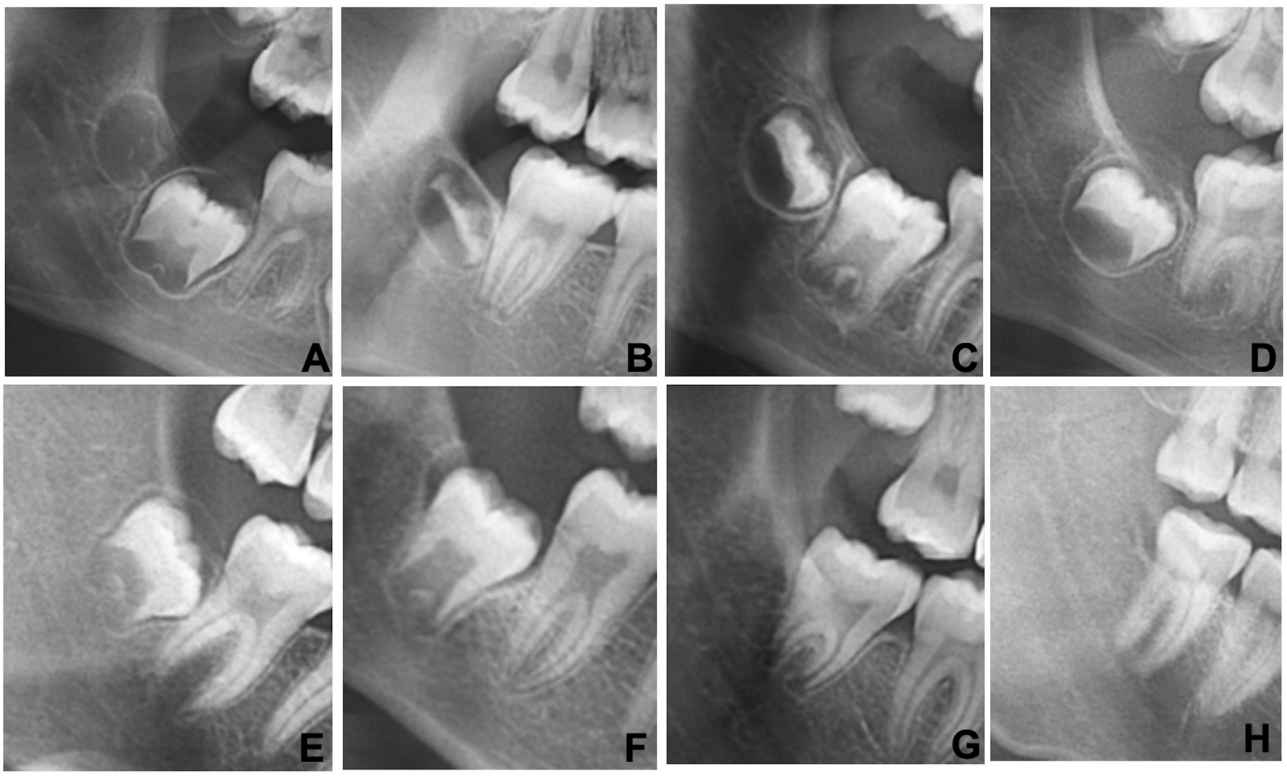

Fig. 1.- Maturation stage of the MTM according to the classification of Demirjian et al. **a.** Stage A: Initial calcification of the cusps; **b.** Stage B: One third of the crown is calcified; **c.** Stage C: Two thirds of the crown are calcified; **d**. Stage D: Complete calcification of the crown; **e.** Stage E: One third of the root is formed; **f.** Stage F: Two thirds of the root are formed; **g.** Stage G: Three thirds of the root are formed, the apex remains open; **h.** Stage H: Apical closure is complete.

