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ORIGINAL ARTICLE

Morphological dental age estimation of adults using the radiographic canine pulp/tooth area ratio

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Summary The reduction of the pulpal space following the deposition of secondary dentin is a radiographically visible morphological feature associated with aging. Currently, there is no reference morphological sample for the Northern Brazilian population when it comes to the radiographically visible dental features for age estimation. This study aimed to test an existing method for age estimation based on the canine pulp/tooth area (PTA) ratio and develop a population-specific equation. The sample consisted of 100 peri-apical radiographs of Brazilian males ($n = 46$) and females ($n = 54$) from the Northern geographic region. The age of the sampled participants was between 18 and 72 years (mean age = 45.43 ± 14.39 years). The estimated age was obtained with the Cameriere's method. A statistically significant negative ($r = -0.595$) association was observed between the permanent canine PTA and the chronological age ($P = 0.0001$). A population-specific equation was structured with a 4-fold (75% × 25%) cross-validation, leading to a mean absolute error of 9.59 years, and root mean square error of 11.66 years ($r^2 = 0.363$). This study provided evidence to support the use of Cameriere's pulp/tooth area ratio for the radiographic dental age estimation of Northern Brazilian adults, especially adjusted with a population-specific equation.

Introduction

Undocumented individuals have restricted civil and human rights. Adults, for instance, are not able to claim retirement, not even financial support from the government, such as State pension [1]. Some of them die without an identity during or after clandestine migration. The ones that reach their destination alive might face the difficulties of being an asylum seeker and later a refugee [2]. Estimating the age of these individuals is a crucial step towards their identity. Dental age estimation may be accomplished by means of (1) invasive and (2) non-invasive techniques. The invasive ones are only applicable to the deceased because

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they include dental extractions from the alveolar socket [3—6]. These techniques can be classified as (1.1) invasive-destructive [3,4] and (1.2) invasive-non-destructive [5,6]. The former consists of the external analysis of the tooth morphology [3,4], while the latter requires tooth sectioning or grinding for an interval visualization [5,6]. The non-invasive techniques are normally radiographic via bidimensional (2D) [7,8] and tridimensional (3D) [9—11] imaging.

The radiographic analysis of adult human teeth for age estimation enables the visualization of the pulpal space [7,8], which has an inversely proportional correlation with the chronological age. In other words, the pulpal space reduces over the time because of the progressive deposition of secondary dentin [7]. In 2004, Cameriere et al. [8] proposed a dental age estimation technique based on the radiographic assessment of 100 permanent maxillary canines obtained from 46 Italian males and 54 females [8]. The canines were selected because of they are robust (and resistant to *ante*- or *postmortem* dental loss) and their pulpal space is easily visible in radiographs [8]. The technique is preferably performed in peri-apical radiographs obtained through parallelism, but because the resulting ratio is a normalized metric between pulp and tooth areas the method can also be performed in panoramic radiographs [12]. Later, Cameriere's method was validated in international samples [13] and became a consolidated resource for dental age estimation of living adults.

One of the countries in which Cameriere's method has been validated is Brazil [14—16]. The Brazilian National Council of the Public Ministry has demonstrated an alarming scenario: about 25% of the individuals in homeless conditions are undocumented [17]. Regarding the history of dental age estimation in the country, samples of adults from the Southeastern [15] and Central-Western [16] regions have been studied, but none was investigated from the Northern region. In opposite to the populations that have been previously studied, the Northern region has geographic borders established with five countries, namely Colombia, Venezuela, Guyana, Suriname, and French Guiana—facilitating clandestine migration. Moreover, the Northern region has a lower populational income compared to Central-Western and Southeastern regions, creating a more challenging condition for civil registration, potentially leading to more undocumented individuals.

This study aimed to test the validity of Cameriere's radiographic canine pulp/tooth area ratio to estimate the age of Brazilian adults from the Northern region, and to propose the development of a country-specific equation.

