Radiographic study of the rhizogenesis in Brazilian adolescents and its contribution to dental age estimation

Estudo imaginológico da rizogênese em adolescentes Brasileiros e sua contribuição para a estimativa de idade dental

Estudio radiográfico de la rizogénesis en adolescentes Brasileños y su contribución a la estimación de la edad dental

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Abstract
When applied to the examination of the living, methods for dental age estimation are based on clinical (visual or direct) or radiographic (radiographic or indirect) analyses. Two-dimensional (2D) techniques, such as panoramic radiography, or three-dimensional (3D) imaging modalities, such as cone-beam computed tomography, enable the visualization of multiple anatomical structures simultaneously. The development of each structure contributes to the age estimation process by providing age information. This study tested the performance of age information from rhizogenesis for age estimation. The sample consisted of panoramic radiographs of 568 female (n = 284) and male (n = 284) individuals, aged between 12 and 17.99 years. Tooth development was classified according to Demirjian et al. (1973) technique, and the age was calculated with the method of Willems et al. (2001). The average chronological age of each individual was compared with the estimated dental age, allowing the quantification of the error of the method for each age group at intervals of one year each. For both sexes, there was an overestimation of the chronological age in the age group of 12 |— 14.99 years, while age was underestimated in the age group of 16 |— 17.99 years (p <

0.0001). Statistically significant differences between sexes were observed in the age group of 15 |— 17.99 years (p < 0.05). The increasing error of the method in the late stages of root formation suggests that age information from the scarce remaining apical development of the permanent dentition may not be appropriate enough for sufficiently accurate forensic examinations.

Keywords: Age; Anatomy; Forensic dentistry; Teeth.

Resumo

Quando aplicados ao exame do vivo, métodos de estimativa de idade dental são fundamentados em análises clínicas (visuais ou diretas) ou imaginológicas (radiográficas ou indiretas). Técnicas bidimensionais (2D), como a radiografia panorâmica, ou tridimensionais (3D), como a tomografia computadorizada de feixe cônico, viabilizam a visualização de múltiplas estruturas anatômicas simultaneamente. Ao se desenvolver, cada estrutura contribui com o processo de estimativa de idade fornecendo informações etárias. Este estudo testou o desempenho de informações etárias da rizogênese para a estimativa de idade. A amostra foi composta por radiografias panorâmicas de 568 indivíduos do sexo feminino (n = 284) e masculino (n = 284), com idades entre 12 e 17,99 anos. O desenvolvimento dental foi classificado de acordo com a técnica de Demirjian et al. (1973), sendo a idade quantificada elo método de Willems et al. (2001). A idade cronológica média de cada indivíduo foi comparada com a idade dental estimada, permitindo o cálculo do erro do método para cada faixa etária em intervalos de um ano cada. Para ambos os sexos, houve uma superestimativa da idade cronológica na faixa etária de 12 |— 14,99 anos, enquanto a idade foi subestimada na faixa etária de 16 |— 17,99 anos (p < 0.0001). Diferenças estatisticamente significativas entre sexos foram observadas na faixa etária de 15 |— 17,99 anos (p < 0.05). O acréscimo do erro do método em fases tardias da rizogênese sugere que a informação etária proveniente do escasso desenvolvimento apical remanescente pode não ser apropriado para exames periciais suficientemente acurados.

Palavras-chave: Idade; Anatomia; Odontologia legal; Dentes.

