# Relation between the Stages of Root Calcification of Third Molars and Chronological Age of Peruvian People from 13 to 23 Years Old 

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#### Abstract

This study investigated the relationship between chronological age and third molar root development for a Peruvian population. In this retrospective analytical study, the sample comprised 1367 panoramic radiographs from people of known chronological age and sex. The root development was classified through Demirjian's stages. Intra-observer agreement was determined using Kappa statistics. Simple and multiple linear regression was used to predict the chronological age from the root development of the third molars.

Symmetry in root development of right and left third molars within the same jaw was $78.54 \%$ (Kappa: 0.72; p<0.05). However, there was only $57.94 \%$ concordance between the maxillary and mandibular third molars (Kappa: 0.47). Root formation was completed earlier in males than in females, in stages F, G and H. The probability that a person was at least 18 years old was high when their third molars were completely formed (stage H), and there was an increased probability that subjects with root development between stages A and E were underage.

These empirical probabilities and regression formulas can be provided for forensic and médicolegal purposes. The mean difference between chronological and dental age was about 1.50 years when any molar was used, this difference decreased to 1.39 years when four molars were assessed. Third molars root development occurred earlier in the Peruvian population than in the German, Japanese, South African and mixed American populations, but later than the Spanish population; it was similar to the Canadian, US Hispanic, and French-Canadian populations.

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## Introduction

Forensic experts make use of post mortem indicators that help with the identification process of skeletonized bodies with unknown identity. Once these indicators are established, they can be compared with ante mortem data gathered

[^0]from families with missing people. ${ }^{1}$
Age estimation is a key parameter for the identification process and both accuracy and reliability are required. ${ }^{2}$ The forensic relevance of these estimation data has dealt for the most part with the likelihood that a person of unknown age is 18 years or older. In Peru and other countries, this is the age one legally becomes an adult, and criminal consequences change significantly. Thus, age estimation is an important indicator for identification, especially if the child is a refugee of uncertain age. ${ }^{2}$

Several indicators are used to estimate the age of skeletonized human remains, such as the
early phases of morphological change in the sternal rib end and pubic symphysis, and dental development. ${ }^{3,4}$

Third molar root development is extensively used for age estimation in sub adults and young adults. Thus, third molar development in combination with skeletal development constitute an adequate method for estimating age. Different studies have found that third molar development was accurate and highly correlated with chronological age. ${ }^{5,6}$ Additionally, several researchers have demonstrated that dental development varies between different populations, making population-specific studies necessary. ${ }^{6}$

Objective to demonstrate the relationship between the root development of third molars and the chronological age of Peruvians from 13 to 23 years.

## Materials and methods

## Research Samples

This study consisted of 1747 panoramic radiographs taken at the Diagnostic Imaging Center in Lima, Peru during the year 2010, which were referred for varying clinical indications. This retrospective cross-sectional study was approved by the Postgraduate Unit of Universidad Nacional Mayor de San Marcos, Lima, Perú and respected the bioethical principles of medical research involving human subjects of the Declaration of Helsinki related to confidentiality and nonmaleficence. Informed consent was not required, as the study was retrospective; however, to ensure confidentiality, the data were coded and stored in an electronic device with a password to which only the principal investigator had access, and at the end of the study, the data were discarded. Sample size calculation was determined using 95\% confidence level, 8\% precision, and 3.21 standard deviation for age. The criteria for inclusion in the sample were: people from 13 to 23 years old (chronological age) including the date of birth (data provided by the patient) and date of the panoramic radiograph acquisition. Dates of birth were verified and confirmed in $68 \%$ of the study sample.

Exclusion criteria were: panoramic radiographs showing obvious dental pathology (abnormalities of dental pulp, alterations in number of teeth, alterations in size of teeth,
alterations in shape of teeth, abnormalities in position of teeth and defects of enamel and/or dentin), and image deformity affecting third molar visualization. The final sample was composed of 1367 panoramic radiographs. Of these, 580 were from male patients and 787 were from females. The age at exposure ranged from 13 to 23 years (Mean age: 17.69, SD 3.21) (Table 1). Panoramic radiographs were taken with a Planmeca Promax 2D (Planmeca, Helsinki, Finland) operated with a CCD sensor with exposure parameters of $76 \mathrm{kV}, 6 \mathrm{~mA}$ and 11 s exposure time.

|  | Age (decimal years) |  |  |
| :--- | :---: | :---: | :---: |
|  | Total | Males | Females |
| Sample | 1,367 | 580 | 787 |
| Mean | 17.691 | 17.590 | 17.766 |
| Std. Dev. | 3.218 | 3.060 | 3.329 |
| Std. Error | 0.087 | 0.127 | 0.119 |
| Minimum | 13.003 | 13.008 | 13.003 |
| Maximum | 23.981 | 23.959 | 23.981 |