Material and methods

Ethical aspects and study design

This study was conducted with the approval of the institutional committee of ethics in human research (protocol: 69017823.2.0000.5374). The study design consisted of an observational analytical and cross-sectional radiographic investigation with retrospective sample collection. The Enhancing the Quality and Transparency of Health Research (EQUATOR) network was consulted and The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement [18] was applied.

Sample and participants

A sample of 100 peri-apical radiographs of Brazilian males ($n = 46$) and females ($n = 54$) from the Northern geographic region. This study equalized the original sample size and sex distribution proposed by Cameriere et al. [8]. The age of the sampled participants was between 18 and 72 years (mean age = 45.43 ± 14.39 years). The radiographs were retrospectively collected from an existing image database and were acquired solely for dental diagnosis and treatment reasons, meaning that no participant was exposed to ionizing radiation for research purposes.

The inclusion criteria were males and females with at least one peri-apical radiograph registered in the archives of a private oral radiology clinic. The exclusion criteria were participants younger than 18 and older than 72 years [8], decayed, fractured, restored and missing permanent canines, endodontically treated permanent canines and canines with peri-apical lesions. Moreover, participants with missing data of birth or date of image acquisition in the oral radiology database were excluded. The eligible radiographs showed 177 permanent canines (84 maxillary and 93 mandibular). The images were exported to a personal computer for metric analyses.

Measurements and variables

All the radiographs were blinded with an alphanumeric code masking the participants sex and age. The metric analyses consisted of the application of Cameriere's pulp-tooth ratio [8] of canines via peri-apical radiographs. The analysis was performed in the permanent canines of the left side. Each radiograph was individually imported in ImageJ software package (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, WI, USA). Using the polygon tool, the area of the pulpal space was calculated, followed by the area of the tooth (covering the full contour of the canine) (Fig. 1). The pulp/tooth area (PTA) ratio was calculated, and the age was estimated with the original Italian equations by Cameriere's [8]. Hence, the first variable of the present study was the estimated age (EA). The second variable was the participants' known chronological age (CA). This continuous variable was changed into a categorical one through the five following age groups: 18—24, 25—34, 35—44, 45—54, 55—64 and ≥ 65 years (Table 1). The third variable was the participants' sex, while the fourth one was the difference between estimated and chronological age expressed in years.

Mitigation of operator-dependent bias

The intra-observer agreement test was performed after the re-analysis (T2) of twenty radiographs initially assessed (T1) from the total sample. The interval between T2 and T1 was of 30 days, and the analyses were accomplished by a Forensic Odontologist (Obs1). A second observer (general dentist,