Resumen

Cuando se aplican al examen de los vivos, los métodos para la estimación de la edad dental se basan en análisis clínicos (visuales o directos) o imaginologicos (radiográficos o indirectos). Las técnicas bidimensionales (2D), como la radiografía panorámica, o las modalidades de imágenes tridimensionales (3D), como la tomografía computarizada de haz cónico, permiten la visualización de múltiples estructuras anatómicas simultáneamente. El desarrollo de cada estructura contribuye al proceso de estimación de la edad al proporcionar información sobre la edad. Este estudio probó el rendimiento de la información de edad de la rizogénesis para la estimación de la edad. La muestra estuvo compuesta por radiografías panorámicas de 568 individuos del sexo femenino (n = 284) y masculino (n = 284), con edades comprendidas entre los 12 y los 17,99 años. El desarrollo dental se clasificó según Demirjian et al. (1973), y la edad se calculó con el método de Willems et al. (2001). Se comparó la edad cronológica promedio de cada individuo con la edad dental estimada, lo que permitió cuantificar el error del método para cada grupo de edad a intervalos de un año cada uno. Para ambos sexos, hubo una sobreestimación de la edad cronológica en el grupo de edad de 12 |— 14,99 años, mientras que la edad fue subestimada en el grupo de edad de 16 |— 17,99 años (p < 0,0001). Se observaron diferencias estadísticamente significativas entre sexos en el grupo de edad de 15 |— 17,99 años (p < 0,05). El creciente error del método en las últimas etapas de la formación de raíces sugiere que la información sobre la edad del escaso desarrollo apical restante de la dentición permanente puede no ser lo suficientemente apropiada para exámenes forenses suficientemente precisos.

Palabras clave: Edad; Anatomía; Odontología forense; Dientes.

1. Introduction

The development of crown and root(s) figures as the main parameter for dental age estimation of children and adolescents (Franco et al., 2013). In the deciduous, mixed, and permanent dentitions, several teeth develop within an overlapped timing (Adserias-Garriga et al., 2018). Each developing tooth provides biological information that can contribute to the process of age estimation. Consequently, more accurate age estimates result from the combination of more developing teeth. This is the reason why age estimation methods have lower error rates in children compared to adolescents. In the latter, age information becomes scarce following progressive rhizogenesis, and third molars usually become the sole sources of age information available (Franco et al., 2020). Additionally, dental development is minimally influenced by intrinsic (physiological) and extrinsic (environmental) factors (Elamin & Liversidge, 2013) – making the teeth reliable anatomic structures for age estimation.

Optimal assessment of dental development requires imaging tools (Franco et al., 2020). Panoramic radiographs and

cone beam computed tomography (CBCT) scans enable the visualization of multiple anatomic structures simultaneously. In other words, the images obtained with these techniques include developing structures that can be combined. When it comes to the imaginological assessment of children and adolescents, optimization and justification are imperative (Oenning et al., 2021). Most of the methods currently used for dental age estimation were trained in panoramic radiographs (Demirjian et al., 1973; Willems et al., 2001; Cameriere et al., 2006; Franco et al., 2021). Moreover, radiation exposure within panoramic radiographs is generally inferior compared to CBCT, and the images obtained are diagnostically acceptable for dental age estimation. These properties make panoramic radiographs a diagnostic mean for dental age estimation that persists over time in the scientific literature.

Dental age estimation is both a clinical and a forensic tool. From the clinical perspective, dentists may investigate patients' biological development through the assessment of teeth. This approach may be more relevant in Orthodontics and Paediatric Dentistry, in which therapeutic and orthopaedic interventions can be planned based on the predicted dental development of the patient. In addition, special care dentistry may benefit from dental age estimation techniques to assess the development of patients and the effects of known systemic diseases (Topolski et al., 2014; Souza et al., 2015; Santos et al., 2017; Gabardo et al., 2020). Usually, studies with (dental or skeletal) age estimation in the clinical scenario have case-control methodological models (Souza et al., 2015; Possagno et al., 2018). In the forensic field, dental age estimation may be used for the examination of the living and the deceased (Senn & Weems, 2013). Applications related to the living include the age assessment of asylum seekers (Nuzzolese & Di Vella, 2008; Roberts et al., 2017), alleged minor offenders (Silva et al., 2013; Goetten et al., 2021), victims and perpetrators of sexual abuse (Augusto et al., 2021; Franco et al., 2021), children for adoption (Roberts et al., 2007), sport's players (Silva et al., 2018), adults applying for retirement and pension (Swetha et al., 2018), and to assure other legal rights. Regarding the deceased, dental age estimation can help to establish the biological profile of the victim, narrowing down lists of missing persons (Dezem et al., 2021; Sartori et al., 2021) – an application with major potential for mass disasters.