Table 1. Age distribution by gender

## Research Methods

The panoramic radiographs were evaluated using the formation stages described by Demirjian et al., ${ }^{7}$ (from stages "A" to " H "). The observation was performed under standardized conditions: dimmed ambient light, with a $29.5-\mathrm{cm}$ monitor (Acer 1410-742G16n, Acer Inc, New Taipei City, Taiwan) with an effective resolution of $1366 \times 768$ pixels. The scores were determined by one observer (forensic dentist) with 15 years of experience in identifying corpses and skeletonized remains, who was also familiar with computer-based procedures. The development of the molars contained in each radiograph were evaluated one by one, starting with the upper right third molar, followed by the lower right, upper left and lower left third molars. This sequence avoided possible biases when assigning a Demirjian developmental stage, since the roots of the upper molars present anatomical differences compared with the lower molars.

## Statistical methods

For inferential statistical analysis, stages were assigned a numeric value, thus stage A: 1, stage $B: 2$, stage C: 3 , stage $D: 4$, stage $E: 5$, stage $F$ : 6 stage $G: 7$ and stage $H: 8$. To assess reliability, 96 randomly selected panoramic

[^1]radiographs were re-examined 3 months after the initial examination by the same observer, and intra-observer agreement was determined using Kappa statistics. The same coefficient was used to determine the agreement between right and left maxillary and mandibular third molar development. Pearson correlation was applied to establish the degree of relationship between third molar root development stages and chronological age. Simple and multiple linear regression was used to predict the chronological age from the root development of the third molars. The simple regression took into account only one molar at a time, while the multiple regression took into account the four third molars. Data analysis was performed using SPSS 25.0 (SPSS, Chicago, IL, USA).

|  | Stage -sex |  |  |  |  |  |  |  |  | N | Mean <br> Age | Std. <br> dev. | Std. <br> error | Minimum | Maximum | 95\% confidence |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | 11 | 13.925 | 1.115 | 0.336 | 13.008 | 16.654 | 13.266 |  |  |  |  |  |  |  |  |
| B-Males | 7 | 15.051 | 1.797 | 0.679 | 13.085 | 18.652 | 13.720 | 14.584 |  |  |  |  |  |  |  |  |
| B-Females | 34 | 14.275 | 1.354 | 0.232 | 13.033 | 17.945 | 13.820 | 14.730 |  |  |  |  |  |  |  |  |
| C-Males | 3.262 | (years) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| C-Females | 42 | 13.878 | 0.946 | 0.146 | 13.041 | 16.561 | 13.592 | 14.164 |  |  |  |  |  |  |  |  |
| D-Males | 62 | 14.302 | 1.143 | 0.145 | 13.061 | 18.039 | 14.018 | 14.586 |  |  |  |  |  |  |  |  |
| D-Females | 97 | 14.424 | 1.278 | 0.13 | 13.003 | 18.37 | 14.169 | 14.679 |  |  |  |  |  |  |  |  |
| E-Males | 74 | 15.421 | 1.423 | 0.165 | 13.02 | 19.756 | 15.098 | 15.744 |  |  |  |  |  |  |  |  |
| E-Females | 112 | 15.35 | 1.643 | 0.155 | 13.055 | 21.452 | 15.046 | 15.654 |  |  |  |  |  |  |  |  |
| F-Males | 45 | 16.311 | 1.35 | 0.201 | 13.263 | 19.331 | 15.917 | 16.705 |  |  |  |  |  |  |  |  |
| F-Females | 58 | 16.562 | 1.624 | 0.213 | 13.444 | 20.901 | 16.145 | 16.979 |  |  |  |  |  |  |  |  |
| G-Males | 26 | 17.608 | 1.575 | 0.309 | 14.654 | 20.167 | 17.002 | 18.214 |  |  |  |  |  |  |  |  |
| G-Females | 24 | 19.59 | 2.411 | 0.492 | 14.871 | 23.808 | 18.626 | 20.554 |  |  |  |  |  |  |  |  |
| H-Males | 220 | 20.216 | 2.013 | 0.136 | 14.915 | 23.959 | 19.949 | 20.483 |  |  |  |  |  |  |  |  |
| H-Females | 234 | 20.724 | 2.21 | 0.144 | 14.28 | 23.981 | 20.442 | 21.006 |  |  |  |  |  |  |  |  |

* Tooth 18 = maxillary right third molar

Table 2. Age distribution by Demirjian's stage and gender (tooth 18).