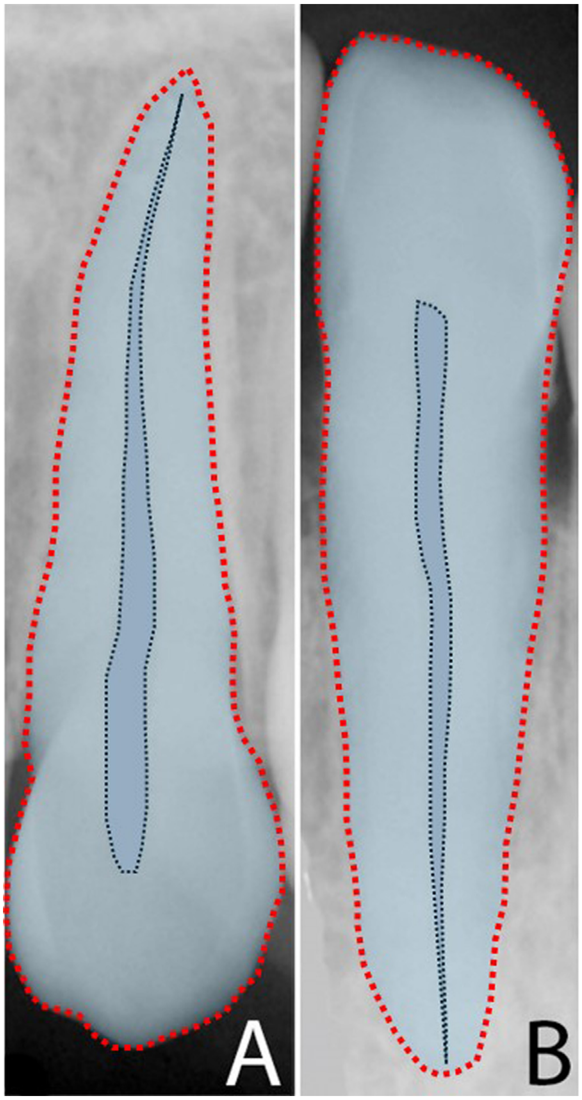

Figure 1 Radiographic outline of the permanent maxillary and mandibular canines considering the area of the tooth and area of the pulpal space.

Obs2) assessed the same radiographs to enable a comparison between Obs1 and Obs2, and to enable the calculation of the inter-observer agreement. The agreements were quantified via Intraclass Correlation Coefficient (ICC).

Statistical analysis

Data normality was assessed with Shapiro-Wilk test. The difference between the PTA of the maxillary and mandibular left permanent canines was tested with a paired *t*-test. Welch's Two Sample *t*-test was used to investigate differences between males and females. Pearson's correlation was assessed between the PTA and the chronological age. Sample linearity was visually assessed by plotting the PTA (y-axis) against the chronological age (x-axis). A linear regression model was built based on k-fold cross-validation with 4-Fold and 2 repetitions. The mean absolute error for a model combining the maxillary and mandibular permanent canines was calculated, as well as the root mean squared error. A visual representation of the difference between estimated and chronological ages by means of Bland—Altman graph was presented. Statistical analyses were performed with R (R Foundation International, Vienna, Austria). Statistical significance was set at 5%, with a 95% confidence interval.

Results

The ICC values for the intra-observer agreement tests were 0.990 for the tooth area measurement, and 0.961 for the pulp area measurement. The inter-observer agreement tests led to ICC values of 0.984 and 0.916 for the tooth and pulp area measurements, respectively. Shapiro—Wilk revealed normal data distribution ($P > 0.9$).

The paired *t*-test assessed the association between the PTA of the permanent maxillary ($n = 84$) and mandibular ($n = 93$) canines, which did not present statistically significant differences ($P = 0.1$). For further analysis, the samples of maxillary and mandibular canines were merged (Table 2). In this process, the PTA of the maxillary canine was prioritized (because it was addressed in the original study [8]), and if not present, the PTA of the mandibular canine was used.

The Welch two sample *t*-test did not show statistically significant differences between the PTA of the permanent (maxillary + mandibular) canines of males and females ($P = 0.4$), indicating no need for sex-specific regression analyses.

The Pearson product-moment correlation revealed a statistically significant negative ($r = -0.595$) association between the permanent canine PTA and the chronological age ($P = 0.0001$). The association indicated a decrease of

Table 1 Sample distribution based on age and tooth position.

Age category	Radiographs (*n*)	Tooth 23 (*n*)	Tooth 33 (*n*)	Mean age	SD
18—24.99	11	10	10	21.7	2.70
25—34.99	16	16	15	31.4	2.84
35—44.99	21	20	20	39.9	3.21
45—54.99	24	19	24	50.0	3.13
55—64.99	18	12	16	60.0	2.99
≥ 65	10	07	08	68.5	2.90
Total	100	84	93		

Age expressed in years. Males = 46; females = 54; SD: standard deviation.

Table 2 Sample distribution based on age merging tooth positions and presenting the PTA.