In Brazil, Law n. 8.069 dated July 13th, 1990, established the Statute of the Child and the Adolescent. According to the Law, individuals below the age of 12 are considered children, while those aged between 12 and 18 (incomplete 18, or 17,99 years) are considered adolescents. Important age thresholds of legal interest are covered by the interval between 12 and 18 years, namely the lower bound of the adolescence (age of 12), the sexual consent (age of 14), relative capacity (age of 16), and the legal of legal majority (age of 18). This age interval (12 |— 17,99) is marked by the root development (rhizogenesis) of the permanent teeth. Alternatively, the only teeth with developing crowns at this point may be the third molars. Studies based on third molar development have revealed error rates of over 18 months (i.e. age interval 15 |— 17,99 years) between chronological (CA) and estimated (EA) ages (Sartori et al., 2021), while studies with the other permanent teeth have demonstrated better outcomes (i.e. age interval of 12 |—15,99 years), such as error rates of less than 8 months of difference between CA and EA (Rocha et al., 2022).

Willems' method (2001), for instance, was developed with a sample of panoramic radiographs of 2.116 Belgian individuals aged 3 |— 18 years. The method was based on Demirjian's (1973) technique for the classification of dental development – within a classification system of 8 progressive stages from A ("beginning of calcification seen in the superior level of the crypt in the form of an inverted cone or cones", without fusion of the calcified cones) to H (apex closure). The system proposed by Demirjian et al. (1973) was set for application to individuals aged 3 |— 17 years (original sample of n = 2.928 French-Canadians). In 2017, three systematic reviews with meta-analyses (Yusof et al., 2017; Wang et al., 2017, Sehrawat et al., 2017) confirmed the applicability of Willems' methods for dental age estimation of children. Given the subsequent reduction of sources of age information with time – as the result of apex closure in late childhood and early adolescence, it is expected that Willems' method would lead to higher error rates in upper age limits (e.g. 15 |— 17,99 years).

The present study aimed to establish a radiographic assessment of the rhizogenesis of adolescents in order to verify if the difference (error rate) between CA and EA progressively increases over time. To this end, Willems' method was applied to a sample of panoramic radiographs of Brazilian individuals.

2. Methodology

Study design and ethical aspects

An observational, analytical, cross-sectional study with retrospective sample selection was performed. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement was considered to structure the present study (Von Elm et al., 2008). Ethical approval was obtained from the institutional committee of ethics in research (protocol: 32848920.7.0000.5374, 4.099.703).

Participants and eligibility settings

The sample consisted of panoramic radiographs previously obtained for dental diagnostic, treatment and follow-up purposes. From an existing image database, a retrospective collection using the following eligibility criteria was accomplished: inclusion criteria – panoramic radiographs of male and female Brazilian individuals with age between 12 |— 17,99 years, and radiographs with a known date of image acquisition and patients' date of birth; exclusion criteria – panoramic radiographs bilaterally missing permanent teeth in the mandible (except third molars), images with visible bone defects suggestive of cysts or tumoral lesions, presence of orthodontic appliances (brackets or retainers) on the mandibular teeth, bilateral presence of crowns, extensive restorations and endodontic treatment in mandibular teeth, presence of orthopaedic devices (removable or fixed surgical appliances), visible deformation of the maxillofacial structures, and radiographs with insufficient quality for the visualization of the mandibular teeth.

Variables

The eligible panoramic radiographs were imported to Windows Photo Viewer™ (Microsoft Corp., Redmond, WA, USA) for visualization with a magnification of 200%. Institutional workstation's personal computer was used. An oral radiologist with over 15 years of experience in the field assessed each of the radiographs and classified the dental development of the seven mandibular left permanent teeth (from central incisor to second molar – 3rd quadrant). The classification was based on Demirjian's technique proposed in 1973, in which eight developmental stages are described: from A (initial mineralization of cusps) to H (apex closure). The EA figured as the first variables considered in this study. In order to reach an EA, Willems' (2001) method was used. According to the method, each of the developmental stages given to the seven mandibular left permanent teeth is converted into age-related values. The sum of the seven values represents the age estimated by Willems' method. The second variable considered in the present study was the CA of the participants. The calculation of the CA was performed by subtracting the date of birth from the date of image acquisition. The obtained outcome (expressed in days) was converted to years. To allow the investigation of developmental differences between males and females, sex was the third variable addressed in this study.