|  | Stage - sex |  |  |  |  |  |  |  |  | Age (years) |  |  |  |  |  |  |  |  | N | Mean <br> Age | Std. <br> dev. | Std. <br> Error | Minimum | Maximum | 95\% confidence |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | B-Males | 12 | 13.781 | 0.601 | 0.173 | 13.008 | 14.86 | 13.442 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Lower limit | Upper limit |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| B-Females | 8 | 14.588 | 2.285 | 0.808 | 13.011 | 19.685 | 13.004 | 16.172 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| C-Males | 25 | 13.988 | 0.863 | 0.173 | 13.085 | 16.457 | 13.649 | 14.327 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| C-Females | 49 | 13.968 | 1.05 | 0.15 | 13.041 | 17.326 | 13.674 | 14.262 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D-Males | 66 | 14.322 | 1.138 | 0.14 | 13.061 | 17.841 | 14.048 | 14.596 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| D-Females | 97 | 14.441 | 1.224 | 0.124 | 13.003 | 18.052 | 14.198 | 14.684 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| E-Males | 73 | 15.277 | 1.388 | 0.162 | 13.02 | 19.619 | 14.959 | 15.595 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| E-Females | 103 | 15.252 | 1.632 | 0.161 | 13.055 | 20.594 | 14.936 | 15.568 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F-Males | 31 | 16.205 | 1.505 | 0.27 | 13.131 | 19.46 | 15.676 | 16.734 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F-Females | 63 | 16.817 | 1.585 | 0.2 | 13.444 | 21.052 | 16.425 | 17.209 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| G-Males | 21 | 18.009 | 1.774 | 0.387 | 15.318 | 23.444 | 17.250 | 18.768 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| G-Females | 27 | 19.16 | 2.077 | 0.4 | 15.732 | 23.655 | 18.376 | 19.944 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| H-Males | 225 | 20.101 | 2.073 | 0.138 | 14.37 | 23.959 | 19.831 | 20.371 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| H-Females | 251 | 20.809 | 2.212 | 0.14 | 14.28 | 23.981 | 20.535 | 21.083 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

* Tooth 28 = maxillary left third molar

Table 3. Age distribution by Demirjian's stage and gender (tooth 28).

## Results

A total of 4370 third molars were evaluated. For males, 316 panoramic radiographs showed all third molars, 139 panoramic radiographs had
three third molars, 99 panoramic radiographs had two third molars and 26 panoramic radiographs had only one third molar. For females, 365 panoramic radiographs had four third molars, 210 panoramic radiographs contained three third molars, 163 panoramic radiographs presented two third molars, and 49 panoramic radiographs had a single third molar.

The intra-observer error was calculated, showing a good agreement between the first and second classification ( $\mathrm{k}=0.77$ ).

|  | Stage - sex |  |  |  |  |  |  |  |  | N | Mean <br> Age | Std. <br> dev. | Std. <br> error | Minimum | Maximum | 95\% confidence |  | L | Lower limit | Upper limit |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| A-Males | 4 | 13.56 | 0.44 | 0.22 | 13.049 | 13.954 | 13.129 | 13.991 |  |  |  |  |  |  |  |  |  |  |  |  |
| A-Females | 2 | 13.637 | 0.839 | 0.593 | 13.044 | 14.23 | 12.475 | 14.799 |  |  |  |  |  |  |  |  |  |  |  |  |
| B-Males | 8 | 13.75 | 0.742 | 0.262 | 13.033 | 14.874 | 13.236 | 14.264 |  |  |  |  |  |  |  |  |  |  |  |  |
| B-Females | 5 | 14.537 | 2.345 | 1.049 | 13.011 | 18.652 | 12.481 | 16.593 |  |  |  |  |  |  |  |  |  |  |  |  |
| C-Males | 52 | 13.933 | 0.781 | 0.108 | 13.008 | 16.457 | 13.721 | 14.145 |  |  |  |  |  |  |  |  |  |  |  |  |
| C-Females | 61 | 13.939 | 1.007 | 0.129 | 13.041 | 17.326 | 13.686 | 14.192 |  |  |  |  |  |  |  |  |  |  |  |  |
| D-Males | 36 | 14.847 | 1.424 | 0.237 | 13.02 | 17.948 | 14.382 | 15.312 |  |  |  |  |  |  |  |  |  |  |  |  |
| D-Females | 81 | 14.682 | 1.462 | 0.162 | 13.003 | 20.471 | 14.364 | 15.000 |  |  |  |  |  |  |  |  |  |  |  |  |
| E-Males | 73 | 15.228 | 1.477 | 0.173 | 13.131 | 20.288 | 14.889 | 15.567 |  |  |  |  |  |  |  |  |  |  |  |  |
| E-Females | 122 | 15.838 | 2.011 | 0.182 | 13.09 | 23.485 | 15.481 | 16.195 |  |  |  |  |  |  |  |  |  |  |  |  |
| F-Males | 71 | 16.506 | 1.372 | 0.163 | 13.531 | 19.507 | 16.187 | 16.825 |  |  |  |  |  |  |  |  |  |  |  |  |
| F-Females | 96 | 17.329 | 2.121 | 0.216 | 13.31 | 23.846 | 16.906 | 17.752 |  |  |  |  |  |  |  |  |  |  |  |  |
| G-Males | 83 | 18.483 | 1.575 | 0.173 | 15.027 | 23.444 | 18.144 | 18.822 |  |  |  |  |  |  |  |  |  |  |  |  |
| G-Females | 125 | 19.955 | 2.141 | 0.192 | 14.28 | 23.896 | 19.579 | 20.331 |  |  |  |  |  |  |  |  |  |  |  |  |
| H-Males | 161 | 20.95 | 1.695 | 0.134 | 16.956 | 23.959 | 20.687 | 21.213 |  |  |  |  |  |  |  |  |  |  |  |  |
| H-Females | 141 | 21.613 | 1.902 | 0.16 | 16.145 | 23.981 | 21.299 | 21.927 |  |  |  |  |  |  |  |  |  |  |  |  |