Age category	Radiographs (*n*)	Canines	Mean age	SD	Mean PTA	SD
18–24.99	11	11	21.07	2.70	0.123	0.024
25–34.99	16	16	31.4	2.84	0.110	0.019
35–44.99	21	21	39.9	3.21	0.104	0.012
45–54.99	24	24	50.0	3.13	0.102	0.018
55–64.99	18	18	60.0	2.99	0.083	0.019
≥ 65	10	10	68.5	2.90	0.078	0.015
Total	100	84				

Age expressed in years. Males = 46; females = 54; SD: standard deviation. The pulp/tooth ratio (PTA) of the maxillary canine was prioritized (because it was addressed in the original study [8]), and if not present, the PTA of the mandibular canine was used.

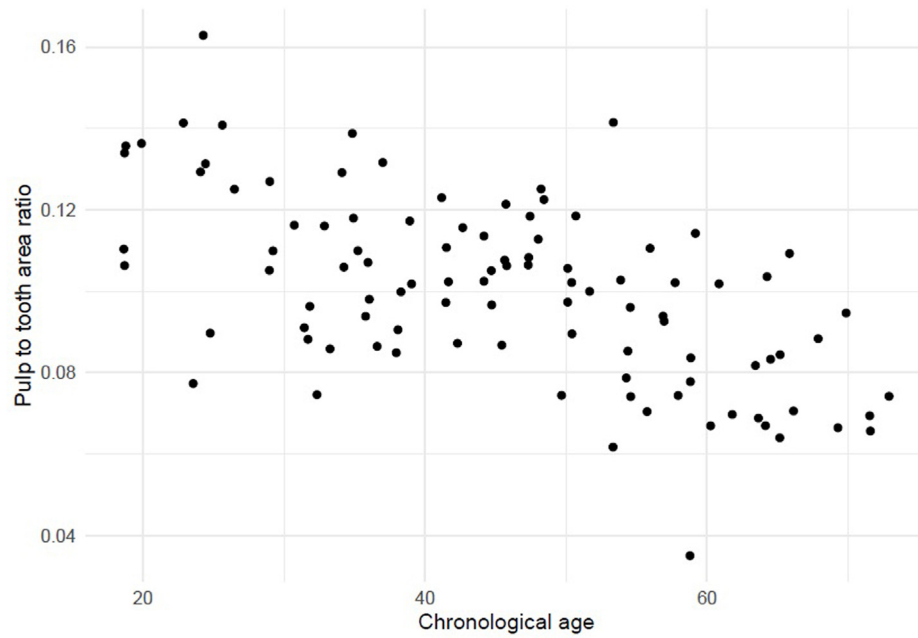

Figure 2 Visual representation of the changes of the pulp to tooth area ratio throughout the time (expressed as the chronological age).

the PTA as the chronological age progresses. Linearity was identified (Fig. 2).

The regression analysis structured with 4-fold (75% × 25%) cross-validation and two repetitions led to the following results after resampling: mean absolute error = 9.59 years, and root mean square error = 11.66 years (r^2 of 0.363). The model equation is written as Age = 84 – 384.7 × PTA (Table 3).

As quality control, the regression was tested individually with the maxillary and mandibular canines to confirm the superiority of the canine combined model. For the maxillary canine, the mean absolute error was 9.74 years and root mean squared error was 12.332 years. For the mandibular canine, the mean absolute error was 10.19 years and root mean squared error was 13.11 years (Table 4). The results were illustrated via Bland–Altman graphs (Fig. 3).

Table 3 Linear regression showing the coefficients, estimates and errors of the model.

Coefficient	Estimate	Std error	*t*	*P*
Intercept	84	5.39	15.6	0.0001
PTA	−384.7	52.45	−7.33	0.0001

Males = 46; females = 54; Std error: standard error; PTA: Pulp/tooth area ratio; t: *t*-test value; *P*: significance set at 5%. The equation that derived from the Northern Brazilian model was written as Age = 84 – 384.7 × PTA. In contrast, the original Cameriere's equations are written as: Age = 99.937 – 532.775*(pulp pixels/upper canine pixels); Age = 89.456 – 461.873*(pulp pixels/lower canine pixels); and Age = 114.624 – 431.183*(pulp pixels/upper canine pixels) – 456.692*(pulp pixels/lower canine pixels) + 1798.377*(pulp pixels/upper canine pixels)*(pulp pixels/lower canine pixels).