Quantified measures and statistics

Willems' (2001) method proposes two tables (one for boys and one for girls) for the conversion of the allocated dental stages into EA. Values within each stage for each tooth position from #31 to #37 are combined (seven values) to quantify the (dental) age. In this study, the error of the method was assessed with the following formula: error = EA – CA. Hence, overestimation was considered when EA > CA (or error = positive value), while underestimation was obtained when EA < CA

(or error = negative value). Errors were quantified per each of the age intervals of one year, for males and females. The outcomes were quantified as mean errors (ME), mean absolute errors (MAE) and root mean squared errors (RMSE). Not only values of central tendency were considered (means) but also dispersion (standard deviation). Variables related to the distribution based on sex were reported as absolute (n) and relative (%) quantification. Normality of distribution was assessed with Shapiro-Wilk test. Paired T tests were used to compared the mean EA and CA for each age interval of one year, for males and females separately. Independent T tests compared the mean ages between females and males for each age interval of one year – this approach was used independently for CA only (in order to assess sample pairing process) and for the AE (in order to assess changes on dental development based on sex). Weighted Kappa statistics was used to calculate intra- and inter-examiner reproducibility. The former was accomplished via the re-examination of 100 radiographs by the main observer within an interval of 30 days from the main analysis. The latter was accomplished with the inclusion of an additional observer for the analysis of the same 100 radiographs – so a comparison could be made. Statistical significance was set at 5%. SPSS software was used for statistical tests (IBM Crop. Armonk, NY, USA).

3. Results

Intra- and inter-examiner reproducibility was above 0.8 according to Weighted Kappa, indicating excellent agreement (Fleiss et al. 2003). Shapiro-Wilk showed normality of sample distribution (p < 0.05).

The sample consisted of 568 individuals, 284 females and 284 males, totalling 3,796 teeth that were classified into stages (568 for each tooth position from #31 to #37). Sample distribution per age interval was similar not only based on age but on sex, ranging from 83 individuals in the age range 17 |— 17,99 years to 100 individuals in the age intervals 14 |— 14,99 years, 15 |— 15,99 years and 16 |— 16,99 years (Table 1).

Table 1 – Absolute (n) and relative (%) sample distribution based on sex (females and males) and age intervals.

Age estimation	Females		Males	
	n	%	n	%
12 –| 12,99 years	46	48,94	48	51,06
13 –| 13,99 years	46	50,55	45	49,45
14 –| 14,99 years	50	50	50	50
15 –| 15,99 years	50	50	50	50
16 –| 16,99 years	50	50	50	50
17 –| 17,99 years	42	50,55	41	49,45
Total	284		284	