* Tooth $38=$ mandibular left third molar

Table 4. Age distribution by Demirjian's stage and gender (tooth 38).

|  | Stage - sex |  |  |  |  |  |  |  |  | N | Mean <br> Age | Std. <br> dev. | Std. <br> Error | Minimum | Maximum | 95\% confidence |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | 8 | 14.273 | 2.266 | 0.801 | 13.033 | 19.816 | 12.703 |  |  |  |  |  |  |  |  |
| A-Males | 3 | 14.377 | 1.413 | 0.816 | 13.044 | 15.858 | 12.778 | 15.843 |  |  |  |  |  |  |  |  |
| A-Females | 5 | 13.817 | 0.639 | 0.286 | 13.124 | 14.86 | 13.256 | 14.378 |  |  |  |  |  |  |  |  |
| B-Males | 6 | 14.78 | 2.443 | 0.997 | 13.148 | 19.685 | 12.826 | 16.734 |  |  |  |  |  |  |  |  |
| B-Females | 48 | 13.999 | 0.826 | 0.119 | 13.008 | 16.457 | 13.766 | 14.232 |  |  |  |  |  |  |  |  |
| C-Males | 48 | Lower limit | Upper limit |  |  |  |  |  |  |  |  |  |  |  |  |  |
| C-Females | 67 | 13.829 | 0.728 | 0.089 | 13.041 | 16.561 | 13.655 | 14.003 |  |  |  |  |  |  |  |  |
| D-Males | 34 | 14.266 | 0.883 | 0.151 | 13.02 | 16.786 | 13.970 | 14.562 |  |  |  |  |  |  |  |  |
| D-Females | 94 | 14.838 | 1.472 | 0.152 | 13.003 | 20.471 | 14.540 | 15.136 |  |  |  |  |  |  |  |  |
| E-Males | 67 | 15.501 | 1.533 | 0.187 | 13.131 | 19.89 | 15.134 | 15.868 |  |  |  |  |  |  |  |  |
| E-Females | 116 | 15.649 | 1.881 | 0.175 | 13.09 | 21.452 | 15.306 | 15.992 |  |  |  |  |  |  |  |  |
| F-Males | 86 | 16.647 | 1.585 | 0.171 | 13.531 | 22.046 | 16.312 | 16.982 |  |  |  |  |  |  |  |  |
| F-Females | 91 | 17.075 | 1.875 | 0.197 | 13.444 | 22.474 | 16.689 | 17.461 |  |  |  |  |  |  |  |  |
| G-Males | 80 | 18.712 | 1.859 | 0.208 | 15.027 | 23.444 | 18.304 | 19.120 |  |  |  |  |  |  |  |  |
| G-Females | 115 | 19.775 | 2.105 | 0.196 | 14.28 | 23.846 | 19.391 | 20.159 |  |  |  |  |  |  |  |  |
| H-Males | 164 | 20.814 | 1.732 | 0.135 | 16.956 | 23.959 | 20.549 | 21.079 |  |  |  |  |  |  |  |  |
| H-Females | 165 | 21.577 | 1.783 | 0.139 | 16.389 | 23.981 | 21.305 | 21.849 |  |  |  |  |  |  |  |  |

*Tooth $48=$ mandibular right third molar
Table 5. Age distribution by Demirjian's stage and gender (tooth 48).

Tables 2-5 show the mean age, standard deviation and standard error of the age as well as the confidence limits for every Demirjian mineralization stage in relation to age and sex. In these tables, the age range for each Demirjian's stage is quite wide, e.g., Table 2 shows that a woman whose third molar is in stage $G$ may be between 14.87 to 23.8 years and the confidence interval was between 18.62 to 20.55 years.

