Science & Justice 59 (2019) 442–447

Contents lists available at ScienceDirect

Science & Justice

journal homepage: www.elsevier.com/locate/scijus

Accuracy of four dental age estimation methodologies in Brazilian and Croatian children

Luany Cristina Pongo da Luz[a], David Anzulović[b], Eduardo N. Benedicto[a], Ivan Galić[b], Hrvoje Brkić[c], Maria Gabriela H. Biazevic[a,*]

[a] Department of Community Dentistry, School of Dentistry, Universidade de São Paulo – USP, São Paulo, SP, Brazil
[b] Departments of Research in Biomedicine, Health and Dental Medicine, University of Split School of Medicine and University Hospital Centre, Split, Croatia
[c] University Hospital Center Zagreb, School of Dental Medicine, University of Zagreb, Zagreb, Croatia

ARTICLE INFO

Keywords:
Forensic anthropology population data
Forensic odontology
Forensic dentistry
Orthopantomograms
Age estimation
Mineralisation

ABSTRACT

Objective: To compare and analyse the accuracy of four age estimation methods using the mineralisation stages of the permanent teeth (Cameriere et al. [16] [CAM], Liliequist and Lundberg [LLH] and Nolla without third molars [NOL7] or with them [NOL8]) in a mixed population of Brazilians and a homogeneous population of Croatians.
Methods: Orthopantomograms of 930 Brazilians (366 males and 564 females) and 924 Croatians (365 males and 556 females) aged between 8 and 14.99 years were analysed using the CAM, LLH, NOL7 and NOL8 age estimation methodologies.
Results: LLH presented the best absolute differences (ADs) among both populations without sex stratification, while CAM presented the worst results. In addition, the mean differences revealed underestimations, except when the LLH and NOL7 methods were used for the Brazilians. When the sample was stratified by sex, the best AD values were found with NOL7 (0.80) for the Brazilians and with LLH (0.98) for the Croatians. When the sample was stratified by sex and age, CAM presented high accuracy at the early ages, and LLH presented high accuracy at the older ages. The results obtained with the Nolla methods (NOL7 and NOL8) were mostly similar, but NOL7 yielded slightly better results.
Conclusions: The values for the Brazilians and the Croatians were relatively similar, and the techniques were properly applied in both population samples. The best method for evaluating both countries was LLH, followed by NOL7, NOL8 and CAM.

1. Introduction

The forensic sciences are facing new challenges, and forensic techniques need to be updated due to the constant changes in population makeup resulting from recent migratory flows. Civil wars and conflicts in nations with low political stability are causing thousands of people to spread throughout the world seeking opportunities for survival. Survivors are being welcomed by several countries, and they are being supported by advances in the discussion and regulation of human rights around the world. This phenomenon may cause a gradual change in the population makeup of the countries that received those refugees, making them more heterogeneous in the future.

The Brazilian population is an example of a heterogeneous population. Historically, miscegenation in Brazil began with the incorporation of Portuguese and African populations into native populations. According to a census organized by the Brazilian Institute of Geography and Statistics [1], the population of Brazil in 2010 comprised 47.52% white individuals, 7.52% black individuals, 1.10% Asian individuals, 0.43% native individuals and 43.42% brown individuals (a mixture of any of the previous combinations). Conversely, countries such as Croatia are not composed of heterogeneous populations as Brazil is. In 2011, a census [2] showed that Croatia was inhabited mostly by Croatians (90.42%), and the second most significant ethnic group (Serbs) represented only 4.36% of the population; both of these groups are classified as white.

In the field of forensic science, age estimation is an important resource for solving both criminal and civil conflicts. Corradi et al. [3] stated that an accurate age estimation for civil law might not satisfy

* Corresponding author at: Community Dentistry Department, School of Dentistry, Universidade de São Paulo (FOUSP), Avenida Professor Lineu Prestes 2227, 05508-000 São Paulo, Brazil.
E-mail address: biazevic@usp.br (M.G.H. Biazevic).

https://doi.org/10.1016/j.scijus.2019.02.005
Received 8 May 2018; Received in revised form 4 February 2019; Accepted 17 February 2019

criminal legal criteria, especially in countries in which decisions are based on proof 'beyond reasonable doubt'. To assist the justice systems, age estimation methods were created and validated for different populations, and diverse techniques have been used with varying quality of the results [4–6]. Researchers have classified those methods as either objective or subjective [7], according to how they are assessed.

The objective method of Cameriere is frequently considered one of the best available [8–10], and this method has already been investigated for both Brazilian [11] (with a reduced sample of only 160 individuals) and Croatian [12] populations (concurrently with two other ethnicities that comprise the population of Bosnia-Herzegovina). The methods developed by Liliequist and Lundberg [13] and Nolla [14] (including or excluding the third molars) are considered subjective. No evidence of the evaluation of the Croatian population using the Nolla method (with or without the third molars) was found in the literature by the authors of this study, and only one article [15] was found that exclusively studied the method using the third molars. In addition, the Liliequist and Lundberg method has never been applied to this population.