Statistical analysis

Inter-observer agreement was assessed using the Kappa test. Association was classified as poor (0.0), slight (0.01-0.20), regular (0.21-0.40), moderate (0.41-0.60), substantial (0.61-0.80) or almost perfect (0.81-1.00) (Landis and Koch, 1977). The data collected were recorded in a Microsoft Office Excel spreadsheet. The normality of the data was analysed using the Kolmogorov-Smirnov test and normality graphs. The Mann-Whitney U test was used to assess differences between sexes, and to assess differences between sexes in MTM maturation stage by side. The correlation between age and maturation stage was analysed by Spearman's correlation coefficient. Pearson's chi-squared test was used to analyse the association between qualitative variables. Ordinal logistic regression (logit model) was used with a generalised linear model (GLM) to estimate the association

Table 2. Maturation states of right mandibular third molars (48) by age and sex.

Stage	Males					Females					p-value
	N	Minimum age	Maximum age	Mean age (SD)	Median age	N	Minimum age	Maximum age	Mean age (SD)	Median age	
A	7	9	19	11.00 (±3.60)	10.00	6	9	11	9.67 (±0.81)	9.50	0.731
B	20	9	12	10.00 (±0.97)	10.00	17	9	13	10.06 (±1.14)	10.00	1.000
C	15	9	15	11.20 (±1.96)	11.00	29	9	17	11.69 (±2.03)	11.00	0.481
D	17	9	18	13.41 (±2.78)	14.00	31	9	23	13.61 (±2.71)	13.00	0.888
E	15	10	19	15.53 (±2.35)	16.00	15	12	20	15.73 (±2.28)	16.00	0.935
F	23	14	22	16.48 (±2.15)	15.00	17	12	22	16.65 (±2.76)	17.00	0.935
G	46	13	24	18.00 (±2.44)	17.00	57	14	24	18.61 (±2.21)	18.00	0.134
H	76	16	24	20.54 (±2.33)	20.50	77	16	24	21.19 (±2.02)	22.00	0.089

Table 3. Maturation states of left mandibular third molars (38) by age and sex.

Stage	Males					Females					p-value
	N	Minimum age	Maximum age	Mean age (SD)	Median age	N	Minimum age	Maximum age	Mean age (SD)	Median age	
A	7	9	11	9.43 (±0.78)	9.00	6	9	11	9.50 (±0.83)	9.00	0.945
B	21	9	17	10.24 (±1.72)	10.00	14	9	15	10.21 (± 1.71)	10.00	0.678
C	10	9	13	11.20 (±1.47)	11.00	30	9	14	11.20 (±1.58)	11.00	1.000
D	20	9	17	12.75 (±2.46)	12.00	30	9	23	13.73 (±2.94)	13.00	0.239
E	13	13	19	15.92 (±1.70)	16.00	19	12	18	15.32 (±2.00)	15.00	0.520
F	21	14	19	15.86 (±1.76)	15.00	18	12	24	16.44 (±2.74)	16.50	0.477
G	45	13	24	17.56 (±2.27)	17.00	48	14	23	18.60 (±2.29)	18.00	0.014*
H	76	16	24	20.47 (±2.27)	20.00	84	16	24	20.95 (±2.03)	21.00	0.165

*Significantly difference between sexes for MTM's maturation stage "G" (significance threshold $p < 0,05$).

between the maturation stages of the MTM with sex, age and side (tooth 38 or tooth 48). The predictive variables were inserted individually in the model. The interaction between sex/age and the maturation stage of the MTM were also analysed. The data were analysed using SPSS 29.0 software (IBM), with a significance threshold of 5%. The graphs were constructed using SPSS 29.0 (IBM) and GraphPad Prism 10.0.3.

RESULTS

No statistically significant differences were found for age in the total sample (p=0.608). Analysis of age against maturation stage of the MTM presented a statistically significant difference between sexes for the variable 'age' only in Stage G, where the median age of females was higher (18 years) than that of males (16 years) (p=0.011). This maturation stage (Stage G) was also the only one that presented a statistically significant difference between sexes for the same variable; but only on the left side, where the median age of females was higher (18 years) than that of males (17 years) (p=0.014) (Table 2 and Table 3). The value of inter-observer agreement was over than 0.9 for all variables.

