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Research Paper

Validation of Cameriere's medical-legal age estimation method using seconds premolars in a Portuguese population

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ABSTRACT

The goal of this project is to validate the Cameriere's method applied to the upper and lower second premolars in a Portuguese population, regarding the forensic estimate of age.

The applied sample consisted in 100 panoramic radiographs, of 60 males and 40 females, patients of the Faculty of Dental Medicine of the University of Lisbon, whose ages vary between 15 and 35 years old. Thus, a total of 400 teeth were investigated (200 upper second premolars and 200 lower second premolars). Each radiograph was analysed using draw and measurement tools featured in Adobe Photoshop, applying the Cameriere's method, and then the pulp/tooth ratio was computed for the 15, 25, 35 and 45 teeth. All data were statistically analysed with the SPSS program, using a significant level of 5%.

It was not observed any kind of relation, linear or not linear, between age and the pulp/tooth ratio. Linear regressions with considerably low values for the coefficient of determination were achieved, which indicates a low reliability for these models.

Accordingly, we conclude that the knowledge of the pulp/tooth ratio does not allow the identification of an individual based on the Cameriere's method, in the scope of a forensic age estimate applied to panoramic radiograph. Further investigations with larger samples and broader age groups are required in order to provide suitable evidence to the legal and social aspects of age estimate in Forensic Dentistry.

1. Introduction

Age estimation plays an important role in forensic medicine for either the living and the death. Regarding the living person, the main gold consists in solving medico-legal civil, penal and social problems, such as cases of adoption of minors or individuals without documents that could prove their age and that came to a foreign country with different jurisdiction. This kind of situations have been increasing in European countries, therefore is necessary to know if the individual should be treated as an adult or a child in criminal processes.[1–5]

The age estimation based on dental hard tissues is justified because these are the most lasting tissue of human body. They are elements with high resistance to the *post mortem* alterations, once they stay intact at a macroscopic, chemical and microbiologic level during long lasting terms. These tissues resist to high temperatures, trauma or severe decomposition. Besides, they are easily analysed when compared with osseous components.[6,7]

Several factors can be used to the age estimation regarding dental analysis, such as mineralization and dental eruption, analysis of the radiologic, biochemical and histological characteristics among others.[6,8]

The methods that are used in adult population can be clinical and radiographic methods.[9–14]

When using radiographic methods is possible to evaluate different characteristics like deposition of secondary dentin at the pulpal wall, which occurs due to the continuous production of the dentinal matrix by odontoblasts in a physiologic way. This manifests by the reduction of the pulpal chamber and therefore permits to estimate the age of a patient without having to use invasive methods.[10,15–17] In 2004, Cameriere et al. present a method using a ratio between the area of the

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pulp and the area of the tooth, using the upper right canine. In this study, Cameriere et al. used orthopantomography and measurements at two-dimensional, computed using computer software.[18]

The aim of this study is to evaluate the application of the above described method, in the Portuguese population, using the upper and lower second pre-molar in orthopantomography.

2. Materials and methods

2.1. Sample

The applied sample consists of 100 individuals between 15 and 35 years old, from both genders, born in Portugal, whom had an orthopantomography taken in Dental Medicine Faculty, University of Lisbon (See Fig. 1). To be included both upper and lower second pre-molars, both left and right, should be on a post-eruptive phase, absence of endodontic or restorative treatments, absence of caries, the teeth should not have morphology and position anomalies.

