

Age estimation in north east Brazilians by measurement of open apices

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ABSTRACT

Dental age (DA) estimation is an extensively investigated resource used by forensic science. This study aimed to evaluate the applicability of the Measurement of Open Apices for DA estimation in north east Brazilians. A total of 429 orthopantomographs of individuals aged 5 to 14.99 years were used. The sample was distributed according to the age groups 5-6.99, 7-8.99, 9-10.99, 11-12.99 and 13-14.99 years, and the data were analyzed descriptively and by linear regression ($\alpha = 5\%$). The majority of the radiographs were from females ($n = 241$; 56.2%), with an overall mean age of 12 years (± 2.12). A significant difference was observed between DA and chronological age (CA) in the total sample and specifically in females and males. The method underestimated CA by 0.31 year (total sample) and by 0.3 and 0.32 year in females and males, respectively. In contrast, the method overestimated CA in the groups 5-6.99 and 7-8.99 years, with a mean difference (MD) of 0.48 year ($p = 0.007$) and 0.17 year ($p = 0.182$), respectively. In the other groups, DA was predicted to be below CA, with a significant difference in the group 13-14.99 (0.75 year). Based on the regression analysis, a correction factor was proposed from the original formula for this population, thereby reaching a predictive power of approximately 80%. To conclude, this method is applicable to the study population aged 5 to 13 years as the estimates obtained did not exceed the error limit of ± 1 year.

INTRODUCTION

Dental age (DA) estimation has been considered a valuable resource in areas such as the forensic disciplines, assisting with human identification, criminal investigations and mass disasters, as well as in biomedical areas such as paediatric endocrinology and orthodontics.^{1,2} Age estimation for forensic purposes is of interest in the civil, criminal and administrative settings. The estimation of age during criminal investigations has been decisive in cases of rape, abduction and identification of the age of criminal responsibility.³ In addition, age estimates can be utilized in cases of child adoption, preparation of documents, illegal immigration, and for retirement purposes. Therefore, age estimation tools have been consistently proven useful for documentation and law enforcement.⁴

The physiological age is determined by the degree of maturation of the individual's biological systems, and that can be used to define the progress of a child towards complete development or maturity. Instead, DA is based specifically on

the maturation of teeth, which is relevant if one considers that children of the same chronological age (CA) may present alterations in the development course of biological systems other than their teeth. Over the last decades, several indices have been developed to estimate age based on body maturation. Of these, DA estimation methods are the most used as teeth are not subject to the same external stimuli as other biological systems.⁵ Accordingly, the literature reports that teeth-related age estimation methods are more reliable, since they are not as much influenced by ethnicity and environmental factors as are skeletal methods.⁶ The method proposed by Cameriere et al.¹ is based on the measurement of open apices. Briefly, it estimates the age of children through the relationship between CA and the measurement of open apices in incompletely formed teeth. These authors proposed a method through a mathematical formula in line with previous studies addressing other age estimation approaches and with the purpose of improving the accuracy of this estimate. They tested the method in a sample of 455 Italian children aged 5 to 15 years and obtained satisfactory results with discrete age underestimation in both males and females, with a residual error of -0.0035 year.¹ Methods that are based on mathematical formulae have a relevant question which is the accuracy that the formula provides in the samples tested. The applicability of an age estimation method is also proportional to the accuracy and precision it demonstrates. Although the terms look similar, in statistics they have different gradations. Accuracy assesses the degree of conformity of a measured or calculated quantity to its actual value, whereas precision defines how many calculated values are the same or similar to the actual value. Thus, accuracy and precision are inherent concepts in order to reach the determination of the applicability of a method. It is known that the same population living in separate regions can present distinct age estimates due to cultural variations, eating habits, environmental factors and ethnic miscegenation, which, in Brazil, can be considered even more complex due to its great territorial area. As a result, the same method may not suit the whole population in distinct geographical regions, which reinforces the need to test several approaches in order to reduce distortions in the method and increase their clinical and/or forensic

usefulness. Thus, the objective of this study was to test the reproducibility and applicability of the Cameriere et al.'s method¹ in a population of north east Brazilians.