For tooth 18, the mean age of stages D, E and $F$ were very similar for both genders, while in stages G and H , the age difference between ages was greater (i.e. 1.9 and 0.5 years younger for men, respectively). For tooth 28 , likewise, the mean age for stages $\mathrm{C}, \mathrm{D}$ and E were very similar for both genders, while in stages $F, G$ and H , the age difference between ages was also greater ( $0.61,1.15$ and 0.7 years younger for men, respectively). Mandibular molars also showed a sexual dimorphism: for tooth 38, the mean age of stages $A, C$ and $D$ were very similar for both genders, while in stages E, F, G and H, the age difference was greater ( $0.61,0.82,1.47$ and 0.66 years younger for men, respectively). Likewise, for tooth 18 the mean age for stages A, $C$ and $E$ were very similar for both genders, while in stages $F, G$ and $H$, age difference was greater ( $0.43,1.06$ and 0.76 years younger for men, respectively). In summary, the formation of roots occurred earlier in males than in females for stages F, G and H; while in earlier stages it was similar for both genders.

The percentile distribution of the age at attainment of stages of third molar formation also shows sexual dimorphism: from the 25th to the 90th percentile, males reached third molar root development stages earlier than females. At the 50th percentile females reached and surpassed legal adult age ( 18 years in Peru) at stage G. Mandibular third molars showed the same tendency, but unlike maxillary molars, it was observed that females turned 18 at stage $F$ (75th percentile). In the case of males, the upper third molars reached adulthood at the 75th percentile when their development was at stage G, and the mandibular molars at the 90th percentile when their development was at stage H .

Tables 2-5 also show differences between root development of upper and lower third molars, since maxillary molars reached a root development stage before mandibular molars. This difference is more pronounced when comparing the mean age of right third molars (tooth 18 vs. 48) as well as comparing the mean age of left third molars (tooth 28 vs. 38), mainly from stage $F$ to stage $H$. There was greater agreement when comparing the mean age of right and left maxillary third molars and right and left mandibular third molars, mainly from stage $D$ to stage H . No significant difference was found between the root development of the upper third molars, as well as between the development of
the mandibular third molars. The symmetry between root development stages of maxillary right and left third molars was 79.8\% and symmetry in the mandible was $77.3 \%$. The overall percentage of concordance, considering both arcs, was $78.5 \%(\kappa=0.72 p<0.05)$. On the other hand, only $57.9 \%$ of maxillary and mandibular third molars had the same development ( $\kappa=0.47$ ).

| Stage | Tooth 18 |  | Tooth 28 |  | Age |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | Males | Females | Males | Females |  |
| A | - | 100 | - | 100 | $<18$ |
|  | - | 0 | - | 0 | $\geq 18$ |
| B | 100 | 85.71 | 100 | 87.5 | $<18$ |
|  | 0 | 14.29 | 0 | 12.5 | $\geq 18$ |
| C | 100 | 100 | 100 | 100 | $<18$ |
|  | 0 | 0 | 0 | 0 | $\geq 18$ |
| D | 98.39 | 97.94 | 100 | 98.97 | $<18$ |
|  | 1.61 | 2.06 | 0 | 1.03 | $\geq 18$ |
| E | 95.95 | 95.54 | 97.26 | 94.17 | $<18$ |
|  | 4.05 | 4.46 | 2.74 | 5.83 | $\geq 18$ |
| F | 91.11 | 81.03 | 83.87 | 82.54 | $<18$ |
|  | 8.89 | 18.97 | 16.13 | 17.46 | $\geq 18$ |
|  | 42.308 | 29.167 | 57.14 | 37.04 | $<18$ |
| H | 13.18 | 14.53 | 42.86 | 62.96 | $\geq 18$ |
|  | 86.82 | 85.47 | 84.56 | 11.95 | $<18$ |

Table 6. Percent probability of an individual being at least 18 years old by Demirjian's stage and sex (teeth 18 and 28).

