Radiographic evaluation of dental and cervical vertebral development for age estimation in a young Brazilian population

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KEYWORDS

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ABSTRACT

Age estimation is guided by the evaluation of events that happen during the processes of bone and dental development. The purpose of this study was to validate the method of age estimation proposed by Lajolo et al. (2013) through oro-cervical radiographic indices in Brazilians. The study aimed to verify the effectiveness of age estimation equations through dental and cervical vertebrae examinations, in addition to including dental and cervical vertebrae data in new age estimation equations. The sample consisted of panoramic radiographs and teleradiographs from 510 subjects (8-24.9 years). Age estimation methods were applied by assessing the development of seven mandibular teeth, cervical vertebrae and third molars. Techniques used previously have been combinations of radiographic indices: Oro-Cervical Radiographic Simplified Score (OCRSS) and Oro-Cervical Radiographic Simplified Score without Wisdom Teeth (OCRSSWWT). In the second phase of the study, dental maturation, vertebral measurements, and real age were estimated by regression equations. OCRSS and OCRSSWWT had success rates of 67.4% (R^2=0.64) and 70.8% (R^2=0.62), respectively. When age estimation equations for tooth evaluations were applied, the average error was 1.3 years, and for cervical vertebrae measurements, the error was 1.9 years. When dental variables and the measurements of cervical vertebrae were included, the average error of equations was 1.0 year. Radiographic indices were easy to perform, and after adequate training, are reliable and can be used in forensic practice. The use of the new equations presented in this study is recommended because including cervical vertebrae and dental data provides greater accuracy for age estimation.

INTRODUCTION

In forensic sciences, estimating the age of living individuals and corpses represents a challenge with significant anthropologic, social, penal, and civil implications. Age estimation supports human identification, provides information of ancestors of a population and assists in situations of birth certificate missing, questionable documentation, resolution of cases involving adoption, criminal responsibility and asylum application. Over the past few years, age estimation cases have become increasingly frequent and relevant worldwide, as economic globalization, associated with increasing migrations related to socio-political issues, has led to a major demand for age estimation in living individuals with no official documentation, particularly children and adolescents.[1,2] To this end, it is necessary to develop highly accurate and reliable non-invasive methods.

Given the current global context, there is an increase in the number of requests for medical and dental experts to establish an individual's chronological age by using age estimation techniques.[3,4] Methods employed are based on determining biological age through the evaluation of teeth and bone structure development.

Tooth eruption and mineralization are widely studied parameters in radiographic images and are successfully applied to estimate chronological age. When tooth development is finishing, dental age estimation becomes much more difficult. The third molar mineralization is the last option of age estimation by teeth, however variability of third molars morphology is usual and it is often an absent tooth. In addition to teeth, modification in the size and shape of cervical vertebrae from birth to bone maturity is an important indicator of bone development, which can be evaluated using lateral teleradiography.

Despite the various studies and age estimation methods already published, there is still great variability between estimated and chronological ages.[5] In this context, association between age estimation methods – dental and bone – leads to better results, the verification of possible discrepancies, and the evaluation of developmental differences between sexes.[6]

To improve results obtained by age estimation studies, research that assesses the developmental stages of different human body structures, and not only teeth, can potentially contribute to a greater reliability of age estimation through x-ray analysis, which is commonly requested in clinical practice. In the pilot study carried out by Lajolo et al. (2013) in Italian caucasians,[5] two new oro-cervical radiographic indices – oro-cervical radiographic simplified score and oro-cervical radiographic simplified score without wisdom teeth – were developed to facilitate, expedite, and increase age estimation reliability.

Given the relevance of assessing more than one anatomical structure and considering the possibility of technical improvements to achieve better results, as well as the importance of validity and reliability tests for age estimation methods in different population groups, the first phase objectives of this study consisted of validating an age estimation method using oro-cervical radiographic indices in a Brazilian mixed population without definition of a specific race, as well as assessing the relationship between chronological and estimated age by using the indices. In the second phase, this study assessed the effectiveness of regression equations for age estimation through the dental examinations described by Chaillet & Demirjian[7] and the equations to estimate age by examining cervical vertebrae developed by Caldas et al.,[8] in addition to devising new equations by including dental records and cervical measurements from previous methods.

MATERIALS AND METHODS

This research was submitted and approved by the Ethics and Research Committee of Faculty of Dentistry of University of São Paulo, and was approved by opinion No. 754.502.