MATERIAL AND METHODS

Study design

This was a documentary, retrospective and descriptive study using secondary data from digital panoramic radiographs obtained between January and December 2016. The images were from individuals aged 5 to 15 years (N = 2,623) and were provided by a private radiology clinic located in the city of João Pessoa, Paraíba, in the north east of Brazil.

Ethical issues

This study was previously approved by the Research Ethics Committee of the Centre for Health Sciences, Federal University of Paraíba, under protocol CAAE 63928516.9.0000.5188, with exemption of the informed consent form.

Intra- and inter-examiner agreement

A pilot study was carried out with fifteen radiographs to check for internal agreement of the examiner (intra-examiner agreement) and comparable agreement with an experienced professional (inter-examiner agreement). Both examiners evaluated the same radiographs at two different moments, with a one week time interval in between. The intra- and inter-examiner concordances were analyzed by Student's paired *t* test and Intraclass Correlation Coefficient (ICC). The results of the pilot study did not indicate a statistically significant difference in the internal and external analyses, hence demonstrating satisfactory agreement. The ICC indicated excellent inter-examiner (0.891, 95% CI: 0.671-0.957) and intra-examiner agreement (0.975, 95% CI: 0.926-0.991).

Sample size and eligibility criteria

The pilot study showed 82.4% agreement between the estimated and the actual CA. Therefore, the magnitude of the effect or the difference between the estimated and the actual age by the complementary value (1 - concordance) was predicted to be 17.6%. A sample calculation with statistical power set at 82% indicated a sample size of 429 radiographs, which were randomly selected by means of a

simple draw with the use of a random number table. An additional 20% of radiographs were included as the sample had yet to be screened for eligibility criteria.

Radiographs of individuals aged 5 to 14.99 years of both sexes, obtained for clinical and/or orthodontic purposes, were included in this study. Distorted radiographic images with noticeable pathological alterations and/or changes in the number or shape of teeth, were excluded from the analysis. In addition, panoramic radiographs with images show pulp involvement in the lower left teeth (except 3rd molars) were also excluded from the study due to the possibility of necrosis with consequent interruption of mineralization.

Data collection

The data were collected by a single previously trained and calibrated examiner. Adobe® Photoshop® CC (PS®CC) image editing software was used to perform the measurements in pixels. After importing the images into the software, brightness, contrast and zoom features were adjusted for better visualization of the lower left teeth, and the pen tool was selected to make markings before measurement. With the pen tool, a line tangent to the incisal border or to the uppermost cusp(s) was marked as well as a line tangent to the lower extremities of the teeth root(s). Next, the midpoints of these two lines were marked and corresponded to the total length of the tooth. This same procedure was used to measure the inner sides of the apices by marking with the pen the distance between the walls of each root. Measurements were taken with the ruler tool after all markings with the pen had been made in pixels. In cases where a tooth on the left side was compromised for any reason, the corresponding tooth on the opposite hemiarch would be used if it was present and in good condition for analysis.

The radiographs were previously coded so that the examiner did not have access to the individual's CA. The seven lower left teeth were considered for analysis, except the third molars. The number of teeth with closed apex was determined (N). In teeth with open apices, the distance between the inner root walls was measured (A_i , where i corresponds to the tooth). In teeth with two roots, the distances of the lateral walls of each root were totaled. To avoid distortions due to the possible difference in

radiographic magnification and angulation, the measure A was divided by tooth length (L), then $X_i = A_i/L_i$.

The measurements obtained were used to estimate CA according to the following formula from Cameriere et al.:¹

$$\text{Age} = 8.971 + 0.375G + 1.631X_5 + 0.674N - 1.034S - 0.176SN$$

Data analysis

The sex, DA and CA data of each individual were tabulated and treated as variables. CA was obtained from the subtraction between the date of birth and the date of the radiographic examination.

The data were analyzed by descriptive and inferential statistics in IBM SPSS software (Statistical Package for Social Sciences) version 20.0 and in R software (Bell Laboratories, version 3.4.2). The Kolmogorov-Smirnov test indicated that DA and CA data did not present a normal distribution (p-value < 0.001), and non-parametric tests were used for data analysis. The Wilcoxon's test was used to compare the means of chronological age with dental age. Linear regression was used in the analysis of age estimation, and in the evaluation of assumptions from the residuals analysis for the equations. To all the tests, a significance level of 5% was adopted.