n: absolute frequency; %: relative frequency. Source: Authors.
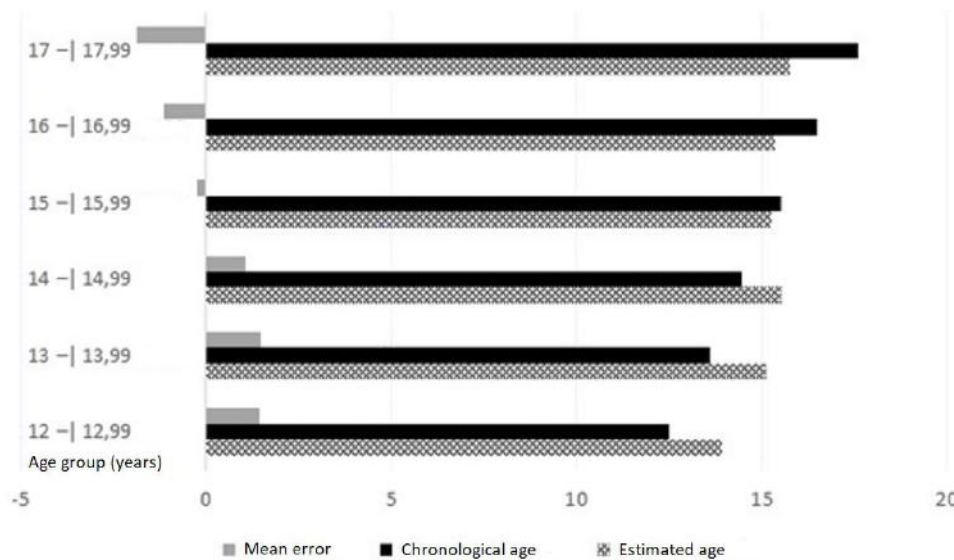
In females, the mean chronological age was 12,49 ± 0,26 years in the age group of 12 –| 12,99 years; 13,61 ± 0,37 years in the age group of 13 –| 13,99 years; 14,47 ± 0,26 years in the age group of 14 –| 14,99 years; 15,5 ± 0,38 years in the age group of 15 –| 15,99 years; 16,48 ± 0,32 years in the age group of 16 –| 16,99 years; and 17,61 ± 0,67 in the age group of 17 –| 17,99 years. The mean estimated age was 13,94 ± 1,68 years; 15,1 ± 0,97 years; 15,53 ± 0,8 years; 15,27 ± 0,88 years; 15,34 ± 0,86 years; and 15,74 ± 0,29 years in the age groups of 12 –| 12,99 years; 13 –| 13,99 years; 14 –| 14,99 years; 15 –| 15,99 years; 16 –| 16,99 years and 17 –| 17,99 years, respectively (Table 2). The mean errors calculated for each of the age intervals were 1,45 years (12 –| 12,99 years); 1,49 years (13 –| 13,99 years); 1,06 years (14 –| 14,99 years); -0,23 years (15 –| 15,99 years); -1,14 years (16 –| 16,99 years) and -1,87 years (17 –| 17,99 years) (Figure 1).

Table 2 – Mean, standard deviation and standard error of the mean quantified between estimated and chronological ages among females.

Age group (years)	Estimated age			Chronological age			p
	Mean	SD	SEM	Mean	SD	SEM	
12 –| 12,99	13,94	1,68	0,24	12,49	0,26	0,03	< 0.0001
13 –| 13,99	15,10	0,97	0,14	13,61	0,37	0,05	< 0.0001
14 –| 14,99	15,53	0,80	0,13	14,47	0,26	0,05	< 0.0001
15 –| 15,99	15,27	0,88	0,12	15,50	0,38	0,05	0.0748
16 –| 16,99	15,34	0,86	0,12	16,48	0,32	0,04	< 0.0001
17 –| 17,99	15,74	0,29	0,04	17,61	0,67	0,10	< 0.0001

SD: standard deviation; SEM: standard error of the mean; statistical significance < 0.05. Source: Authors.

Figure 1 – Visual representation of estimated and chronological ages and the mean error. A trend of overestimation was observed in the age group from 12 to 14,99 years. Underestimation occurred from 15 to 17,99 years.
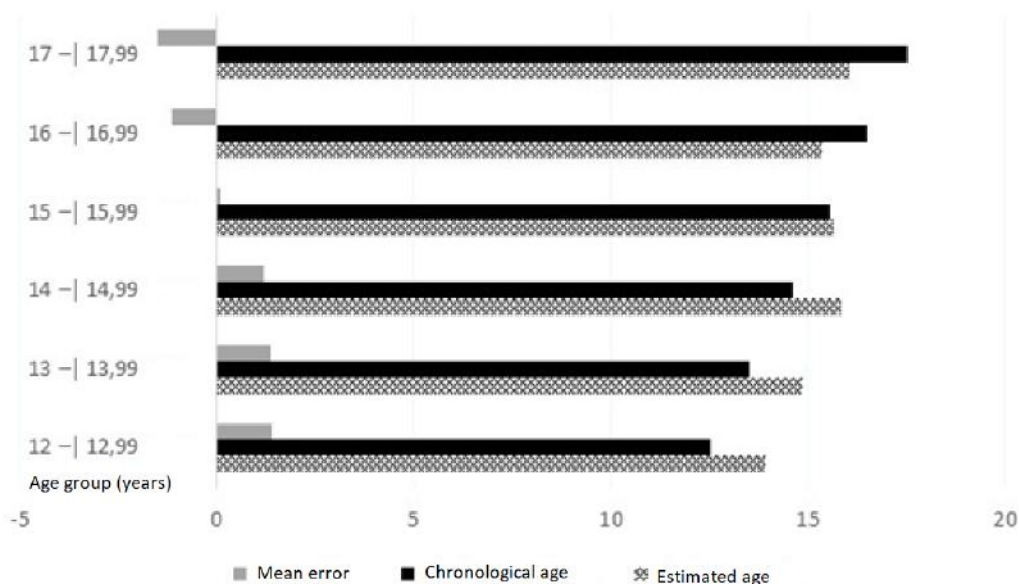