The study sample consisted of a total of 1020 radiographic images (510 panoramic radiographs and 510 lateral teleradiographs) from 510 Brazilian individuals aged between 8 and 24.9 years, stratified by sex (female and male) and age group, a total of seventeen groups, each one with the same number of female and male. All radiographic images were obtained previously at Rizzo Clinic – Digital Dental Radiology – located in the city of Feira de Santana (Bahia) – for clinical diagnosis and orthodontic treatment reasons. Panoramic radiographs and teleradiographs were selected from each participant, simultaneously gathered from the collection of images and assessed in this study exclusively for the purpose of age estimation.

Use of oro-cervical radiographic indices – Phase I

In order to apply the radiographic index methodology proposed by Lajolo et al.,[5] the following age estimation methods were initially applied: Demirjian's Method (DM)[9] to estimate the age of the seven lower teeth from the left side; the Cervical Vertebral Maturation Method (CVM);[10] and the Demirjian method modified by Mincer et al.[11] to estimate the age of third molar development (TMD). Following the evaluation of the radiographs and the application of the previously described methods, the two oro-cervical radiographic scores proposed by Lajolo et al.[5] were applied. The Oro-Cervical Radiographic Simplified Score (OCRSS) is based on the sum of the scores adopted for the three previously applied techniques. The second index – Simplified Oro-Cervical Radiographic Score Without Third Molar (OCRSSWWT) – is derived from the combination of the first two methods mentioned above.

Therefore, the sum of the values for the simplified scores obtained for each method corresponds to the radiographic index scores. Based on the simplified values of the oro-cervical radiographic scores, the study sample was divided into three (3) groups (A, B, and C), which represented the increase in chronological age. Group A includes individuals aged 8-13.9 years, group B includes adolescents aged 14-17.9 years, and group C includes individuals aged 18-24.9 years (Figure 1). In order to compare the classification generated by the scores, chronological ages of the studied population were also distributed into groups A, B, and C to allow for the assessment of the accuracy rate of the scores provided by the radiographic indices.

Use of regression equations for age estimation – Phase II

For age estimation using equations, the model established by Chaillet and Demirjian[7] was used. Eight lower teeth from the left side (central incisor to the third molar) were assessed and a score was given for the maturation of each tooth according to tables recommended by the technique. Next, the scores of each of the 8 teeth were summed to obtain a Total Maturity Score (S). The S value was used in regression formulae and chronological ages were estimated for all study participants.

To estimate age through cervical vertebrae using the equations by Caldas et al.,[8] digital lateral teleradiographs were analyzed using the ruler tool in Adobe Photoshop CS6 (Adobe Systems Inc., USA) and, according to the technique, the following measurements were carried out: anterior height of the vertebral body (AH), vertebral body height (H), posterior vertebral body height (PH), and antero-posterior vertebral body length (AP), in the third and fourth cervical vertebrae. After all measurements were taken, regression equations were applied, and chronological ages were estimated for all study patients.

Reliability assessment and statistical analysis

Prior to the analysis of the radiographic images, examiners underwent a period of study and improvement of techniques. The inter-examiner concordance test used a blind methodology and two independent and experienced examiners to apply the age estimation methods.

The data for statistical calculations were entered in Microsoft Excel (Microsoft Co., Redmond, USA) and analyzed using MedCalc v16.4.3 (MedCalc Software, Ostend, Belgium). To assess the reliability of radiographic indices, the Kappa coefficient was calculated for intra and inter-examiner data. A descriptive statistical analysis of the sample was carried out using mean, standard deviation, minimum, and maximum values. Determination coefficients (R^2) for the age estimation methods, accuracy rates of radiographic scores, mean error, and regression equations were also determined. The level of significance for the tests was set at 5%.

RESULTS

Oro-cervical radiographic indexes results

Overall intra-examiner and inter-examiner reliability for the OCRSS was 0.91 ($p<0.001$; CI 95% = 1.0–0.71) and 0.85 ($p<0.001$; CI 95% = 1.0–0.66), respectively. For the OCRSSWWT, intra-examiner Kappa coefficient was 0.83 ($p<0.001$; CI 95% = 1.0–0.61) and inter-examiner Kappa coefficient was 0.73 ($p<0.001$; CI 95% = 0.92–0.53).

Table 1 . Characterization of the sample studied by chronological age group, OCRSS, and OCRSSWWT.