RESULTS

A total of 600 radiographs were randomly selected and screened for the eligibility criteria, resulting in a final sample of 429 radiographs. The sample had a mean CA of 12.02 years (± 2.06), with 56.18% (n = 241) of female subjects. The reasons for excluding some of the images (n = 171) were diverse, mainly: older than 14.99 years (n = 99), low image sharpness (n = 48) and, to a lesser extent, atypical number of teeth (n = 1) (Table 1).

The distribution of the sample by sex and CA group (in years) can be found in Table 2.

The mean CA by sex is shown in Table 3.

There was a statistically significant difference between DA and CA in the total sample (Wilcoxon test, p-value < 0.001), as well as specifically in females (p-value < 0.001) and males (p-value < 0.001) (Table 4). The method underestimated the actual age by 0.3 year in females and by 0.32 year in males.

Table 1. Absolute and relative frequencies of the reasons for sample exclusions

Exclusion reason	N	%
Older than 14.99 years	99	57.89
Poor image quality	48	28.07
Missing tooth	16	9.36
Pulp calcification	3	1.6
Abnormal tooth shape	2	1.2
Abnormal tooth position	2	1.2
Atypical number of teeth	1	0.5
Total	171	100.0

Table 2. Sample distribution by sex and age group (chronological age is expressed in years)

Chronological age (years)	Sex				Total	
	Female		Male		Total	
	n	%	n	%	n	%
5	0	--	1	0.5	1	0.2
6	5	2.1	4	2.1	9	2.1
7	6	2.5	3	1.6	9	2.1
8	20	8.3	7	3.7	27	6.3
9	17	7.0	14	7.4	31	7.2
10	19	7.9	20	10.6	39	9.1
11	26	10.8	24	12.8	50	11.6
12	58	24.1	42	22.3	100	23.3
13	47	19.5	37	19.7	84	19.6
14	43	17.8	36	19.1	79	18.4
Total	241	100.0	188	100.0	429	100.0

Table 3. Mean dental and chronological ages expressed in years by sex

	Number of cases	Mean chronological age (\pmstandard deviation)	Mean dental age (\pmstandard deviation)	Mean difference	p-value
Male	188	12.09 (\pm 1.99)	11.77 (\pm 1.95)	-0.32	0.000*
Female	241	11.97 (\pm 2.12)	11.68 (\pm 1.90)	-0.30	0.000*
Total	429	12.02 (\pm 2.06)	11.71 (\pm 1.93)	-0.31	0.000*

*Statistically significant difference (Wilcoxon test)

Table 4. Mean dental and chronological ages expressed in years by age group

	Number of cases	Mean chronological age (\pmstandard deviation)	Mean dental age (\pmstandard deviation)	Mean difference	p-value
5-6.99 years	10	6.34 (\pm 0.46)	6.82 (\pm 0.50)	0.48	0.007*
7-8.99 years	36	8.28 (\pm 0.48)	8.45 (\pm 0.1)	0.17	0.182
9-10.99 years	70	10.07 (\pm 0.62)	10.01 (\pm 0.99)	-0.06	0.302
11-12.99 years	150	12.13 (\pm 0.53)	12.02 (\pm 1.1)	-0.11	0.174
13-14.99 years	163	13.94 (\pm 0.8)	13.19 (\pm 0.8)	-0.75	0.000*
Total	429	12.02 (\pm 2.1)	11.71 (\pm 1.93)	-0.31	0.000*

*Statistically significant difference (Wilcoxon test)

As shown in Table 5, the analysis of the age groups revealed that DA was above CA in the groups 5-6.99 and 7-8.99 years, with a statistically significant difference in the former (p -value $<$ 0.001). On the other hand, DA was found to be below CA in the other groups, with a statistically significant difference found only in the group 13-14.99 years (p -value $<$ 0.001).

Overall, the method underestimated the age in 89.28% of the cases as opposed to only 11.72% of overestimation. The mean differences observed in Table 5 indicate overestimation in groups 5-6.99 and 7-8.99 years and underestimation in

the other groups. The method considerably underestimated the age of individuals within the range 13 to 14.99 years, which clearly shows reduced applicability near the age of 15 (Figure 1). Table 6 shows the correction factors for age estimation in both sexes and specifically in females and males, with the corresponding determination (R^2) and correlation (r) coefficients and estimation error of the models. The coefficients of determination presented satisfactory values for age estimation, with approximately 80.0% accuracy. The model for both sexes and the one for males can be

considered more accurate as they showed higher R^2 and less significant estimation errors.