Source: Authors.

In females, the mean chronological age was 12,51 ± 0,27 years in the age group of 12 –\| 12,99 years; 13,51 ± 0,25 years in the age group of 13 –\| 13,99 years; 14,62 ± 0,29 years in the age group of 14 –\| 14,99 years; 15,56 ± 0,40 years in the age group of 15 –\| 15,99 years; 16,48 ± 0,30 years in the age group of 16 –\| 16,99 years; and 17,53 ± 0,26 in the age group of 17 –\| 17,99 years. The mean estimated age was 13,91 ± 1,54 years; 14,87 ± 1,15 years; 15,84 ± 0,55 years; 15,64 ± 0,80 years; 15,34 ± 0,86 years; and 16,03 years in the age groups of 12 –\| 12,99 years; 13 –\| 13,99 years; 14 –\| 14,99 years; 15 –\| 15,99 years; 16 –\| 16,99 years and 17 –\| 17,99 years, respectively (Table 3). The mean errors calculated for each of the age intervals were 1,4 years (12 –\| 12,99 years); 1,32 years (13 –\| 13,99 years); 1,22 years (14 –\| 14,99 years); 0,08 years (15 –\| 15,99 years); -1,14 years (16 –\| 16,99 years) and -1,5 years (17 –\| 17,99 years) (Figure 2).

Table 3 – Mean, standard deviation and standard error of the mean quantified between estimated and chronological ages among females.

Age group (years)	Estimated age			Chronological age			p
	Mean	SD	SEM	Mean	SD	SEM	
12 –| 12,99	13,91	1,54	0,22	12,51	0,27	0,03	< 0.0001
13 –| 13,99	14,87	1,15	0,17	13,51	0,25	0,03	< 0.0001
14 –| 14,99	15,84	0,55	0,10	14,62	0,29	0,07	< 0.0001
15 –| 15,99	15,64	0,80	0,11	15,56	0,40	0,05	0.5074
16 –| 16,99	15,34	0,86	0,09	16,48	0,30	0,04	< 0.0001
17 –| 17,99	16,03	0,00	0,00	17,53	0,26	0,04	< 0.0001

SD: standard deviation; SEM: standard error of the mean; statistical significance < 0.05.
Source: Authors.

Figure 2 – Visual representation of estimated and chronological ages and the mean error. A trend of overestimation was observed in the age group from 12 to 14,99 years. Underestimation occurred from 15 to 17,99 years.

Source: Authors.

An overview of the mean errors and their interpretation for each age group is presented in Table 4. Tables 5 and 6 provide a comparison between females and males considering the chronological and estimated ages, separately. The former was performed to assess sample pairing (balance) based on age between the different sex groups. The latter was performed to investigate advanced/delayed dental development between females and males. The comparison of chronological ages between groups revealed a balanced age distribution and pairing process between females and males (p>0.05) (Table 5). The comparison of estimated ages revealed statistically significant differences in the age groups from 15 to 17,99 years (p<0.01). However, the differences were all < 4 months between females and males and were not considered clinically significant (Table 6).