	N	Mean	SD	Minimum	Maximum
Real age group A	180	10.970	1.6979	8.000	13.920
Real age group B	120	15.991	1.1265	14.000	17.920
Real age group C	210	21.510	1.9905	18.000	24.920
OCRSS A	98	10.003	1.4052	8.000	14.000
OCRSS B	256	15.976	3.6285	9.170	24.830
OCRSS C	156	21.342	2.3187	15.830	24.920
OCRSSWWT A	167	11.092	2.1303	8.000	19.420
OCRSSWWT B	114	17.393	3.8411	9.580	24.830
OCRSSWWT C	229	19.933	3.0727	12.750	24.920

A = 8 – 13.9 years, B = 14 – 17.9 years, C = 18 – 24.9 years, SD = standard deviation, OCRSS = oro-cervical radiographic simplified score, OCRSSWWT = oro-cervical radiographic simplified score without wisdom teeth.

The characteristics for the studied population and its distribution based on chronological ages using OCRSS and OCRSSWWT, are reported in Table 1. Distribution of the number of individuals in both the OCRSSWWT and the real age groups A, B, and C was more similar when compared to the distribution of individuals in the OCRSS groups. However, the mean estimated ages using OCRSS were closer to the mean chronological age.

Figure 1. Three radiographic methods of age estimation with their respective stages and scoring for obtaining the simplified score proposed by Lajolo et al. (2013)[5].

Age estimation method	Dental development stage		Score
Demirjian's Method to age estimation of 7 mandibular teeth (DM)	A to G	→	0
	H	→	1
Cervical Vertebral Maturation Method for skeletal age estimation (CVM)	I to III	→	0
	IV	→	1
	V to VI	→	2
Demirjian method modified by Mincer to estimate the age of third molars development (TMD)	A and B	→	0
	C to F	→	1
	G and H	→	2

Oro-cervical radiographic simplified score (OCRSS)	Simplified scores
OCRSS A	0-1
OCRSS B	2-4
OCRSS C	5

Oro-cervical radiographic simplified score without wisdom teeth (OCRSSWWT)	Simplified scores
OCRSSWWT A	0-1
OCRSSWWT B	2
OCRSSWWT C	3

For the assessment of the entire study sample, the accuracy using OCRSS was 54% for the group aged 8-13.9 years, 86.8% for individuals aged 14-17.9 years, and 67.8% for the group aged 18-24.9 years. The success rate was 67.4% (R^2 = 0.64) across all groups when applying OCRSS. Regarding OCRSSWWT, the accuracy of the method was 85% for group A, 38.5% for group B and 77.4% for group C. The overall accuracy for the OCRSSWWT method was 70.8% (R^2 = 0.62).

The analysis of results using the OCRSS method for females and males shows a higher accuracy for males (70.7%), except for group B in which the results for females had an accuracy of 90.3% compared to 83.3% for males. According to the OCRSSWWT results, age estimation for males was more accurate for all age groups (A, B, and C), with an average accuracy of 75.4% for males and 66.1% for females (Table 2).

Table 2. Distribution of the sample based on OCRSS and real age, and OCRSSWWT and real age.

OCRSS		REAL AGE		
	A	B	C	
A	97	1	0	98
B	83	106	67	256
C	0	15	141	156
Total	180	122	208	510
Hit rate (%)	54%	86.8%	67.8%	67.4%
OCRSSWWT		REAL AGE		
	A	B	C	
A	153	12	2	167
B	22	47	45	114
C	5	63	161	229
Total	180	122	208	510
Hit rate (%)	85%	38.5%	77.4%	70.8%
OCRSS (Females)		REAL AGE		
	A	B	C	Total
A	42	0	0	42
B	48	56	37	141
C	0	6	65	71
Total	90	62	102	254
Hit rate (%)	46.6%	90.3%	63.7%	64.2%
OCRSSWWT (Females)		REAL AGE		
	A	B	C	Total
A	67	2	0	69
B	18	23	24	65
C	5	37	78	120
Total	90	62	102	254
Hit rate (%)	74.4%	37.1%	76.5%	66.1%
OCRSS (Males)		REAL AGE		
	A	B	C	Total
A	55	1	0	56
B	35	50	30	115
C	0	9	76	85
Total	90	60	106	256
Hit rate (%)	61.1%	83.3%	71.7%	70.7%
OCRSSWWT (Males)		REAL AGE		
	A	B	C	Total
A	86	10	2	98
B	4	24	21	49
C	0	26	83	109
Total	90	60	106	256
Hit rate (%)	95.5%	40%	78.3%	75.4%

Group A = 8 – 13.9 years, Group B = 14 – 17.9 years, Group C = 18 – 24.9 years, OCRSS = oro-cervical radiographic simplified score, OCRSSWWT = oro-cervical radiographic simplified score without wisdom teeth.