Figure 2 presents a graph panel on the predictive diagnoses of the models generated by linear regression analysis for age estimation in the total sample ($n = 429$) and only in females ($n = 241$) and males ($n = 188$).

The homoscedasticity analysis of the different models indicated that the points in the graphs are distributed randomly and uniformly, therefore the errors are independent and have constant variance. The Kolmogorov-Smirnov normality test indicated that the residues have a normal distribution. The Durbin-Watson test confirmed what can be seen in the homoscedasticity graphs – the errors are independent. Lastly, the analysis of Figure 2 suggests that the residues are distributed

linearly, that is, the proposed models have adequate linear adjustments.

Once the proposed models are consistent with all assumptions, one should opt for those with higher coefficients of determination (R^2) and less significant errors. There is a greater number of errors near 14 to 15 years of age in the correction factor generated for both sexes and only for females or males. Therefore, as shown in Table 6, age estimation in females should be performed preferably through the general equation for both sexes, despite the small difference in the method's accuracy between the models for both sexes and females only. In contrast, age estimation in males should preferably be performed through the equation generated specifically for males.

Table 5. Equations generated for estimation of age in the Brazilian population

Population	Equation	Determination coefficient (R^2)	Correlation coefficient (r)	Error
Total	Age = 0.82 + 0.95X + 0.93	79.32%	0.89	0.93
Female	Age = 0.39 + 0.99X + 0.97	78.95%	0.88	0.97
Male	Age = 1.35 + 0.91X + 0.89	80.06%	0.89	0.89

X = age estimated by the Cameriere et al.'s method (2006).

All estimates meet the assumptions of normality, linearity, homoscedasticity and independence of errors.

Figure 1. Mean differences between the dental and chronological ages expressed in years