The objective of this study was to compare two populations, one with a high degree of miscegenation (Brazilian) and one with a low degree of miscegenation (Croatian), to verify the differences in age estimation and the accuracies of the four age estimation methodologies. These results may help professionals in the fields of orthodontics, forensic dentistry or legal medicine to understand the effects of miscegenation and ethnicity on teeth mineralisation chronology in two highly different populations.

2. Materials and methods

Orthopantomograms (OPGs) of 930 Brazilians (366 males and 564 females) and 924 Croatians (365 males and 556 females) with ages ranging from 8 to 14.99 years were used (Table 1). The difference in the total numbers used in each method was due to the need to include as many OPGs as possible; thus, an X-ray that was considered appropriate for a certain technique might have been used in one case but excluded in another case. The Brazilian radiographs were obtained from a private radiology clinic (Campina Verde-MG, Brazil) and were acquired using an Orthophos XG 3D CEPH – (PAN/CEPH/TOMO®– Sirona/Germany). The Croatian radiographs were obtained from the archives of the School of Dental Medicine of the University of Zagreb, and some of the radiographs were not digital.

The OPGs included in the study included data regarding the patient's sex and date of birth and the date when the X-rays were taken. The study excluded all OPGs of individuals who presented anomalies (systemic diseases, growth disorders or syndromes), any X-rays with a low quality, as well as distorted or elongated images. At the end of the selection, the OPGs were submitted for analysis using the age estimation methods of Cameriere et al. [16] (CAM), Liliequist and Lundberg [13] (LLH) and Nolla [14] (NOL7–Nolla without the third molars; and NOL8–Nolla with the third molars).

The CAM method [16] is fully explained in the original article. Briefly, the method was applied by measuring the panoramic X-rays using Adobe Photoshop CS6® and adding the numbers in the formula that was developed by the authors. For teeth with one root, the distance (A_i, i = 1 to 5) between the inner sides of the open apex was measured. For teeth with two roots (A_i, i = 6 and 7), the values were the sum of the distances between the inner sides of the two open apices. When considering the possible differences in magnifications and angulations among the X-rays, it was necessary to normalize the data by dividing it by tooth length (L_i, i = 1 to 7). Finally, dental maturity was evaluated according to the sum of the normalized open apices (S).

The LLH method [13] consisted of separating the panoramic X-rays according to sex and then classifying the lower left teeth (with one or more roots) according to calcification scores. Afterwards, a small adjustment was necessary. The authors showed the results by age intervals, an approach that is not commonly used in the present day. To adapt these results to a specific age, the method required a table that was developed by Hägg and Matsson [17].

The Nolla methods [14] were developed to use in two different ways according to the original article. One way involves using all of the upper and lower teeth from one side, except for the third molars (NOL7), and the other way involves using all of teeth included in the NOL7 method plus the third molars (NOL8). These methods involve the observation and identification of the mineralisation stages of the teeth based on a reference table [14], and the teeth are classified according to 11 phases. The phases include no calcification but range from the identification of calcification at the crown (phase 0) to the presence of a completely closed apex (phase 10). After the classification of each tooth, the phases are summed, and the results are viewed on a reference table that provides the dental age (DA) estimation [14]. The same sex-specific tables used by Nolla were used for the DA estimation in the present study.

This study was initiated after three examiners (ENB, DA and LCPL) underwent training and calibration for the previously mentioned methods. After calibration, intra- and inter-examiner agreement for 20% of the sample was analysed at a 2-month interval using the intraclass correlation coefficient (ICC). Adobe Photoshop CS6® was used to view the images.

Both DA and chronological age (CA) were checked for normality using a skewness and kurtosis test. The results were checked using either a paired *t*-test or a Wilcoxon test for paired samples, depending on their normality. To evaluate whether the methodologies under- or overestimated the ages, the differences of the means (DA - CA) were applied. To obtain absolute accuracy, the mean absolute differences (ADs) were calculated. All of the data are presented in the following ways: without sex stratification; with sex stratification; and with sex stratification and age cohorts.

The study used Microsoft Excel® (Microsoft, Redmond, WA, USA), STATA® 13.0. (StataCorp, College Station, Texas, USA), and Medcalc® (Medcalc® Software, Mariakerke, Belgium) to analyse all of the data, and the level of significance for all tests was 95%.

The University of São Paulo (FOUSP) Ethics and Research Committee approved this research under process number 1.556.100. In addition, all ethical standards defined in the Helsinki Declaration on human experimentation were observed.