The lowest age of complete MTM maturation was 16 years in both sexes (Stage H). In 8% of individuals this stage occurred at an age of less than 18 years. For Stage D, 7.1% were aged 18 years or more; for Stage E, 21% were aged 18 years or more; for Stage F, 25.3% were aged 18 years or more and for Stage G, 51.5% were aged 18 years or more. For Stage G, an association was found between age and sex [$X^2(1) = 8.038$, p=0.005], the age of 18-24 years being associated with female sex (Fig. 2). No association was found for any other stage (p>0.05).

Table 4. Spearman's correlation between stage and age, by side and sex.

Sex	Tooth	Spearman's Rho	p-value	Classification
Males	48	0.821	<0.001	Significant, positive, high
Males	38	0.857	<0.001	Significant, positive, high
Females	48	0.861	<0.001	Significant, positive, high
Females	38	0.859	<0.001	Significant, positive, high

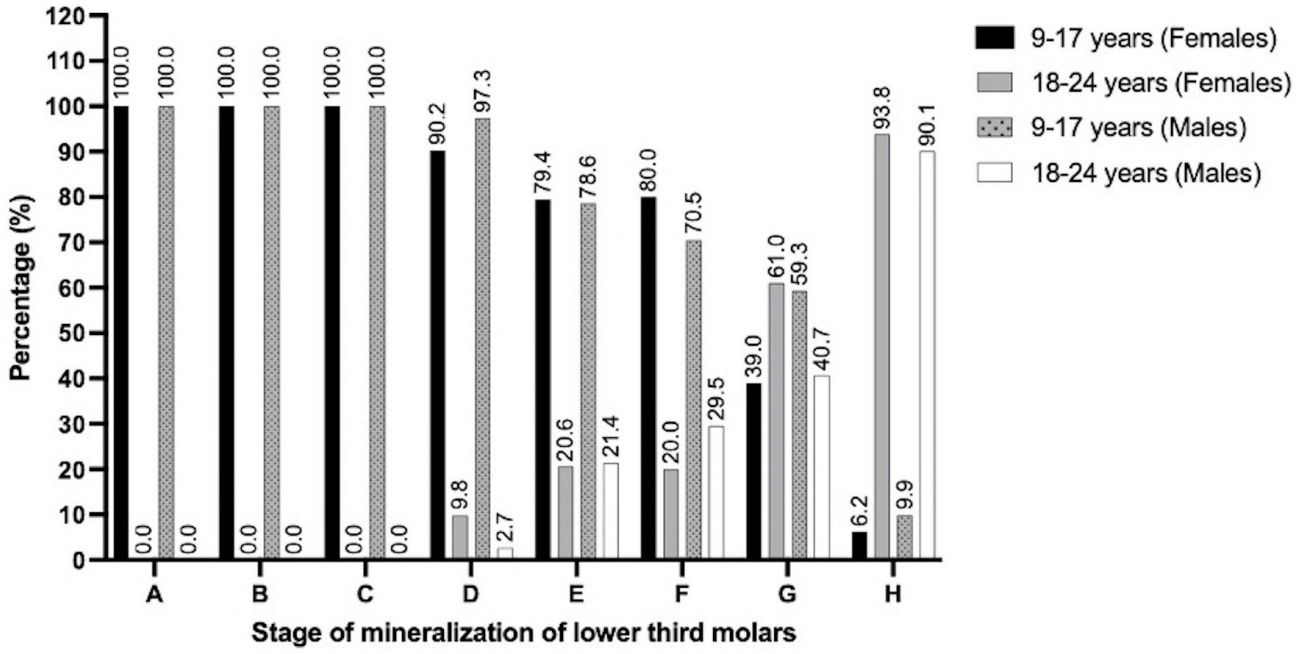

Fig. 2.- Maturation stage of the MTM by sex and age (age greater or less than 18 years).

A statistically significant difference was found between sexes by age only in Stages G (p=0.005) and H (p=0.029), where the median age of females was higher than that of males (Fig. 3).

The correlation between maturation stage and sex was significant, positive, and high for both sexes, in both tooth 48 and tooth 38, showing that increasing age and maturation stage are correlated (Table 4).