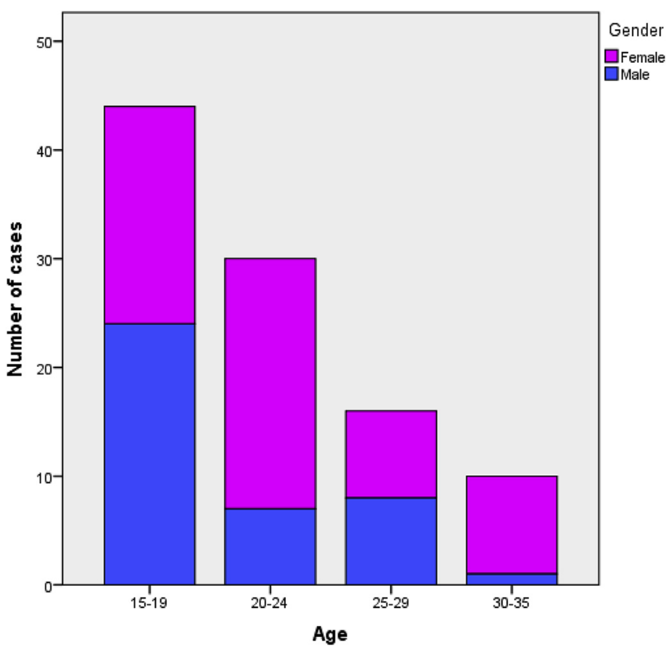

Fig. 1. – Distribution of the sample according to age and gender.

2.2. Methodology

Radiographic analysis was performed according to the measurements purposed by Cameriere et al.[18] using the computer software *Adobe Photoshop®*. All measurements of the tooth 15, 25, 35 and 45 (according to the Federation Dentaire International tooth numbering system) were made using at least 20 points to measure the tooth area and using at least 10 points to measure the pulp area.

The data were recorded in the computer software *Microsoft Excel®* and the ratio pulp/tooth was calculated.

2.3. Intra- and inter-observer calibrations

One observer collected all the data considering the upper pre-molars while another observer collected the data from the lower pre-molars. Moreover, in order to test the intra-observer variability, each observer repeated the measurements of 10% of the total sample (randomly selected), 3 months after the first measure.

In order to test the inter-observer variability the measurement of 10% of the total sample was performed by the other observer.

The inter- and intra-observer calibration were analysed by the intraclass correlation coefficient (ICC) based on the Fleiss classification.[19] Thus, the reliability is considered poor if ICC is lower than 0.40, satisfactory to good if ICC is between 0.40 and 0.75, and excellent if ICC is greater than 0.75.

2.4. Statistical analysis

The data considering tooth area and pulp area was first inserted in the *Microsoft Excel* program. Then, this same data was exported to the program IBM® *SPSS Statistics* 21 *(Statistical Package for the Social Sciences),* in which the statistical analysis was performed. A significance level of 5% was used in all the performed tests. This value indicates the probability to refuse the null hypothesis when this is true. A linear regression model was applied in order to obtain the age estimation according to gender and ratio pulp/tooth from all pre-molars. In addition, the analysis of covariance (ANCOVA) was applied to study possible interactions of gender in the linear regression model, considering age and pulp/tooth ratio as co-variables. The last two variables were analysed separately. To evaluate the regression estimates, a comparison between chronological age and estimated age was made using the coefficient of determination (R^2), the standard deviation of estimation (SE) and the mean absolute error (ME). These correspond to the three measures used by Cameriere et al.[20] Finally, the linear regression was evaluated considering each gender separately.

3. Results

The applied sample is consisted by 100 individuals with the distribution displayed in Table 1.

The ICC was used to evaluate the intra-observer variability. In each tooth under analysis, this coefficient was applied to the three measures of the study: tooth area, pulp area and ratio pulp/tooth. The lowest obtained value corresponds to the ratio pulp/tooth in the tooth 15 (0.298) which reveals a poor reliability in this measure. Nevertheless, all the remaining ICC values are greater than 0.6, which reveals a good or excellent reliability (cf. Table 2).

Similarly, the inter-observer variability was evaluated, where the ratio value of the tooth 15 also showed a low value (0.227) which means a poor reliability. Nevertheless, all the other values (cf. Table 3) reveal a good or excellent reliability.

In fact, both the tooth and the pulp area are measured twice, and the reliability of these measures is at least reasonable, being in most cases excellent. The pulp/tooth ratio is obtained by dividing these two measurements, thereby it amplifies the errors of their measurements and, therefore, has a lower reliability level. Even so, only in the tooth 15 the intra and inter-observer ICC values are effectively low.