| Stage | Tooth 38 |  | Tooth 48 |  | Age |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | Males | Females | Males | Females |  |
| A | 100 | 100 | 87.5 | 100 | $<18$ |
|  | 0 | 0 | 12.5 | 0 | $\geq 18$ |
| B | 100 | 80.00 | 100 | 83.33 | $<18$ |
|  | 0 | 20.00 | 0 | 16.67 | $\geq 18$ |
| C | 100 | 100 | 100 | 100 | $<18$ |
|  | 0 | 0 | 0 | 0 | $\geq 18$ |
| D | 100.00 | 96.30 | 100 | 96.81 | $<18$ |
|  | 0 | 3.70 | 0 | 3.19 | $\geq 18$ |
| E | 94.52 | 86.07 | 94.03 | 90.52 | $<18$ |
|  | 5.48 | 13.93 | 5.97 | 9.48 | $\geq 18$ |
|  | 11.27 | 64.58 | 35.42 | 17.44 | 67.03 |
| $<18$ |  |  |  |  |  |
| G | 38.554 | 21.6 | 37.50 | 23.97 | $\geq 18$ |
|  | 61.446 | 78.4 | 62.50 | 76.52 | $\geq 18$ |
| H | 3.73 | 5.67 | 4.88 | 4.24 | $<18$ |
|  | 96.27 | 94.33 | 95.12 | 95.76 | $\geq 18$ |

Table 7. Percent probability of an individual being at least 18 years old by Demirjian's stage and sex (teeth 38 and 48).

Tables 6 and 7 show the probability that a person is of legal adult age (18 years) with respect to the development of maxillary and mandibular third molars for males and females. The probability that a person was at least 18 years old was high when their third molars were completely formed. There was an increased probability that subjects with root development between stages D and E were underage. The correlation analysis between each third molar and chronological age was around 0.8 for the total sample (male and female) and the coefficient of determination (r2) was around 0.6. Simple and multiple regression analysis allowed finding a formula for each of the third molars and a formula that included the four third molars, which were derived from the data contained in Table 8. Thus, multiple linear regression using the best regression model improved the prediction of chronological age by this formula:
Age $=7.69+0.55(\mathrm{t} 18)+0.69(\mathrm{t} 38)+0.37(\mathrm{t} 48)$.

* $t=$ tooth

The formula above can be used with corpses and human skeletons whose sexes cannot be determined, for example, when skeletonized human remains are very damaged with absent sexual indicators.

The best regression model analysis for males and females is shown below.
Male:
Age $=8.24+0.49(\mathrm{t} 18)+1.02(\mathrm{t} 38) \quad * \mathrm{t}=$ tooth
Female:
Age $=7.20+0.60(\mathrm{t} 28)+0.59(\mathrm{t} 38)+0,56(\mathrm{t} 48)$

* $\mathrm{t}=$ tooth

| Simple linear regression | Intercept | Regression Coefficient | F | r | $\mathrm{r}^{2}$ | Resid | $\mathrm{p}^{\star}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Tooth 18 | 8.95 | 1.40 | 2027.00 | 0.81 | 0.66 | 1.53 | <0.05 |
| Tooth 28 | 8.86 | 1.42 | 2106.62 | 0.82 | 0.67 | 1.52 | < 0.05 |
| Tooth 38 | 8.78 | 1.50 | 2215.00 | 0.82 | 0.66 | 1.51 | < 0.05 |
| Tooth 48 | 8.96 | 1.47 | 2313.93 | 0.82 | 0.67 | 1.49 | < 0.05 |
| Multiple linear Regression |  | Regression Coefficient | F | r | $\mathrm{r}^{2}$ |  | $\mathrm{p}^{*}$ |
| Constant |  | 7.67 | 505.83 | 0.87 | 0.75 |  | $<0.05$ |
| Tooth 18 |  | 0.43 |  |  |  |  | $<0.05$ |
| Tooth 28 |  | 0.18 |  |  |  | 1.39 | 0.15 |
| Tooth 38 |  | 0.67 |  |  |  |  | < 0.05 |
| Tooth 48 |  | 0.34 |  |  |  |  | <0.05 |
| Best regression model |  | Regression Coefficient | F | r | $\mathrm{r}^{2}$ |  | $\mathrm{p}^{*}$ |
| Constant |  | 7.69 | 672.763 | 0.87 | 0.75 |  | $<0.05$ |
| Tooth 38 |  | 0.69 |  |  |  |  | $\begin{gathered} \ll \\ 0.05 \end{gathered}$ |
| Tooth 18 |  | 0.55 |  |  |  |  | <0.05 |
| Tooth 48 |  | 0.37 |  |  |  |  | <0.05 |

* $p<0.05$ implies significance

Table 8. Correlation and regression coefficients between Demirjian's stages and chronological age.

Accuracy to predict age was evaluated through the difference between chronological and dental age, this latter based on the degree of development of the third molars. This difference was positive for stages $C, D$ and $H$ (dental age was below chronological age), but negative for stages E, F and G (dental age was above chronological age). Tooth 18 showed minor differences between chronological and dental age (Table 8). When the sign of the difference was ignored - absolute difference -, the average difference between chronological age and dental age was about 1.5 years. However, the multiple regression analysis reveals a statistical improvement in the prediction of chronological age when the right third molars and the upper left third molar were used (Table 8), since the difference decreased to 1.39 years.