In both males and females, the maturation stage of the MTM was associated with the age of 18 years (females: [X^2(7) 294.557, p<0.001]; males: [X^2(7) 225.041, p<0.001]). The fit of the ordinal logistic regression model was significant [X^2(14)1170.700, p<0.001], showing that it has greater predictive power than the null model. Nagelkerke's pseudo R^2 explained 73.5% of the variations of the model.

The variables 'sex' and 'side' were not able to predict the maturation stage of the MTM (p=0.121 and p=0.215, respectively). The variables 'sex/age' and 'age' were shown to influence the stage of maturation of the MTM (p=0.006 and p=<0.001, respectively). The variable 'sex/age' presented predictive power only in individuals aged 11-12 years, where females were shown to have a 7.04 times greater likelihood of belonging to higher maturation stages than males (p=0.009). 'Age' was the only variable to influence the maturation stage in all the age bands (p<0.001).

DISCUSSION

In the present study, we analysed the maturation stage of MTM in Chilean individuals by age, sex and side, using the classification proposed by Demirjian et al. (1973). Previous studies have indicated that the maturation stage of third molars is not influenced by sex (de Oliveira et al., 2012; Khosronejad et al., 2017); the same was observed in the present study, except in younger individuals where the interaction 'sex/age' determined more advanced stages of maturity in females than in males. Our study also showed that the variable 'side' does not influence the maturation of MTM, indicating that it occurs similarly in both sides; a similar finding was reported by Meinl et al. (2007) in their study in an Austrian population.

In our study, Stage D of maturation (complete calcification of the crown) occurred at 13 years for the total sample; although females presented a lower median age for this stage, there was no

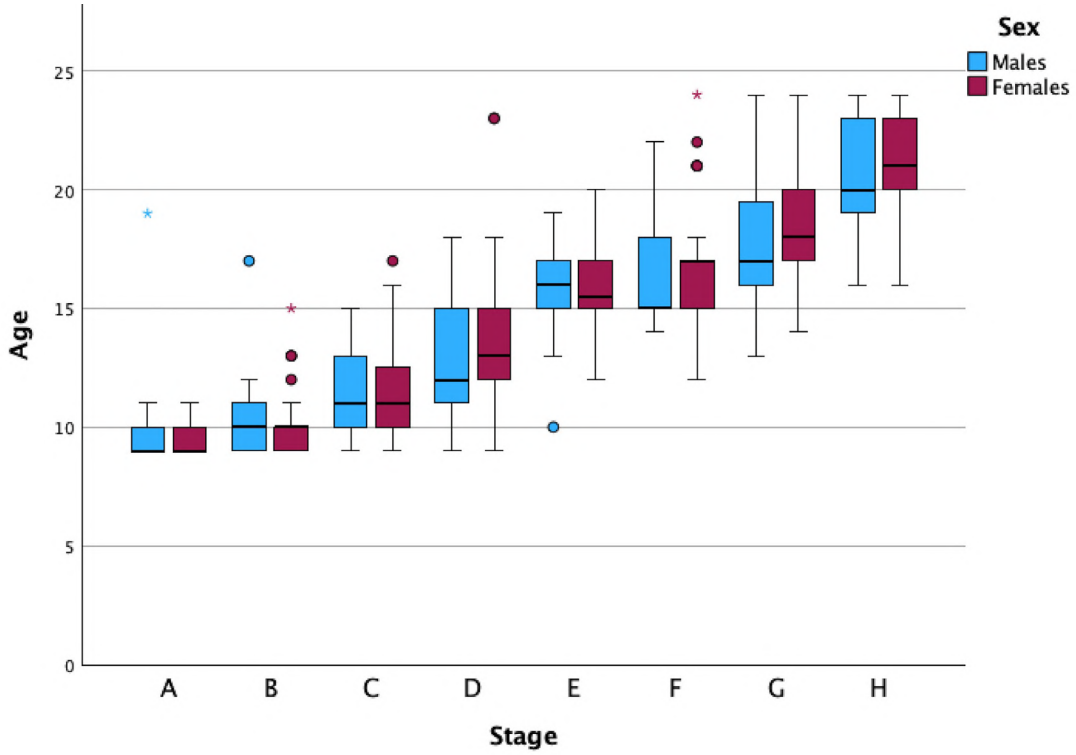

Fig. 3.- Box plot showing the relation between age and maturation stage of MTM. A. Males, B. Females. Outliers ( ) atypical data (*).