3. Results

The inter-examiner evaluation for the CAM, LLH, NOL7 and NOL8 methods resulted in ICCs with average measures of 0.8951, 0.9225, 0.9176 and 0.9413, respectively. The intra-examiner evaluations for the CAM, LLH, NOL7 and NOL8 methods yielded ICCs of 0.9166, 0.9202, 0.9866 and 0.9856, respectively.

Table 1 presents the differences in the means and the ADs for the two countries, both with separation by sex and without separation by sex (or age). In terms of accuracy (AD), the method with the worst results for Brazilians was CAM (1.26 years), and the method that best evaluated the Croatians was LLH (0.99); the latter method showed no statistical significance between DA and CA. Furthermore, the results of the NOL7 and NOL8 methods for Brazilians did not differ significantly.

Additionally, when the sample was not separated by sex, there was no statistically significant difference in the results of the NOL7 method for Brazilian and Croatian males, the LLH method for Brazilian females and the NOL7 method for Brazilian females. The ages were underestimated in all of the females except for the Brazilian females when LLH and NOL7 were used. In males, age underestimation occurred only with the CAM method for both populations and for with NOL7 method for the Croatians.

Table 2 shows the accuracy of the age estimation methods according to country, sex and age cohort. Among 9.00- to 9.99-year-old and 10.00- to 10.99-year-old females, no differences were found in the

Table 1
Results of mean differences in years (DA - CA) between the Dental Age (DA) and Chronological Age (CA) for each method and country, for the sexes analysed together and separately.

Method	Country	n	Sex	CA		DA		DA-CA		95% CI of DA-CA		AD		AD median	Test Z[a]	Test t[b]	P[a]	P[b]
				Mean	SD	Mean	SD	Mean	SD			Mean	SD					
CAM	Brazil	930	M + F	12.17	1.76	11.13	1.47	−1.05	1.13	−1.12	−0.97	1.26	0.89	1.11	21.45	−28.18	< 0.001*	< 0.001*
CAM	Croatia	902	M + F	12.17	1.79	10.98	1.60	−1.19	1.32	−1.28	−1.11	1.38	1.12	1.13	21.50	−27.17	< 0.001*	< 0.001*
LLH	Brazil	820	M + F	11.94	1.73	12.10	1.40	0.16	1.08	0.08	0.23	0.88	0.65	0.75	−3.86	4.20	< 0.001*	< 0.001*
LLH	Croatia	850	M + F	12.03	1.77	11.98	1.47	−0.05	1.27	−0.14	0.03	0.99	0.81	0.81	−0.35	−1.17	0.727	0.242
NOL07	Brazil	930	M + F	12.17	1.76	12.23	1.81	0.05	1.16	−0.02	0.13	0.91	0.72	0.75	−0.74	1.43	0.458	0.153
NOL08	Brazil	930	M + F	12.17	1.76	12.17	2.00	−0.01	1.32	−0.09	0.08	1.05	0.80	0.83	−0.23	−0.16	0.820	0.874
NOL07	Croatia	924	M + F	12.18	1.78	11.87	1.76	−0.31	1.44	−0.41	−0.22	1.07	1.01	0.80	4.96	−6.65	< 0.001*	< 0.001*
NOL08	Croatia	921	M + F	12.19	1.78	11.88	1.96	−0.31	1.53	−0.41	−0.21	1.18	1.02	0.93	5.53	−6.18	< 0.001*	< 0.001*
CAM	Brazil	366	M	12.10	1.82	11.02	1.54	−1.08	1.13	−1.20	−0.96	1.27	0.91	1.06	−18.34	13.81	< 0.001*	< 0.001*
CAM	Croatia	357	M	12.09	1.83	10.88	1.53	−1.20	1.25	−1.33	−1.07	1.38	1.05	1.16	−18.20	13.80	< 0.001*	< 0.001*
LLH	Brazil	336	M	11.94	1.79	12.27	1.38	0.33	1.07	0.21	0.44	0.91	0.65	0.75	5.62	−5.38	< 0.001*	< 0.001*
LLH	Croatia	352	M	12.04	1.84	12.21	1.37	0.17	1.20	0.04	0.29	0.99	0.70	0.86	2.59	−3.19	0.010*	0.001*
NOL7	Brazil	366	M	12.10	1.82	12.19	1.56	0.09	0.97	−0.01	0.19	0.80	0.56	0.74	1.80	−1.71	0.073	0.088
NOL8	Brazil	366	M	12.10	1.82	12.45	2.02	0.34	1.20	0.22	0.47	1.03	0.71	0.91	5.45	−5.57	< 0.001*	< 0.001*
NOL7	Croatia	365	M	12.11	1.84	11.98	1.65	−0.13	1.43	−0.27	0.02	1.08	0.94	0.85	−1.71	0.75	0.088	0.452
NOL8	Croatia	365	M	12.11	1.84	12.30	2.05	0.19	1.56	0.03	0.35	1.23	0.98	1.02	2.29	−2.82	0.023*	0.005*
CAM	Brazil	564	F	12.22	1.73	11.19	1.42	−1.03	1.14	−1.12	−0.93	1.26	0.88	1.13	−21.41	16.40	< 0.001*	< 0.001*
CAM	Croatia	545	F	12.23	1.76	11.04	1.64	−1.19	1.36	−1.30	−1.07	1.39	1.16	1.11	−20.32	16.48	< 0.001*	< 0.001*
LLH	Brazil	484	F	11.94	1.69	11.98	1.41	0.04	1.08	−0.06	0.14	0.86	0.64	0.74	0.84	−0.42	0.402	0.677
LLH	Croatia	498	F	12.02	1.72	11.82	1.52	−0.20	1.30	−0.32	−0.09	0.98	0.88	0.76	−3.51	2.33	0.001*	0.020*
NOL7	Brazil	564	F	12.22	1.73	12.25	1.96	0.03	1.27	−0.07	0.14	0.99	0.79	0.75	0.57	0.31	0.570	0.760
NOL8	Brazil	564	F	12.22	1.73	11.98	1.97	−0.23	1.34	−0.35	−0.12	1.06	0.86	0.83	−4.13	4.21	< 0.001*	< 0.001*
NOL7	Croatia	559	F	12.23	1.75	11.80	1.83	−0.44	1.44	−0.56	−0.32	1.07	1.05	0.75	−7.20	5.85	< 0.001*	< 0.001*
NOL8	Croatia	556	F	12.24	1.74	11.60	1.85	−0.64	1.41	−0.76	−0.52	1.15	1.05	0.85	−10.64	9.65	< 0.001*	< 0.001*