To determine how much of the chronological age variability could be explained by the individual and combined age estimation methods, the adjusted determination coefficients were calculated. The R^2 value of the DM was 69%, higher than the value of 46% found for CVM.
The method that detected maturation stages of the third molar showed the highest determination coefficient (R^2 = 0.83). For the radiographic indices associated with the previously mentioned methods, the R^2 value for OCRSS was 0.64 and 0.62 for OCRSSWWT.

Results concerning the use of linear regression equations for age estimation
The use of age estimation equations described by Chaillet and Demirjian[7] for the whole study sample (510 participants), showed a maximum age prediction limit of 16 years using this technique. For the method of Caldas et al.,[8] the maximum value of estimated age was approximately 19 years. Although the equations were used for the whole sample (8-24.9 years), for analyzing the data, the group corresponding to the age group of 8 to 18 years (300 individuals) was selected.
A comparison between chronological age and age estimated by the two equations (scoring eight lower teeth on the left side and measuring cervical vertebrae) was carried out using the Mean Squared Error (MSE) for each method, calculated as follows:

$$MSE = \frac{1}{n}\sum\left(Chronological\,Age - Age\,predicted\,by\,the\,equation\right)^2$$

The MSE for the equations of Chaillet and Demirjian[7] was 1.65. A Mean Error (ME) of 1.29 was obtained by applying the square root (√), i.e., on average, this method showed an error of 1.3 years. For the equations developed by Caldas et al.,[8] the MSE was 3.57 and ME was equal to 1.9 years older or younger than the chronological age.
With the aim of developing new age estimation equations, a linear regression model was used, with chronological age as the dependent variable and using the same variables adopted by Caldas et al.[8] and Chaillet and Demirjian[7] as independent variables, in addition to their interactions with the variable 'sex'. The estimated parameters of the final model are reported in Table 3.
The new equations developed from the combination of variables obtained by dental measurements and cervical vertebrae are:

Chronological age (female) = - 285.6183 + 2.699277 x (AH3/AP3) + 0,451021 x (AH4/AP4) + 0,0007371 x (S value^3) - 0.1637731 x (S value^2) + 12.05215 x (S value).

Chronological age (male) = - 215,7111 + 1.755609 x (AH3/AP3) + 1.564029 x (H4/AP4) + 0,0005675 x (S value^3) - 0,1251991 x (S value^2) + 9.181172 x (S value).

For this proposed model, the MSE was 1.016; with a square root of 1.008 years. Thus, the new equations in this study showed an average error of 1.04 year (Table 4). Therefore, the equations produced estimated ages with high accuracy when compared to the equations which used individual age markers.

Table 3. Estimated parameters for the new proposed model for age estimation by including dental and cervical vertebrae data.

Estimated coefficients	Estimated	Standard error	t value	P value
Intercept: Female	-285.6183	38.9638	-7.33	0.000
(AH3 / AP3)	2.699277699	1.569079	0.28	0.087
(AH4 / AP4)	0.451021	1.623739	9,61	0.000
(S value^3)	0.0007371	0.0000767	-8.79	0.000
(S value^2)	-0.1637731	0.0186311	8.10	0.000
(S value)	12.05215	1.487366	-7.33	0.000
Intercept: Male	-215.7111	38.9147	-5.54	0.000
(AH3 / AP3)	1.755609	1.160963	1.51	0.132
(H4 / AP4)	1.564029	2.413496	0.65	0.518
(S value^3)	0.0005675	0.0000809	7.02	0.000
(S value^2)	-0.1251991	0.0192922	-6.49	0.000
(S value)	9.181172	1.511698	-6.07	0.000

AH3 = distance from the top of the front part to the tangent of the third vertebrae lower part, AP3 = Antero-posterior distance at middle of third cervical vertebral body; AH4 = distance from the top of the front part to the tangent of the fourth vertebrae lower part, AP4 = Antero-posterior distance at middle of fourth cervical vertebral body, S value = Total maturity score of the eight lower teeth.

Table 4. Results regarding the mean square error (MSE) and mean error (ME) of the age estimation equations applied in this study.

Age range	Equations of Chaillet and Demirjian (2004)		Equations of Caldas et al. (2007)		Equations with methods association	
	MSE	**ME**	**MSE**	**ME**	**MSE**	**ME**
8 – 18 years (n = 300)	1.65	1.29	3.57	1.89	1.01	1.04

MSE = mean square error, ME = mean error.