3.1. Tooth 15

Concerning the tooth 15, no association between age and pulp/tooth ratio was found, as Fig. 2 shows.

Using the linear regression model to estimate the age as a function of the pulp/tooth ratio, the equation presented in Table 2 was obtained. This model presents a R^2 value of 0.043, SE = 5.186, ME = 4.1709,

Table 1
Sample distribution according to age and gender.

Age Gender	Number of orthopantomographies	
	Female	Male
[15–19] years old	20	24
[20–24] years old	23	7
[25–29] years old	8	8
[30–35] years old	9	1

Table 2
ICC for intra-observer variability assessment.

Tooth area	15	25	35	45
tooth	0.869	0.711	0.982	0.983
pulp	0.714	0.747	0.968	0.923
ratio	0.298	0.612	0.955	0.916

Table 3
ICC for inter-observer variability assessment.

Tooth area	15	25	35	45
tooth	0.928	0.941	0.951	0.932
pulp	0.544	0.824	0.824	0.712
ratio	0.227	0.543	0.454	0.723

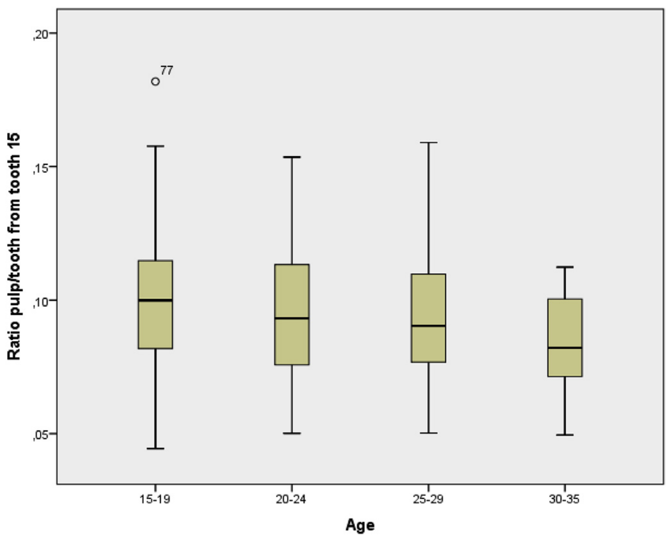

Fig. 2. – Ratio pulp/tooth Boxplot by age for tooth 15.

with a p-value = 0.037 for the F test.

By ANCOVA it seems to be an interaction between gender and the linear relation between age and ratio pulp/tooth 15 (p-value = 0.029). Thus, considering the gender separately, the p-value for the F test in the feminine gender is 0.064 and in the male gender is 0.392. Therefore, with a significant level of 0.05, it seems that the pulp/ratio is not suitable for age estimation in both genders.

3.2. Tooth 25

Concerning the tooth 25, Fig. 3 seems to show correlation between age and pulp/tooth ratio.

The equation obtain by the linear regression model is also presented in Table 2. This model presents a R^2 value of 0.033, SE = 5.214, ME = 4.1161 and p-value = 0.07 for the F test.

By ANCOVA it seems to exist an interaction by the covariate gender (p-value = 0.013). Thus, considering the gender separately, for the feminine gender the p-value is 0.356 and for the male gender is 0.041 and, therefore, the liner model is more suitable to age estimation on males.

3.3. Teeth 15 and 25

Considering as independent variables R_{15}*R_{25}, R_{15} and R_{25} and keeping the age as dependent variable, the variance inflation factor (VIF) has high levels and, as such the variable R_{15}* R_{25} was removed from the model to avoid multicollinearity problems.