Table 4 – Mean error between estimated and chronological age quantified for each age group (expressed in years) and distributed based on sex.

Sex	Age group (years)	Mean EA	Mean CA	Mean error	Interpretation
F	12 –| 12,99	13,94	12,49	1,45	Overestimated
	13 –| 13,99	15,1	13,61	1,49	Overestimated
	14 –| 14,99	15,53	14,47	1,06	Overestimated
	15 –| 15,99	15,27	15,5	-0,23	Underestimated
	16 –| 16,99	15,34	16,48	-1,14	Underestimated
	17 –| 17,99	15,74	17,61	-1,87	Underestimated
M	12 –| 12,99	13,91	12,51	1,4	Overestimated
	13 –| 13,99	14,87	13,51	1,36	Overestimated
	14 –| 14,99	15,84	14,62	1,22	Overestimated
	15 –| 15,99	15,64	15,56	0,08	Overestimated
	16 –| 16,99	15,34	16,48	-1,14	Underestimated
	17 –| 17,99	16,03	17,53	-1,5	Underestimated

F: female; M: male; EA: estimated age; CA: chronological age; Mean values expressed in years.
Source: Authors.

Table 5 – Mean age, standard deviation and standard error of the mean quantified between the chronological ages of females and males.

Age group (years)	Estimated age			Chronological age			p
	Mean	SD	SEM	Mean	SD	SEM	
12 –| 12,99	12,49	0,26	0,03	12,51	0,27	0,03	0.7541
13 –| 13,99	13,61	0,37	0,05	13,51	0,25	0,03	0.1436
14 –| 14,99	14,47	0,26	0,05	14,62	0,29	0,07	0.07
15 –| 15,99	15,50	0,38	0,05	15,56	0,40	0,05	0.483
16 –| 16,99	16,48	0,32	0,04	16,48	0,30	0,04	0.9317
17 –| 17,99	17,61	0,67	0,10	17,53	0,26	0,04	0.4783

SD: standard deviation; SEM: standard error of the mean. Source: Authors.

Table 6 – Mean age, standard deviation and standard error of the mean quantified between the estimated ages of females and males.

Age group (years)	Estimated age			Chronological age			p
	Mean	SD	SEM	Mean	SD	SEM	
12 –| 12,99	13,94	1,68	0,24	13,91	1,54	0,22	0.9083
13 –| 13,99	15,10	0,97	0,14	14,87	1,15	0,17	0.3008
14 –| 14,99	15,53	0,80	0,13	15,84	0,55	0,10	0.1105
15 –| 15,99	15,27	0,88	0,12	15,64	0,80	0,11	0.0296
16 –| 16,99	15,34	0,86	0,12	15,34	0,86	0,09	0.0139
17 –| 17,99	15,74	0,29	0,04	16,03	0,00	0,00	< 0.0001

SD: standard deviation; SEM: standard error of the mean. Source: Authors.

4. Discussion

Brazil is a country with a continental area. Geographically, the territory is divided into five regions – North, Northeast, Central-west, Southeast and South. Most of the population lives in the Southeast region – the economic center of Brazil. Nearly 22% (46.5 million) of Brazilians live in the State of Sao Paulo. By 2018, forty-seven out of the 220 dental schools in Brazil were in the State of Sao Paulo (San Martin et al., 2018), justifying the high number of dental age estimation studies with South-eastern populations. Recently, Franco et al. (2021) performed a systematic literature review and meta-analysis with 13 eligible studies collected from 2,527 initially identified. The authors investigated the methods with the best performance for dental age estimation in Brazilian children and found out that Willems' method (2001) emerged as the one with the smallest difference between chronological and estimated ages (pooling together females and males and different age

categories, the method reached a difference of less than a month between ages). The outcomes related to the method, however, were based on a single study with over 900 children (aged 5-15 years) from the South of Brazil (Franco et al., 2013). Subsequentially, other studies using Willems' method were performed in the country (Frítola et al., 2015; Souza et al., 2015; Gabardo et al., 2020; Machado et al., 2020; Gonçalves et al., 2021; Rocha et al., 2022). Those specifically sampling populations from the Southeast corroborated the good performance of the method.