Discussion

Dental age estimation of adults based on the morphological features of the permanent teeth is a topic of forensic odontology since the 50's [3]. This method evolved over the last years and changed from an invasive and destructive approach into image-based analyses. The method proposed by Cameriere et al. [8] has demonstrated proper applicabil-

Table 4 Mean absolute errors and root mean squared errors for the models based on tooth positions.

Model	*n*	MAE	RMSE
Tooth 23 only	84	9.741	12.332
Tooth 33 only	93	10.191	13.115
Teeth 23 or 33	177	8.966	11.715

23/33: International Dental Federation tooth notation; *n*: sample size; MAE: mean absolute error; RMSE: root mean squared error.

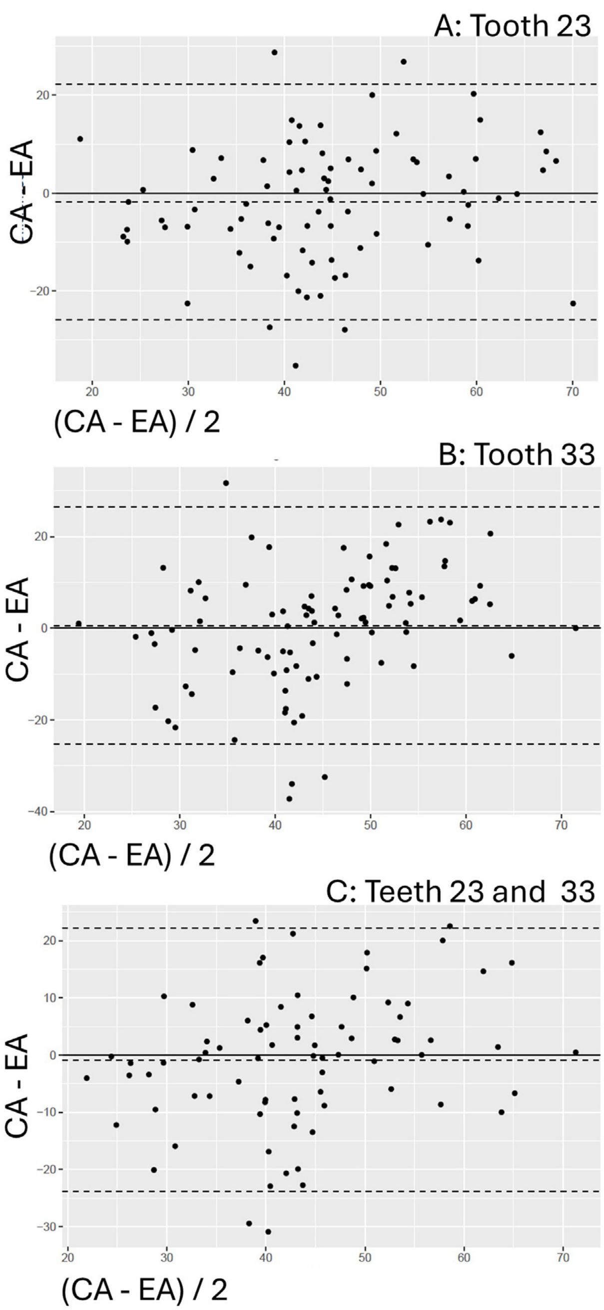

Figure 3 Bland-Altman plots designed for the teeth #23 (A), 33 (B) and 23 + 33 (C) considering the chronological (CA) and estimated (EA) ages.

ity worldwide [13] but has never been tested in a Northern Brazilian population.