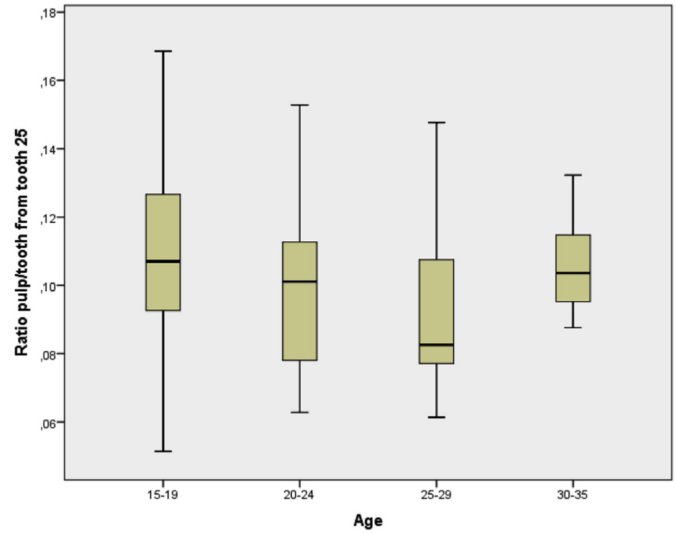

Fig. 3. Ratio pulp/tooth Boxplot by age for tooth 25.

Using the linear regression model, the value obtained for the adjusted R^2 was 0.032, SE 5.189 and ME 4.1057 with p-value of 0.167 for the tooth 15 and of 0.352 for the tooth 25 (Table 4).

Considering the gender separately, the obtained p-value for the F test in feminine gender was 0.184 and in the male gender was 0.126.

3.4. Tooth 35

Concerning the tooth 35, there also seems to exist no correlation between age and ratio pulp/tooth, as showed in Fig. 4.

Using the linear regression model an equation was obtained with R^2 = 0.08, SE = 5.085 and ME = 4.1032 (Table 4).

ANCOVA indicates the existence of an interaction between gender and the remaining variables (p-value = 0.017). Considering the genders separately, two equations (Table 4) were obtained to estimate the age. For the feminine gender the R^2 had a value of 0.080 and SE of 5.225, with a p-value of 0.028 for the F test, and for the male gender the R^2 had a value of 0.088 and SE of 4.598, with a p-value of 0.063 for the F test.

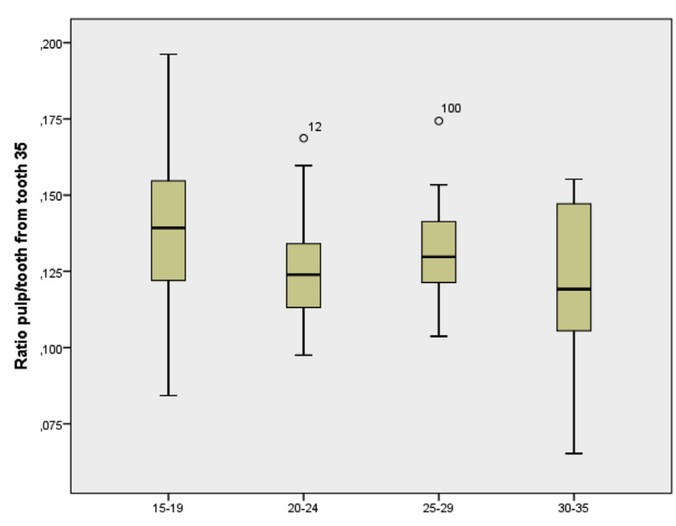

Fig. 4. Ratio pulp/tooth Boxplot by age for tooth 35.

Table 4
Equations obtained by the application of the linear regression model.

Tooth	Equation	R^2	SE	ME	*p-value*
15	Age = 25,484–40,976*R_{15}	0,043	5186	4171	0,037
25	Age = 25,526–39,216*R_{25}	0,033	5214	4116	0,07
35	Age = 29,605–61,469*R_{35}	0,08	5085	4103	0,004
45	Age = 32,493–83,890*R_{45}	0,128	4950	3935	0,0001