AD: Absolute Difference.
M – Male; F – Female; CAM – Method of Cameriere et al. [16]; LLH – Method of Liliequist & Lundberg [13] with the table of Hagg & Matsson [17]; NOL7 – Method of Nolla without third molars; NOL8 – Method of Nolla with third molars; CI –Confidence Interval; SD – Standard Deviation; n – Sample number.
[a] Paired Test t.
[b] Paired Wilcoxon test.
* Statistically significant (5%).

accuracy of the methods; the same was observed among 10.00- to 10.99-year-old and 11.00- to 11.99-year-old males. Among males in the age range of 12.00–12.99 years, the LLH technique results were similar to the NOL7 and NOL8 results for both the Croatian and Brazilian samples. The Online Supplementary Figs. S1-S4 show detailed comparisons of the relationship between DA and CA in the groups according to sex and country.

4. Discussion

Both examination evaluations (intra- and inter-examiner) indicated excellent reliability [18], thus showing that all the methods are reproducible.

Regarding the accuracy for the two populations, when both the male and female groups were evaluated together, the best results were obtained with the LLH method (0.88 years) for the Brazilians and the worst were obtained with the CAM method (1.38) for the Croatians. The AD values for the Croatians (1.07) with the NOL7 method were similar to those reported in the study by Kumaresan et al. [9] (1, 1) but were inferior to those reported by Mohammed et al. [19] (0.65). The non-sex distinction also determined that only the LLH and NOL7 methods for Brazilians resulted in age overestimations, similar to the findings of other studies [9,19,20] that used NOL7, while the CAM values for both countries resulted in age underestimations, which were

Table 2
Accuracy of the age estimation techniques according to the country, sex and age cohort.