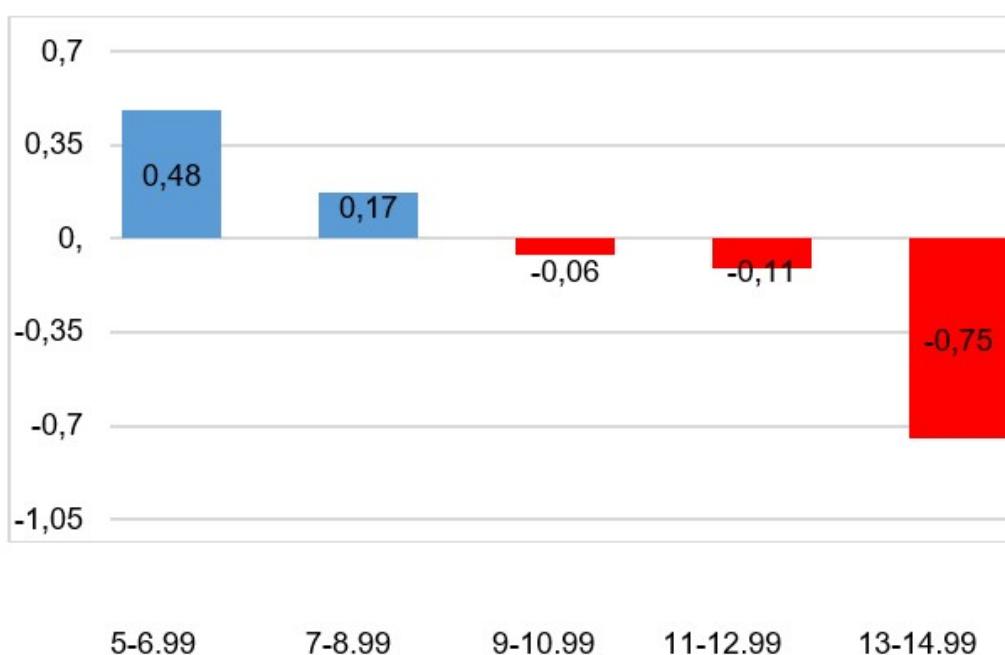
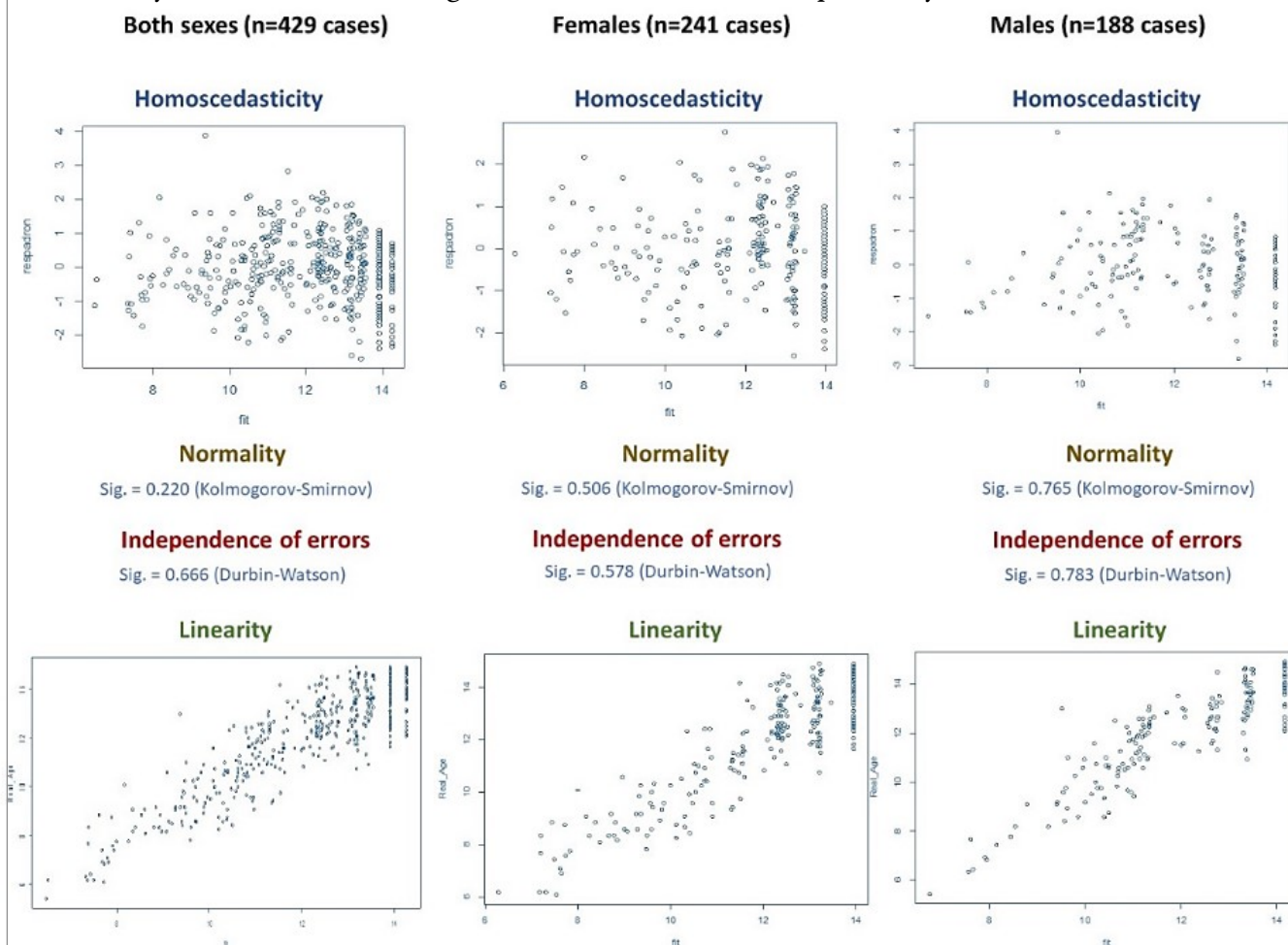


Figure 2. Graphical representation of the assumptions (homoscedasticity, normality and linearity) for correction factors generated for both sexes, and specifically for females and males