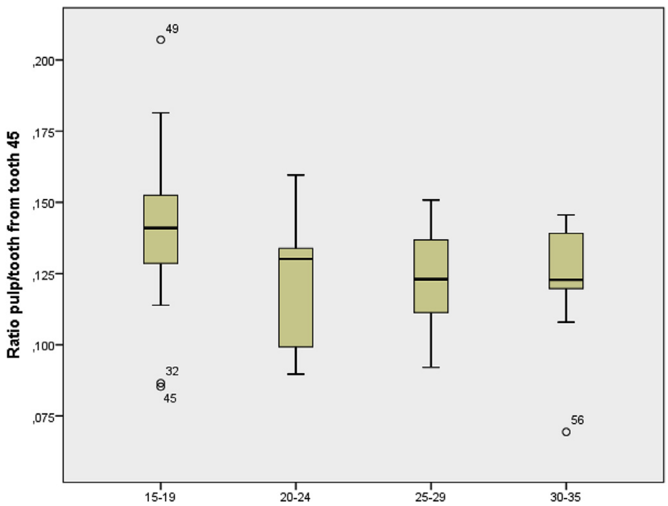

Fig. 5. Ratio pulp/tooth Boxplot by age for tooth 45.

3.5. Tooth 45

In the same way as previous teeth, there is no correlation between age and ratio pulp/tooth for the tooth 45 (Fig. 5).

The obtained equation after applying the linear regression model is showed in Table 4. This model presents a R^2 value of 0.128, SE of 4.950, ME of 3.9351, with a p-value of 0.0001 for the F test.

By ANCOVA is possible to infer that there is no interaction between the gender and the remaining variables (p-value = 0.076), in contrast with what happened with previous teeth. Nevertheless, considering the genders separately two equations were obtained (Table 4) and for the feminine gender the p-value was 0.119 and for the male gender was 0.0001 for the F test.

3.6. Teeth 35 and 45

Considering as independent variables R_{35}*R_{45}, R_{35} and R_{45} and age as dependent variable, the VIF presents a high value and, as such, the variable R_{35}*R_{45} was removed from the model to avoid multicollinearity problems.

The linear regression with the R_{35} and R_{45} as variables, an equation presented in Table 5 was obtained, with adjusted R^2 of 0.119, SE of 4.952 and ME of 3.9019, with a p-value of 0.340 for the *t*-test of tooth 35 and of 0.013 for the tooth 45. Thus, it seems that the tooth 35 has no additional information to age estimation when the information from tooth 45 is already included in the model.

In order to evaluate if there is any differences on the results, the linear regression was determined for each gender (Table 5) with a p-value of 0.075 for the female gender and 0.001 for the male gender for the F test.

Table 5
Equations obtained by the application of the linear regression model associating the two upper and lower pre-molars.

Teeth	Equation	R^2	SE	ME
15 + 25	Age = 26,851–30,928*R15 – 22,794*R25	0,032	5189	4,1057
35 + 45	Age = 33,686–68,585*R_{45} – 24,247*R_{35}	0,119	4952	3,9019

4. Discussion

The usage of age estimation methods has increased due to the augment of emigration and to the legal necessities associated to this event.[2,21,22]

Considering tooth 15, it is possible to verify that there is no distinction between the different age groups using the ratio pulp/tooth. What was expected would be that the ratio of the tooth would decrease with the augment of age, just like is showed in Fig. 2 from the article of Cameriere et al.[20]

By ANCOVA it is possible to identify that the variable gender is significant (*p-value* = 0.029) for the tooth 15. The estimated model only explains 4.3% of age variability. Moreover, when considering separately the genders, with tooth 15, both models are not reliable.

Analysing tooth 25, the results are similar to those obtained for tooth 15 according to the distribution of the ratio pulp/tooth of tooth 25 through the different age groups.

The ANCOVA permits to identify that the variable gender is significant (p-value = 0.013). After applying the linear regression model it is possible to conclude that this model only explains 3.3% of the age variability.

Considering the genders separately, the obtained model for the male gender explains 10.6% and for the female gender explains only 1.5% of the age variability. However, in this last case, the p-value is 0.356 so it is possible to remove this variable from the model without losing much information.

When analysing the data referring to both teeth 15 and 25, the VIF reveal values higher than 10, so the variable R_{15}*R_{25} was removed from the model. After removing the variable, the estimated model presents a p-value of 0.352 for the tooth 25 and 0.167 for the tooth 15. Therefore, these are variables that could be removed from the model without losing much information.