Finally, Table 9 shows mean ages and standard deviations per each Demirjian stage from prior studies within several countries. Three aspects are highlighted: (1) the similarity between mean ages of the Peruvian population and the Canadian, US Hispanic, and FrenchCanadian populations. (2) The Peruvian population reached the Demirjian stages before the German, Japanese, South African and American (white 80\%, 19\% were black and 1 \% consisted of other races or was unspecified) populations. This difference was greater with respect to the German and Japanese populations but more subtle with respect to the American and South African populations. (3) The formation of the roots of the third molars occurred at earlier ages in the Spanish population than the other populations. However, the difference between the mean ages per stage of the Peruvian and Spanish populations was minimal.

## Discussion

Several studies carried out in different countries use the development of third molars to predict the age of people from the midadolescence to the early 20's. The results of these studies can be applied to different forensic contexts such as the identification of people and corpses whose identity is unknown. The correlation between the root development stage and the chronological age was very good in our study, the determination coefficient for every molar was above 0.6 and the determination coefficient was above 0.7 (r2=0.76) when using
the four third molars for multiple regression. Other authors such as Mincer et al,. ${ }^{8}$ Prieto et al., ${ }^{9}$ Engström et al., ${ }^{10}$ and Demisch et al., ${ }^{11}$ showed determination coefficients around those found in our study. Another important aspect was the accuracy, that is to say, the difference between chronological age (decimal age) and dental age (root development stages). This difference ranged between 1.49 and 1.53 years using the simple linear regression formula for each third molar. However, a difference of 1.39 years was obtained when using the best regression model (four thirds molars); that is an accuracy increase of 0.12 years. This result does not represent a significant increase when estimating the age of an unknown person. Even so, the results obtained in this research were similar to those obtained by Mincer et al., ${ }^{8}$ and Arany et al., ${ }^{12}$ They reported an absolute difference around 1.6 years. Solary \& Abramovitch ${ }^{13}$ reported a difference of 3 and 2.6 years for females and males, respectively. These last figures are very different from those obtained in this research. The highest accuracy was reported by Prieto et al., ${ }^{9}$ they found a difference of -0.10 for left lower third molars and 0.07 for right lower third molars.

Our research revealed the tendency of males to reach development stages before females. This finding has been shared by other researchers which obtained similar results. ${ }^{3,8,9,12-}$ 18 However, Lutalo et al., ${ }^{19}$ This found no significant differences between the sexes, which disagrees with our research peculiarity was important when developing the regression formula for males and females. Our results showed that males reached stage $D$ at the age of 14.43 and females at the age of 14.59 which is similar to the ages obtained by Bolaños et al., who found complete crown formation of third molars at the age of $14 .{ }^{20}$ However, her results were classified according to the Nolla method. Although their research aimed to predict the presence or absence of mandibular third molars, its findings regarding the age at which the complete formation of the crown and the entire length of root occurs (18 years) were similar to those obtained in this research. Likewise, other studies showed similar results in relation to the complete formation of crown and root. ${ }^{8,9,12,13,20-25}$ The 14 years is important in our legislation because adolescents from this age can enter into sexual relations with other adolescents of similar
ages without being penalized. The 18 years of age also has equal importance that will be seen in the next paragraph.

Our research also showed that the probability that an individual is an adult increase when the last molars have completed their development (stage H). Nevertheless, there will always be the possibility to find cases which do not meet this condition. Similarly, the probability that a person is under 18 also increases when its wisdom teeth are in stages A, B, C, D and E. Thus, Mincer et al., ${ }^{8}$ concluded that if an individual shows a development between stages A and D, the probability to be $\geq 18$ years old is low; but if root apices are closed (H stage), the probability to be adult ( $\geq 18$ years) increases. Other researchers agreed with Mincer et al., ${ }^{8}$ when they mention that a fully formed third molar indicates that it is a person over 18 years of age. ${ }^{18,26,27}$ Prieto et al., obtained similar results with respect to age prediction in people around 18 years old; but, unlike Mincer et al., ${ }^{9}$ they mentioned that the probability that a person was under the age of 18 coincided when root development was between A and E stages. Solary \& Abramovitch mentioned that people whose third molars are in stages D, E and F are more likely to be younger than 18 years old. However, this study subdivided F and G stages into two stages ( $F$ and $F 1, G$ and $G 1$ ). This allowed him to conclude that from stage F1 the probability that a person is under 18 decreases dramatically. ${ }^{13}$ In the Japanese population, Arany et al., ${ }^{12}$ deduced that people in stages A, B, C and $D$ are under 20; but if a person shows root development in stage $F$, there is $97 \%$ of probability that it is above 14 years. Also, if roots are fully developed, there is a $99 \%$ probability that a person is $\geq 16$ years old and $98 \%$ probability that it is $\geq 18$ years old. Therefore, it is important to take into account that there will always be a margin of error that opens the probability to find cases which do not match the statistical predictions found by several researchers, as well as in this investigation. ${ }^{28}$