DISCUSSION

In a pilot study with sixty Italians,[5] the intra and inter-examiner reliability of radiographic indices were not evaluated, being one of limitations presented by the authors. In the present study, the reproducibility tests of the method were performed and it was found that the OCRSS and OCRSSWWT showed a satisfactory intra and inter-examiner agreement.

The accuracy of the OCRSSWWT method for group A (8-13.9 years) was 85%, higher than the 54% accuracy of the OCRSS method, confirming the hypothesis that in many cases, the assessment of the development stage of third molar results in an over-estimation of the chronological age due to its advanced maturation, which can be compatible with an age above the real age of a child or adolescent. Aynsley-Green et al.[12] emphasized the possibility of attributing a more advanced age to individuals who presented early maturation and, nevertheless, a lower chronological age regarding their development stage.

In general, the index which did not evaluate third molars (OCRSSWWT), produced more accurate results. However, it is important to note that accuracy was greater for OCRSS group B, which suggests the importance of assessing third molar mineralization for the age group between 14-17.9 years. For OCRSSWWT group C, accuracy was 77.4 %, with some cases of individuals in group B and a few in group A. In the pilot study,[5] ages of individuals between 18-24.9 years were also under-estimated. One possibility to improve results for individuals between 18 and 21 years of age consists of examining both the third molars and the clavicle, so that combination of these two methods produces better results.[13] Clavicular epiphysis can be easily examined in dry bone or through imaging with computed tomography.[14,15]

Martrille et al.[16] recommend that a large number of dental and skeletal indicators should be used to estimate chronological age. However, to increase the potential of each method, the final assessment should consider the method or methods with higher accuracy for a particular age group.

According to the guidelines of previous studies,[17,18] to improve the performance of research in age estimation, a study sample should consist of females and males distributed in age groups with equal numbers in order to compose a more homogeneous sample group, thus favoring more reliable statistical results. However, the radiographic indices proposed by Lajolo et al.[5] categorize individuals into groups A (8 to 13.9 years), B (14 to 17.9 years), and C (18 to 24.9 years), that is, combine several age groups into a single group. For many individuals over 21 years of age, dental and vertebral maturation have already been completed. Moreover, age estimation by these intervals has the complicating factor of the analysis of individuals who are close to the cut-off points between groups, since it is common for these individuals to have their ages over-estimated for the adjacent age group.[19]

Females ages were over-estimated more often and therefore had lower accuracy compared to male ages, which can possibly be explained by hormonal factors that result in an earlier maturation of females, leading to an over-estimated age.[5] These findings corroborate previously published results,[20,21] in which all variables for age estimation showed higher accuracy in males in comparison with females, since females reached maturation stages earlier than males.

The accuracy of each method in estimating chronological age through the stages of development of biological characteristics is limited, since it is necessary to consider biological variability and variation of maturation rates among individuals of the same age. Acceleration or delay of biological development of the different structures of the human body does not proceed in perfect agreement, the combination of more than one age estimation technique can provide results of estimated ages with more accuracy when compared to the practice of an isolated method, where error variance tends to be greater.[13,22] Association of methods can improve results to discriminate whether an individual is under or over 18 years old, this critical age represents the imputability age in Brazil and other countries in South America. In this sense, a method of age estimation with regression equation seems to be better to predict age than only scores classification, since difficulty is on the threshold of 18 years.

In this work, equations that evaluated dental characteristics presented an average error lower than the equations that only analyzed measurements of the cervical vertebrae. When we tested a new model with the combination of the data from the vertebrae and the teeth, the error was attenuated and the model produced age estimates with an average error equal to 1 year. Therefore, the new equations presented in this work produced estimated ages with greater accuracy when compared to the equations that used individual age markers. This result is in accordance with previous studies,[23-25] which state that the use of regression equations using a combination of data from different anatomical structures has the ability to reduce errors in the estimated age group by reducing global variability. It is important to mention that the newly developed formulae including dental and skeletal variables were adjusted according to the sample in our study, which favours the results. Thus, it is recommended that future research apply these equations to a new, independent sample.

CONCLUSIONS

This study represents the first research on the applicability of the method proposed by Lajolo et al.[5] in Brazilians, using a large sample. The simplicity of the method, following adequate training, confirms the possibility that it can be used in forensic practice. However, it is suggested that the method can be used to support other techniques which produce an estimated age value, not only to classify an individual into a comprehensive age group. Thus, it is possible to apply the new equations proposed in this study, which include data from cervical vertebrae with dental development information, to obtain age estimation with greater accuracy.

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