Considering the gender separately, the adjusted coefficient of determination is 0.025 for the female gender and 0.058 for the male gender, but in both genders the values are too low so the explanatory capability of the model is very low.

Hence, it is possible to conclude that both tooth 15 and tooth 25 present little information about age estimation.

After analysing the extremes and quartiles diagram of tooth 35 (Fig. 4), is possible to verify that the ratio pulp/tooth doesn't allow to classify an individual in a certain age group, once individuals from different groups can present the same ratio value, contrary to the results obtained by Cameriere et al.[20] when using the same tooth.

With tooth 35, the variable gender revealed to be statistically significant with a p-value < 0.05, contrary to most of the previous studies referring the canine.[4,18,22–24]

Even when analysing each gender separately, the obtained linear regressions present a value of R^2 lower than the one obtained by Cameriere et al.[20]. Besides, the p-value associated to the ratio pulp/tooth in male gender is higher than 0.05, which means that we can remove this variable from the model without losing much information. Thus this means that the obtained model presents a low reliability, explaining only 8% of the age variability.

Despite this, the obtained values for the difference in years between chronological and estimate age were similar to those obtained by Cameriere et al.[20] for the tooth 35 (SE = 7.59 years). However, is important to refer that the sample used in this study only included individuals between 15 and 35 years old, when Cameriere et al.[20] included individuals between 18 and 75 years old. Therefore, obtaining an error of 4 or 5 years on a sample with lower range is not as good when comparing with a sample with a bigger age range. Thus, the difference between the results obtained in this study and the results obtained by Cameriere et al.[20] can be related to this factor and to the size of the sample.

Similar to the results obtained to the tooth 35, it is not possible to include an individual in an age group using the ratio pulp/tooth of tooth 45 and none relation was obtained between the ratio and age (Fig. 5).

The ANCOVA test showed that gender is not statistic significant to tooth 45 as other authors had showed.[4,18,22–24] Just like to tooth 35 no relation was verified even when each gender was evaluated separately. The power of explanation of this model reveals values like 12.8% that is greater than to tooth 35, but still very low when compared to other authors.[20]

When the variable gender is evaluated separately, the model obtained to the male gender presents an explanation power of age of 28.1% and to female gender 4.1%. However the p-value for the *t*-test on female gender is 0.119, so this variable could be removed from the model without losing much information. In this way, the ratio pulp/tooth of tooth 45 does not present much information about age but still presents the higher R^2 value of this study, even if is lower to values obtained by other authors, being specially significant to the male gender.

The SE and ME values are satisfied, assuming lower values compared to those achieved by Cameriere et al.[20] for tooth 45 (SE = 7.42 and ME = 6.02 years).

Considering teeth 35 and 45 together and the obtained results, it is possible to confirm that it is not useful to add the information of tooth 35 to the model once this variable does not present statistical significance (p-value = 0.340) and the value for R^2 was lower (0.119) to the one obtained just for tooth 45.

As to the analysis to both genders separately, this model revealed some relevance considering male gender, even when considering both teeth 35 and 45. To the female gender the *p-value* showed a value higher than 0.05.

In conclusion, the ratio pulp/tooth to tooth 45 presents some information about age in male gender but still pretty low comparing to previous studies using the same tooth[20].

When comparing both upper and lower pre-molars it is possible to infer that the adjusted determination coefficient is lower in the upper pre-molars (0.032 vs. 0.119). On the other side, when considering both genders separately it is possible to verify that the value is always higher to the lower pre-molars either to the female and male gender.

5. Conclusion

The obtained results in this study do not permit the identification of a Portuguese population when using Cameriere method in orthopantomography in age estimation, once this same method presented a low reliability so it is not possible its validation.

Therefore, it is important to carry out more studies in this field, with larger samples and higher age range, in order to increase the scientific evidence and to contribute to the progress and harmonization in Forensic Dentistry area and, thus, to ensure scientific integrity on forensic dental age estimation.

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