Age groups	Method	Brazil - Female					Croatia - Female					Brazil - Male					Croatia - Male				
		n	AD	SD	SE	AD median	n	AD	SD	SE	AD median	n	AD	SD	SE	AD median	n	AD	SD	SE	AD median
8.00–8.99	CAM	34	0.57	0.70	0.12	0.36	34	0.51	0.43	0.07	0.36	28	0.50	0.32	0.06	0.51	28	0.52	0.41	0.08	0.42
	LLH	34	1.17	0.79	0.14	0.83	34	0.91	0.73	0.12	0.76	28	1.60	0.53	0.10	1.63	28	1.47	0.46	0.09	1.47
	NOL7	34	0.80	0.73	0.13	0.67	34	0.68	0.55	0.09	0.58	28	0.73	0.62	0.12	0.62	28	1.06	1.25	0.24	0.75
	NOL8	34	0.71	0.71	0.12	0.59	33	0.59	0.47	0.08	0.44	28	0.66	0.54	0.10	0.58	28	1.10	1.51	0.28	0.61
9.00–9.99	CAM	33	0.70	0.50	0.09	0.67	33	0.85	0.47	0.08	0.73	32	0.71	0.42	0.07	0.63	30	0.71	0.65	0.12	0.54
	LLH	33	1.12	0.59	0.10	1.00	33	0.89	0.63	0.11	0.68	32	1.31	0.60	0.11	1.29	32	1.17	0.84	0.15	1.05
	NOL7	33	0.66	0.68	0.12	0.42	33	0.74	0.61	0.11	0.50	32	0.87	0.68	0.12	0.66	32	1.21	0.87	0.15	1.10
	NOL8	33	0.80	0.71	0.12	0.50	33	0.91	0.76	0.13	0.59	32	0.96	0.65	0.11	0.87	32	1.14	1.07	0.19	0.92
10.00–10.99	CAM	62	0.82	0.66	0.08	0.63	59	0.82	0.59	0.08	0.77	40	0.77	0.47	0.07	0.73	40	0.84	0.65	0.10	0.79
	LLH	62	0.70	0.66	0.08	0.50	61	0.86	0.73	0.09	0.60	40	0.89	0.45	0.07	0.83	40	0.85	0.56	0.09	0.74
	NOL7	62	0.82	0.61	0.08	0.74	61	0.97	0.80	0.10	0.75	40	1.01	0.59	0.09	1.13	40	1.00	0.79	0.12	0.79
	NOL8	62	0.82	0.65	0.08	0.67	60	0.96	0.62	0.08	0.93	40	1.08	0.60	0.09	1.17	40	1.01	0.80	0.13	0.80
11.00–11.99	CAM	94	1.12	0.71	0.07	1.02	91	1.15	0.91	0.10	0.93	51	1.11	0.68	0.09	0.98	52	1.06	0.80	0.11	0.83
	LLH	93	0.79	0.64	0.07	0.67	93	0.88	0.65	0.07	0.75	50	0.63	0.64	0.09	0.46	52	0.90	0.54	0.07	0.85
	NOL7	94	0.75	0.59	0.06	0.67	93	0.77	0.71	0.07	0.58	51	0.82	0.55	0.08	0.75	52	0.90	0.64	0.09	0.91
	NOL8	94	0.91	0.72	0.07	0.79	93	0.94	0.75	0.08	0.77	51	0.92	0.72	0.10	0.74	52	1.18	0.83	0.11	1.07
12.00–12.99	CAM	119	1.13	0.77	0.07	1.00	112	1.39	1.12	0.11	1.31	70	1.31	0.90	0.11	1.18	68	1.56	1.01	0.12	1.53
	LLH	111	0.83	0.56	0.05	0.67	113	0.83	0.68	0.06	0.71	68	0.80	0.62	0.08	0.59	68	0.71	0.61	0.07	0.63
	NOL7	119	0.95	0.91	0.08	0.67	118	1.09	0.99	0.09	0.76	70	0.68	0.50	0.06	0.59	68	0.83	0.61	0.07	0.74
	NOL8	119	1.03	0.83	0.08	0.75	118	1.11	1.04	0.10	0.68	70	1.03	0.65	0.08	0.95	68	1.25	0.81	0.10	1.05
13.00–13.99	CAM	121	1.47	0.88	0.08	1.46	117	1.58	1.19	0.11	1.31	90	1.50	0.91	0.10	1.32	88	1.70	1.12	0.12	1.74
	LLH	93	0.75	0.53	0.05	0.66	95	0.91	0.92	0.09	0.65	79	0.79	0.53	0.06	0.67	82	0.93	0.61	0.07	0.84
	NOL7	121	1.15	0.80	0.07	1.09	119	1.08	1.20	0.11	0.65	90	0.61	0.42	0.04	0.59	90	1.10	1.13	0.12	0.70
	NOL8	121	1.09	0.80	0.07	0.91	118	1.19	0.99	0.09	0.90	90	1.13	0.71	0.07	1.13	90	1.43	0.96	0.10	1.37
14.00–14.99	CAM	101	1.96	0.86	0.09	1.90	99	2.18	1.40	0.14	1.89	55	2.08	0.97	0.13	2.01	51	2.19	1.01	0.14	2.21
	LLH	58	1.08	0.74	0.10	1.01	69	1.65	1.31	0.16	1.48	39	0.92	0.80	0.13	0.67	50	1.31	0.91	0.13	1.27
	NOL7	101	1.34	0.81	0.08	1.34	101	1.59	1.36	0.14	1.16	55	1.09	0.59	0.08	1.17	55	1.54	0.98	0.13	1.39
	NOL8	101	1.55	1.08	0.11	1.50	101	1.70	1.47	0.15	1.34	55	1.15	0.90	0.12	0.83	55	1.21	1.04	0.14	0.83

AD – Absolute difference; SD – Standard Deviation; SE – Standard Error; CAM – Method of Cameriere et al. [16].
LLH – Method of Liliequist & Lundberg [13] with the table of Hagg & Matsson [17]; NOL7 – Method of Nolla without third molars; NOL8 – Method of Nolla with third molars.

also similar to the reports of previous studies [9,21,22].