DISCUSSION

As previously reported by other authors, the limitations of this study comprise cases of age underestimation or overestimation. According to literature reports, the acceptable estimation error margin of the Cameriere et al.'s method¹ is up to one year. Beyond this, the method is considered not applicable to the study population and should therefore be combined with other approaches to estimate the individual's age.^{7,8} Initially tested in a population aged 5 to 15 years, the Cameriere et al.'s method¹ underestimated the age of individuals between 12 and 15 years. Hence, this method is not universally applicable to individuals of any age, which may also be considered a shortcoming of the study. Another possible limitation refers to the sample, which was defined by a completely random probabilistic method, yet not balanced for age. This is because in Brazil it is uncommon to request orthopantomographic examinations of young

children, generating a situation with few young children's radiographs.

In the present study, the method overestimated the individuals' age within the range 5-6.99 and 7-8.99 years, whereas it underestimated the age in the other groups. These findings corroborate the study by Fernandes et al.,² who investigated the applicability of the Cameriere et al.'s method¹ in a population of south east Brazil aged 5-15 years. The authors did not find a statistically significant difference between CA and DA ($p=0.603$), which disagrees with our findings showing statistical difference in the total sample ($p<0.001$). In Fernandes et al.'s study,² DA was found to be significantly above CA in 5-10-year-olds (overestimation) and significantly below in 11-14-year-olds (underestimation). These results corroborate ours with respect to the groups in which age was over- or underestimated, except in 10-year-old individuals whose DA was underestimated herein. The mean difference

between CA and DA indicated an overestimate of 0.48 year among 5-6.99-year-olds and of 0.17 year among 7-8.99-year-olds. In the subsequent age groups, underestimates of 0.06 and 0.11 years were observed in the groups 9-10.99 and 11-12.99 years, respectively. The highest age underestimate (0.75 year) was found in the group 13-14.99 years. By using the same method, Fernandes et al.² reported that the CA of Brazilian south east individuals was under- and overestimated in 54.4% and 45.6% of the cases, respectively. Here, the method underestimated the individuals' age in 89.28% of the cases and overestimated it in only 11.72% of the cases. Despite agreeing with the previous report, this study showed a predominance of underestimates in more than 75% of the sample. This discrepancy may be a result of the number of radiographs examined, that is, 160 panoramic radiographs analyzed by Fernandes et al.² and 429 analyzed in our study; or due to sample size, which may lead to statistical significance or non-significance; in addition to individual and regional differences in each population sample.

Cameriere et al.¹ reported a mild CA underestimation and pointed out that 90% of the absolute value of residual errors obtained were less than 1 year. This fact corroborates the findings of the present study, since the largest mean difference found was -0.75 year in the group 13-14.99 years.

When comparing two age estimation methods, one of which being the Cameriere et al.'s method,¹ Wolf et al.⁹ observed DA overestimation in males aged 6 to 11 years and underestimation at 12 to 14 years. In females, DA overestimation was observed in individuals aged 6 to 10 years and underestimation at 11 to 14 years. Cameriere et al.¹⁰ showed a clear lack of accuracy of the method for individuals aged 12 to 14 years. Gulsahi et al.¹¹ reported that DA decreases progressively as CA increases. However, this could not be confirmed by Wolf et al.,⁹ since the authors observed loss of accuracy of the method from the age of 12, i.e., DA remained stagnant and did not continue to decrease with the increase of CA. This fact was also observed in our study, from the age of 14 though.

Gulsahi et al.¹¹ found an overall underestimate of 0.35 year in a Turkish sample of both sexes. The age underestimate was 0.24 years in females and 0.47 year in males. They observed that the difference between DA and CA in different age

groups was accompanied by a greater underestimate with the increase of CA. The authors also stated that the Cameriere et al.'s method¹ is more accurate for females than it is for males. The authors reasoned that pre-puberty and puberty phases may take place at the age of 8 to 15, with particular growth changes occurring in females. With this, the variation in maturity and development can affect both sexes differently, including tooth development. The present study is in line with what has been described by Gulsahi et al.¹¹ regarding age underestimation in the total sample, with a mean difference of 0.31 year between CA and DA.