Liversidge reported about people of certain age groups with extensive dental maturity scores (classified according to Demirjian's stages) and people with a dental maturity score covering a wide range of age. ${ }^{29}$ In other words, it is possible to find young people whose teeth are in the last maturity stages and older people whose teeth are in the first stages. This characteristic found by

Liversidge in her study sample (9,371 people from 2 to 18 years old) is consistent with our research, in which we found 17 -year-old people with A, B and C development stages. Although Liversidge's research did not work with wisdom teeth, our investigation found third molars with the first root development stages in people under 13 years old.

When comparing mean ages of this population with other populations (Table 9), many similarities and differences were found; this finding was also reported by Prieto et al., ${ }^{9}$ The most obvious difference was between the Peruvian and Japanese population ranging from 0.92 to 3.60 years, ${ }^{12}$ this may be construed as a difference in dental maturity between these populations or maturity patterns for each population. However, Liversidge \& Marsden concluded that characteristics of a reference sample such as sample size, shape, range of the age distribution and selection of radiographs are more important than the ethnic groups or populations. ${ }^{24,25}$ The research - conducted by Liversidge - that included an extensive database composed by radiographs of 2-to-18-year-old children ( 4,710 males and 4,661 females) from Australia, Belgium, Canada, England, Finland, France, Sweden and South Korea. This investigation showed a wide range of age for each of Demirjian's stages. It also stated that describing dental formation at the population level requires a large sample with a wide range of ages that includes children in different degrees of development: regular, advanced and slow. Her research found - in 7 years-old children - a large number of different Demirjian's stages for the same age ( 7 years-old). ${ }^{29}$ This fact explains the wide age ranges for every Demirjian's stage on our investigation (tables 2-5). Accordingly, the differences mentioned above should not be interpreted as different maturity patterns, since the high age variation for a particular development stage is a pathognomonic characteristic of the population, for example, an eighteen years-old person with an immature dentition. These two conditions, mentioned in this and the previous paragraph, are not found very often in samples collected by different researchers. However, this does not mean that there are different maturity patterns in the world.

Therefore, it is highly likely that future studies, using a large sample of Peruvian data, will find similar results to Liversidge's research
and to our research. ${ }^{30}$
Another important finding on third molar development was the right-left symmetry of maxillary third molars and the right-left symmetry of mandibular third molars. However, when comparing the symmetry of root development antagonist molars, only $58 \%$ of the cases exhibited the same grade of root development. Similar results on symmetry of development of third molars were reported by other authors. ${ }^{3,9,12,13}$ These findings indicate that all four molars (18, 28, 38, and 48) should be used to predict age more accurately in an unidentified person or corpse. ${ }^{31,32}$

This research was performed on 1367 panoramic radiographs distributed in age groups from 13 to 23 years old. Although an attempt was made to maintain equity in this distribution, the highest number of radiographs corresponded to 13 -year-old children (16.09\%) and the lowest number of them to 22 and 23 -year-old young adults ( 6.22 and $6.36 \%$ respectively). This could constitute a bias whose magnitude cannot be calculated with certainty. On the other hand, the panoramic radiographs were obtained from apparently healthy people. However, it is not possible to determine if there were people who have suffered diseases that alter dental development, which could become another bias derived from this last condition. The points described above should be considered as limitations of this investigation that forensics should keep in mind before applying them in their cases. ${ }^{33}$

## Conclusion

Our findings confirm a positive correlation between chronological age and the development of the third molars root that allowed us to find the regression formulas aimed at estimating the age of unidentified persons and bodies between 13 and 23 years of age.

## Future Scope / Clinical Significance

This study provides some future scopes/clinical sigificances, such as: an age range for each stage of root development for forensic purposes, root development of maxillary third molars occurred before mandibular third molars, stages $\mathrm{A}-\mathrm{E}$ and stage H indicate with high probability whether a person is underage or overage, and this is an inexpensive, non-invasive method for estimating age.

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## Ethical policy and institutional review board statement

This retrospective cross-sectional study was approved by the Postgraduate Unit of Universidad Nacional Mayor de San Marcos, Lima, Perú and respected the bioethical principles of medical research involving human subjects of the Declaration of Helsinki related to confidentiality and non-maleficence. Informed consent was not required, as the study was retrospective; however, to ensure confidentiality, the data were coded and stored in an electronic device with a password to which only the principal investigator had access, and at the end of the study, the data were discarded.

## Declaration of Interest

The authors report no conflict of interest.

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