When the sample was separated by sex, the best accuracy was found with the NOL7 (0.80) method for Brazilians, and the worst accuracy was found with the CAM (1.39) method for the Croatians. Both countries tended to present values close to 1.00, in agreement with one previous study [9] and contradicting studies that showed an accuracy near 0.53–0.75 [12,19]. In the same stratification, the ages were underestimated for both sexes when the NOL7 method was used for the Croatians and for both sexes and both countries when the CAM method was used. The CAM method results were in agreement with previous reports [9,21,23,24] of age underestimations, but these results were contradicted by other studies [20,22,25].

The distinction between sex and age revealed interesting results (Table 2). In both populations, the values with the best accuracy for both sexes were obtained with the CAM method for 8-year-olds, and the worst values were obtained with the CAM method for 14-year-olds. Other studies have shown different accuracies, with one study observing the best age for the CAM was 9 years [26], another study reporting that the best ages for the CAM were 10 years for males and 12 years for females [12], and still another study indicating that the best age for the CAM was 13 years for females [27].

Using other methodologies and the same stratification, the males showed the worst accuracy when the LLH and NOL7 methods were used. For the females, there were only coincidences among the populations in terms of the best accuracy of the NOL8 method for 8-year-olds and the worst accuracy for the NOL7 and NOL8 methods for 14-year-olds.

The difference in the means in both populations (with sex and age stratifications) revealed that the ages were underestimated for 14-year-olds with all the methods. The CAM method underestimated the ages of males at all ages (except for 8-year-old Croatians). For the 9- to 12-year-olds, the CAM method underestimated the age, and the LLH, NOL7 and NOL8 methods presented overestimations. Among 13-year-old Brazilians, only the NOL8 method overestimated the age.

When the sample was stratified by sex and age, it showed that for 8-year-old females from both countries, overestimation occurred with all methods. For 9- to 10-year-olds, ages were underestimated by CAM for all countries and by NOL8 for Brazilians. The relationship between the under- and overestimations was the same in both countries for children aged 8, 11, 13 and 14 years old.

Once sex and age stratifications were applied (Supplementary Figs. S1-S4), the results revealed a statistically non-significant result when CAM was used for males aged 8 and 9 years and a statistically non-significant result when LLH was used for 12-year-old males. For the females, the same result occurred with both CAM and NOL8 for 8-year-olds; with both NOL7 and NOL8 for 9-year-olds; with NOL8 for 10-year-olds; and with LLH, NOL7 and NOL8 for 11-year-olds.

Moreover, when the data were stratified between sex and age, there was a clear tendency of the CAM method to more accurately evaluate early ages, from 8 to 10 years old; however, the results worsened beyond 10 years. The AD for both countries tended to progressively rise, except in the case of female Croatians aged 9–9.99 years, which showed a similar behaviour to that described in the study of Gulsahi et al. [26] for both sexes. Conversely, these results contradicted the results of other studies [12,27], which showed inconsistent increasing and decreasing values. in regard to inconsistent increasing and decreasing values.

Fernandes et al. [11] were the first to investigate the accuracy of the CAM method in Brazilians, but the values were restricted to mean errors (differences of the means) and two-years age cohort intervals. This is in contrast with our study, which presents both the differences of the means and the absolute difference/mean absolute errors and evaluates 1-year age cohorts.

Table 2 shows that LLH initially yielded the worst results for all populations; however, at older ages, LLH had high accuracy. Both NOL7 and NOL8 appeared to have similar results most of the time; however, NOL7 yielded slightly better results, possibly because of the high variability of the mineralisation stage of the third molar, which is considered in NOL8 but not in NOL7. Additionally, the NOL7 method underestimated age in both populations and sexes at the ages of 8–12 years, except in the case of NOL7 for 12-year-old Brazilians. These data contrast with data reported by other studies [25], wherein all values were underestimations.

For males, the over- and underestimation patterns were the same for the ages 9–12 years and 14 years, whereas for females, the patterns were similar for 8-, 11-, 13- and 14-year-olds. The hypothesis that tooth development is affected by miscegenation might not be true because our study examined two different populations and found that both populations presented similar results, especially in the patterns of over- and underestimations. Other studies that have focused on Brazilian samples and different DA estimation techniques [28,29] have reached the same conclusions.

When comparing our results with a previous study [29] (underestimation = −0.58 and AD = 0.97) of Brazilians using the LLH method without separating by sex, our study overestimated the age (0.16) and presented better accuracy (0.88). When only the male subjects were observed, the other study [29] showed contrasts (underestimated = −0.45 and AD = 0.91) in for DA-CA. For females, there was also a contrast in the DA-CA and AD results (underestimated = −0.67 and AD = 1.01 for the previous study): the present study produced an overestimated value = 0.04 and an AD value of 0.86.