significant difference between sexes. De Oliveira et al. (2012) and Nicodemo et al. (1974) reported similar results for the Brazilian population. In the Austrian population, Stage D occurred at a more advanced age, in individuals aged 15.5 years (Meinl et al., 2007). Lewis et al. (2015) and de Oliveira et al. (2012) say that if an individual presents Stage D development of the third molar, he/she is unlikely to have reached the age of 18, similar to the findings of the present study.

Kachan et al. (2021) and de Oliveira et al. (2012) say that Stages F and G help to identify individuals aged over 16 years. In the present study, the median age for Stage F was 16 years (minimum 12 years); and for Stage G, 18 years (minimum 13 years). Thus, Chilean individuals with maturation Stage F or G are not always aged over 16 years, although this is the case in the majority of the sample.

The present study shows that both Stage G and Stage H individuals may be older than 18 years, similar to the findings reported by Khosronejad et al. (2017). However, our study showed that a larger number of females than males may present a chronological age of 18 years in Stage G. For Stage H, the two sexes present the same probability of being aged over 18 years.

Our results agree with studies reported previously, with only 8% of the individuals presenting complete maturation of the MTM before the age of 18. In the present study, the minimum age for Stage H was 16 years for both sexes, which is lower than the age reported in the literature. De Oliveira et al. (2012) reported that the minimum age for complete maturation was 17 years; however, in another study in Brazilians the age range was between 18 and 20.5 years (Nicodemo et al., 1974). Our results show that age can be correlated with maturation of the MTM, both showing a similar pattern of increase. Furthermore, analysis by multinomial logistic regression showed that age influenced all the maturation stages of the MTM. Our findings also agree with those reported by Lewis et al. (2012), when they state that individuals who presented complete root formation (Demirjian's Stage H) have probably attained legal majority (18 years). Analysis of stage H (complete apical closure) helps in assessing the maturity of the third molar and is a very precise indicator for identifying whether the individually is legally of age (Kanchan et al., 2021), being more sensitive in males than in females (Santiago et al., 2018). The probability that an individual with maturation of the third molar at Stage H has attained the age of 18 or over is 96.8% for males and 98.6% for females in the Brazilian population (de Oliveira et al., 2012), 99.5% and 99.3% respectively for Austrian males and females (Meinl et al., 2007), and greater than 94% for the population in India (Lewis et al., 2015). Lewis et al. (2015) reported that only 4.4% of individuals presented complete root development before the age of 18 years. Although various studies have used Demirjian's method for age estimation in different populations, this is the first study to analyse the precision of this method for this purpose in the Chilean population.

Our study presents certain limitations that should be considered. First, the study may not be representative of the whole Chilean population, since it was carried out only in individuals who attended the Dental Teaching Attention Clinic, La Frontera University, in Temuco (La Araucanía Region). Future studies should consider including individuals from other regions in order to obtain more solid results that can be used at national level. Secondly, it must be considered that no previous study was carried out on the accuracy of Demirjian's method in the Chilean population. However, this method has been described in the literature as being reliable for estimating age in different populations.

CONCLUSIONS

Demirjian's method of age estimation is simple and easy to apply and can be used as a support tool for the identification of Chilean individuals. Our results showed that for the contemporary Chilean population it is very unlikely that an individual is aged 18 years or over if the maturity of the MTM is at a stage prior to complete calcification of the crown (Stage D). On the other hand, it is highly probable that a Chilean individual with complete root formation of the MTM is legally of age, regardless of sex.

AUTHOR CONTRIBUTIONS

CL, NA: project development; CL, IG, FC: data collection; NA, NFD: data analysis; NA, NFD: manuscript

writing and manuscript editing; All authors have read and agreed to the published version of the manuscript.

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Ethical Approval

The informed consent was obtained from all participants and/or their legal guardians. The study was approved by the Scientific Ethics Committee of La Frontera University, Folio Nº120_21.

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