Our findings agree with other studies in the literature. In a sample of Malaysian children, DA was underestimated by 0.41 year in the total sample, 0.44 year in males and 0.39 year in females, thus indicating a slight difference between the sexes, although with greater underestimation observed in males.¹² When analyzing 259 individuals aged 5-15 years in India, Rai, Cameriere and Ferrante⁷ observed that the method overestimated by 1 year the age of 20% and 25% of the female and male samples, respectively.

In Egypt, El-Bakary, Hammad and Mohammed¹³ also showed similar findings, with an underestimate of 0.26 year in females, 0.49 year in males, and 0.29 year in the total sample. In a Mexican sample aged 5 to 15 years, De Luca et al.¹⁴ found a significant positive correlation ($p = 0.001$) between DA and CA in both sexes. The authors reported mild underestimation of age by 0.10 year in females and 0.00 in males (100% accuracy). In Italy, Pinchi et al.⁸ reported underestimates of 0.96 year and 1.07 year in a sample of female and male children, respectively. These data are not consistent with those of Balla et al.,³ who observed age underestimates of 0.51 year in males and 0.7 year in females. Guo et al.¹⁵ reported underestimates of 0.03 year in females (non-significant, $p > 0.05$) and of 0.43 year in males (significant, $p < 0.05$). In the present study, a mean DA underestimate of 0.32 year was observed in males ($p < 0.05$) as compared to 0.3 year in females, which indicates accuracy due to their proximity to CA.

When analyzing 2,630 panoramic radiographs of an Italian population aged 4 to 17 years, Cameriere et al.¹⁰ observed an overestimate of 0.72 year in males and 0.73 year in females. In 2006, Cameriere et al.¹ found different results,

that is, underestimation of the total sample of 0.035 year. In the 2006 study, the authors analyzed an Italian sample (213 males and 242 females) aged 5 to 15 years. The study by Cameriere et al.¹⁰ differs from the present study since the former observed age overestimation in the total sample. This can be explained due to variations in different countries, such as race and culture, hormonal factors, climatic factors, genetic influence, among others. The method is not considered universal; therefore, it needs to be tested in a certain population before it is deemed valid.

Mazzilli et al.¹⁶ applied the Cameriere et al.'s method[†] in a population of south east Brazilians aged 4 to 16 years. The authors observed a mean DA of 8.76 years, which did not match the CA of 10.00 years, indicating total underestimation of 1.24 year. When the authors applied a correction for the European formula, the mean DA was found to be 10.04, thus demonstrating greater similarity to the CA and better adaptation to the use of the adjusted formula. Here, a regression analysis was performed to generate predictive power data, which represented the possibility of predicting DA in approximately 80% of the total sample.

The results observed in this study using the measurement of open apices corroborate others reported in the literature. The variations between under- and overestimation of age can be explained by existing regional differences, even within the same country. Racial miscegenation is a factor that may contribute to different region-based outcomes.^{2,11} Different findings from age estimation studies with several populations have to do with the accuracy and relevance of the selected method, ethnic diversity, sample age, sample size, biological variation, and statistical approach.¹⁷ In forensic practice, the knowledge

about all these variables is of utmost importance, given that previous information on an age estimation method allows dental experts and anthropologists to make the choice for a reliable tool and thus prevent the occurrence of technical errors.¹⁸

The quest for an accurate age estimation method should consist of the validation of several existing methods in the literature. Importantly, the study of the applicability of estimation tools should contemplate the rich miscegenation existing in each country. In clinical and forensic practice, a single method does not provide reliable information, that being the reason for validation of the Cameriere et al.'s method[†] in this Brazilian population. Herein, a correction factor was proposed from the original formula, but it still needs to be further tested for cross-validation.

CONCLUSIONS

The Cameriere et al.'s method[†] can accurately estimate the age of the Brazilian north east population aged 5 to 14.99 years, since the mean difference between the estimated dental age and the actual chronological age was close to zero. Although the method underestimated age in 89.28% of the cases and overestimated it in 11.72% of the cases, the mean difference between the dental and chronological age indicated an acceptable error of 0.3 year (underestimate) and 0.32 year (overestimate). For the age group 13-14.99 years, specifically, it is recommended to combine the method with other approaches that have been validated in this population in order to improve the accuracy of the age estimate. Furthermore, the correction factor proposed from the original formula for this study population may provide a greater predictive power and lower estimation error.

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