When Brazilians were compared using the Nolla method (NOL7), a recent article [28] found under- and overestimations only for the cohort and non-sex distinctions. Our study presented the DA-CA for all sexes without separation by sex and by separating the sexes without a cohort distinction. Despite these differences, it was possible to observe that all values were overestimations.

The difference in the total number of radiographs is explained by both the differences in the methods themselves and the quality of the non-digital X-rays provided by the Croatian sample. The differences in the methods led to the differences in the total number because after the age of 11 years, the LLH table begins to classify some subjects into a higher age group (over 14.99 years old), which caused these subjects to be excluded from our target sample (below 14.99 years old).

The quality of the Brazilian panoramic X-rays was more uniform because these X-rays were digital, and one panoramic device was used to record all of the Brazilian X-rays. Because the Croatian sample was obtained from different archives, it included several scanned X-rays and several panoramic X-rays that, after being included at the beginning of the study, could not be used, especially for the CAM method, which requires direct measurements. Overall, however, these differences in sample characteristics did not interfere with the results because our objective was simply to analyse the accuracy of each method for each country, and there was no statistical approach for comparing the methods.

As a limitation of the study, the methods could not be applied to people who were missing one of the teeth required by the methodologies. When the investigation began, it was expected that the results for the Brazilian individuals would be slightly better than the Croatians because the X-rays of the Croatian individuals were of an inferior quality compared with the Brazilian X-rays, all of which were digital; however, the results of the investigation suggest that this issue did not influence the results. Another limitation concerns the application of the CAM methodology. This method requires measurements of the opening of the root apices, and these measurements may be difficult to perform when the apex is about to close. The Adobe Photoshop CS6® software allows a high zoomed view of the region, but improvements in imaging software may aid this procedure and help it become more reliable.

When we perform research on ethnic traits, it is a common to observe large differences between some populations. We normally find traits that conclusively separate the ethnic patterns of one individual from those of another. In this study, we found that dental development is not one of these differences. The results for the miscegenated population of Brazil were similar to the results for the subjects of the original studies (in which the subjects were not part of a miscegenated population).

In conclusion, this study shows that while the Brazilian and Croatian populations differ in their diversity, the values for these populations are relatively similar. The best evaluation methods for both countries are LLH, followed by NOL7, NOL8 and CAM.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scijus.2019.02.005.

Acknowledges and source of funding

The authors recognize the scholarship support provided by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, IC 2016). Also, this study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 (Demanda Social - CAPES DS and CAPES Ciências Forenses -25/2014).

Conflicts of interest

None.