A recent systematic review with meta-analysis endorsed the pulp/tooth area ratio as a reliable technique for adult dental age estimation [13]. The outcomes of the present study followed the same recommendations for the specific Brazilian Northern population. More specifically, a moderate [19], negative and statistically significant correlation was observed between the pulp/tooth area ratio and the chronological age — meaning the area (especially from the pulpal space) progressively reduced with time (as the participants get older). This phenomenon is explained by the continuous deposition of secondary dentin along the pulpal space during life [20].

Some authors claim that the secondary dentin is uniformly deposited in the pulpal cavity (including the pulp chamber and root canal) [20]. Conversely, Nudel et al. [21] explain that the primary and secondary dentin are produced by the same type of odontoblasts, and that the secondary dentin follows a nonhomogeneous formation. In this context, the deposition of secondary dentin may differ between tooth groups, but normally starts in the most superior aspect of the pulp chamber after apex closure [21]. The secondary dentin is distinguished from the primary one based on the direction of dentin tubules and is not influenced by sex [21].

The lack of sex-related influence on secondary dentin formation was observed in the present study, since differences were not statistically significant. These findings enabled the developed of a single regression formula combining males and females. Similarly, we also detected lack of statistically significant differences between the pulp/tooth area ratio of the maxillary and mandibular canines — suggesting that the deposition of secondary dentin might be different across tooth groups, but similar within the same group (canines) even if the dental arches are different.

Interesting, when the deposition secondary dentin was considered a morphological parameter for dental age estimation [3], other parameters were considered together — such as attrition, periodontosis, root resorption, cementum apposition, and root translucency [3]. While on one hand these parameters are (positively or negatively) correlated with age, on the other hand they also may be confounding factors between each other in dental age estimation studies. Authors [21] have demonstrated that attrition, for instance, may be associated with the deposition of secondary dentin. They argument that a thicker layer of secondary dentin may be observed below the cementum-enamel junction of teeth with attrition [21]. In this context, the formation of secondary dentin would be a biological response to strengthen the tooth structure specifically in a region of intense force load. Theoretically, this would be more evident in teeth with reduced structure, such as those with attrition.

Cameriere's method is mainly focused on the reduction of the pulpal space after the deposition of secondary dentin [8]. A deeper look, however, will highlight that attrition is also considered in this method. When the outline of the tooth area is accomplished, the entire contour of the tooth structure is selected and considered in the authors' equation. Hence, a tooth with attrition would allow the selection an area that is smaller than the one observed if the tooth had no attrition. This feature of the method also can be interpreted for the inclusion of root resorption and cementum apposition as parameters involved in the outlining of the dental area. The combination of morphological features in the radiographic dental analysis makes this method a valuable resource for the non-invasive dental age estimation of adults.

Based on the subsequential analyses of tooth position and sex, which pointed out the lack of statistically significant differences, a single population-specific equation was developed for the Northern Brazilian population. The development of population-specific solutions for dental age estimation has been proposed to enable a more representative practical tool for a target sample [22,23]. However, it must be noted that this rationale is disputable, especially when validation studies use deficient material and methods to develop population-specific equations. The idea behind the present study's equation was to fill the gap left by the null literature about Cameriere's method [8] in the Northern Brazilian population. As a result, the developed equation's error rate fell within the range of what has been normally accepted for the adult population in the scientific literature [6,15].

From a global perspective, future studies in the field should consider the unification of country-specific equations that could serve as a more macroscopic tool for regional (continental) purposes. Nationally, the existing Brazilian equations should be tested more often and in different external validation samples. This way, a decision could be made between their unification, or the development of subsequent regional equations.

Conclusion

This study provided evidence to support the use of Cameriere's pulp/tooth area ratio for the radiographic dental age estimation of Northern Brazilian adults. The specific outcomes indicated lack of difference between the area ratio of maxillary and mandibular canines, as well as between males and females. An inversely proportional correlation between the permanent canine pulp/tooth ratio and the chronological age was observed. A population-specific equation was developed.

Disclosure of interest

The authors declare that they have no competing interest.

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