References

[1] IBGE, Brazilian Institute of Geagraphy and Statistics, (2011) http://seriesestatisticas.ibge.gov.br/ (Accessed Fev 13 2017).
[2] Statistical Yearbook of the Republic of Croatia, Croatian bureau of statistics, Zagreb (2016) 2016.
[3] F. Corradi, V. Pinchi, I. Barsanti, R. Manca, S. Garatti, Optimal age classification of young individuals based on dental evidence in civil and criminal proceedings, Int. J. Legal Med. 127 (2013) 1–8.
[4] E. Ferguson, C. Wilkinson, Juvenile age estimation from facial images, Sci. Justice 57 (2017) 58–62.
[5] A.B. Márquez-Ruiz, M.C. Treviño-Tijerina, L. González-Herrera, B. Sánchez, A.R. González-Ramírez, A. Valenzuela, Three-dimensional analysis of third molar development to estimate age of majority, Sci. Justice 57 (2017) 376–383.
[6] P. Streckbein, I. Reichert, M.A. Verhoff, R.H. Bödeker, C. Kähling, J.F. Wilbrand, H. Schaaf, H.P. Howaldt, A. May, Estimation of legal age using calcification stages of third molars in living individuals, Sci. Justice 54 (2014) 447–450.
[7] L. Kullman, G. Tronje, A. Teivens, A. Lundholm, Methods of reducing observer variation in age estimation from panoramic radiographs, Dentomaxillofac Radiol. 25 (1996) 173–178.
[8] I. Galić, M. Vodanović, S. Janković, F. Mihanović, E. Nakaš, S. Prohić, E. Galić, H. Brkić, Dental age estimation on Bosnian–Herzegovinian children aged 6–14 years: evaluation of Chaillet's international maturity standards, J. Forensic Legal Med. 20 (2013) 40–45.
[9] R. Kumaresan, N. Cugati, B. Chandrasekaran, P. Karthikeyan, Reliability and validity of five radiographic dental-age estimation methods in a population of Malaysian children, J. Investig. Clin. Dent. 7 (2016) 102–109.
[10] V. Pinchi, F. Pradella, G. Vitale, D. Rugo, M. Nieri, G.A. Norelli, Comparison of the diagnostic accuracy, sensitivity and specificity of four odontological methods for age evaluation in Italian children at the age threshold of 14 years using ROC curves, Med. Sci. Law 56 (2015) 13–18.
[11] M.M. Fernandes, R.L.R. Tinoco, D.P.P. de Braganca, S.H.R. de Lima, L.F. Junior, E.D. Junior, Age estimation by measurements of developing teeth: accuracy of Cameriere's method on a Brazilian sample, J. Forensic Sci. 56 (2011) 1616–1619.
[12] I. Galić, M. Vodanović, R. Cameriere, E. Nakaš, E. Galić, E. Selimović, H. Brkić, Accuracy of Cameriere, Haavikko, and Willems radiographic methods on age estimation on Bosnian–Herzegovian children age groups 6–13, Int. J. Legal Med. 125 (2011) 315–321.
[13] B. Liliequist, M. Lundberg, Skeletal and tooth development. A methodologic investigation, Acta Radiol Diagn. (Stockh) 11 (1971) 97–112.
[14] C.M. Nolla, The development of the permanent teeth, J. Dent. Child. 27 (1960) 254–266.
[15] M. Legović, A. Sasso, I. Legović, G. Brumini, T. Ćabov, M. Šlaj, I. Vančura, M. Lapter, The reliability of chronological age determination by means of mandibular third molar development in subjects in Croatia, J. Forensic Sci. 55 (2010) 14–18.
[16] R. Cameriere, L. Ferrante, M. Cingolani, Age estimation in children by measurement of open apices in teeth, Int. J. Legal Med. 120 (2006) 49–52.
[17] U. Hägg, L. Matsson, Dental maturity as an indicator of chronological age: the accuracy and precision of three methods, Eur. J. Orthod. 7 (1985) 25–34.
[18] J.L. Fleiss, Reliability of measurement, in: J.L. Fleiss (Ed.), The Design and Analysis of Clinical Experiments, John Wiley & Sons, Inc., 1999.
[19] R.B. Mohammed, P. Sanghvi, K.K. Perumalla, D. Srinivasaraju, J. Srinivas, U.S. Kalyan, S.K.M.I. Rasool, Accuracy of four dental age estimation methods in southern Indian children, J. Clin. Diagn. Res. 9 (2015) HC01–HC08.
[20] B. Rai, S. Anand, Tooth developments: an accuracy of age estimation of radiographic methods, World J Med Sci. 1 (2006) 130–132.
[21] S.B. Balla, P. Venkat Baghirath, B. Hari Vinay, J. Vijay Kumar, D.B.G. Babu, Accuracy of methods of age estimation in predicting dental age of preadolescents in south Indian children, J. Forensic Legal Med. 43 (2016) 21–25.
[22] R. Cameriere, L. Ferrante, H.M. Liversidge, J.L. Prieto, H. Brkic, Accuracy of age estimation in children using radiograph of developing teeth, Forensic Sci. Int. 176 (2008) 173–177.
[23] T. Lauc, E. Nakaš, M. Latić-Dautović, V. Džemidžić, A. Tiro, I. Rupić, M. Kostić, I. Galić, Dental age in orthodontic patients with different skeletal patterns, Biomed. Res. Int. 2017 (2017) 8976284.
[24] V. Pinchi, G.A. Norelli, F. Pradella, G. Vitale, D. Rugo, M. Nieri, Comparison of the applicability of four odontological methods for age estimation of the 14 years legal threshold in a sample of Italian adolescents, J Forensic Odontostomatol. 30 (2012) 17–25.
[25] T.G. Wolf, B. Briseno-Marroquin, A. Callaway, M. Patyna, V.T. Muller, I. Willershausen, V. Ehlers, B. Willershausen, Dental age assessment in 6- to 14-year old German children: comparison of Cameriere and Demirjian methods, BMC Oral Health 16 (2016) 120.
[26] A. Gulsahi, R.E. Tirali, S.B. Cehreli, S. De Luca, L. Ferrante, R. Cameriere, The reliability of Cameriere's method in Turkish children: a preliminary report, Forensic Sci. Int. 249 (2015) (319.e311-319.e315).
[27] M. Rivera, S. De Luca, L. Aguilar, L.A. Velandia Palacio, I. Galic, R. Cameriere, Measurement of open apices in tooth roots in Colombian children as a tool for human identification in asylum and criminal proceedings, J. Forensic Legal Med. 48 (2017) 9–14.
[28] L.J. Lopes, H.A.R. Nascimento, G.P. Lima, L.A.N. Santos, D.P. Queluz, D.Q. Freitas, Dental age assessment: which is the most applicable method? Forensic Sci. Int. 284 (2018) 97–100.
[29] E.N. Benedicto, A.C.S. Azevedo, E. Michel-Crosato, M.G.H. Biazevic, Validity and accuracy of three radiographic dental age estimation methods in Brazilians, Forensic Sci. Int. 283 (2018) 128–135.