From a methodological perspective, the study of Rocha et al., 2022, was superior compared to the remaining literature given the high and balanced sample of children. The authors studied a population of 1,000 females (n = 500) and males (n = 500) equally distributed per age interval of one year, from 6 to 15.99 years. Their outcomes showed that the Willems method estimate age in Brazilian South-eastern children with an overall error rate of <4 months for both females and males. These findings corroborate the usefulness of the method even after 20 years from its origin. According to the authors, the worse age estimates came from patients in the older age intervals (close to 15.99 years). In the present study, interest behind the older age groups was raised and a study with South-eastern Brazilian children aged 12 –| 17,99 years was performed. The rationale behind the upper age bound (17,99 years) relied on the original age limit established by Willems et al. (2001) in their study, while the lower bound (12 years) was considered based on the current Brazilian age of legal interest to distinguish children and adolescents (which is the age of 12 according to the Statute of the Child and the Adolescent.

Despite the smaller sample size of the present study compared to Franco et al., 2013 (n = 1,357) and Rocha et al., 2022 (n = 1,000), our sample (n = 568) was considerably higher than that of Frítola et al., 2015 (n = 398), Souza et al., 2015 (n = 160; study with HIV children), Gabardo et al., 2020 (n = 81; study with children with amelogenesis imperfecta); Machado et al., 2020 (n = 180) and Gonçalves et al., 2021 (n = 220). Moreover, our age interval of interest (12 –| 17,99) consisted of 6 age groups (12 –| 12,99; 13 –| 13,99; 14 –| 14,99; 15 –| 15,99; 15 –| 15,99; 16 –| 16,99 and 17 –| 17,99 only – which allow sample to populate each age group with more individuals (in this study sample distribution per age group ranged from 41 to 50). Clearly, more age groups would enable a more comprehensive understanding of the dental development in the early childhood, but in this study our aim was to focus on an age interval that could allow us to assess ages of legal interest to the Brazilian judicial system, namely the ages of 12, 14, 16 and 18 (represented by the limit of 17,99).

The outcomes of this study showed that Willems et al. (2001) method reached an overall absolute error of 1.2 years in females and 1.11 in males – values that are different (higher) from all the studies published with Brazilian populations so far. Results of high absolute error rates (close to 1 year) for Willems' method were observed in a few studies; for instance, within the Japanese (Ramanan et al., 2012) and Central Southern Chinese Han (Yang et al., 2019) population. While there are differences in dental development across populations worldwide, they seem to be discrete in most of the cases (Thevissen et al., 2010). Hence, given the previous (good) performance of the method in the Brazilian population, especially in the study of Rocha et al., (2002) – with a population from the same geographic region, the outcomes of the present study should be (re)analyzed in detail with strategies to enhance methodology, namely an equally balanced sample and more examiners. Intra- and inter-examiner tests were performed in the present study and reached excellent outcomes, but the inclusion of more examiners could fit as a quality-control strategy to double-check the process of image analysis. The inclusion of three or more examiners was already established in previous dental age estimation studies (Gonçalves et al., 2021) and seems to be promising. A direct comparison between our outcomes and the previous study with multiple examiners is not possible, however, because the authors used intraclass correlation coefficient (ICC) to assess the reproducibility of dental age estimation based on a staging technique (Demirjian's –technique which is originally dependent on categorical variables [instead of continuous, such as age]). In other words, the comparisons were based on age estimates instead of the decision-making process of development stages (which is the primary variable of interest of staging techniques).

An additional phenomenon observed in this study was the predominance of overestimation detected in the younger

age groups (12 –| 14,99) and underestimation in the remaining age groups. The fact is that higher error rates were indeed expected in older age groups because with progressive rhizogenesis fewer developing teeth are observed. By the age of 16, most of the permanent teeth are fully developed and do not contribute directly to the age estimate with proper age information. This is the reason why most of the methods designed for adolescents rely on third molar development (or the combination of developing permanent teeth and third molars). Since Willems' method was originally designed for children (scientifically proved to be useful for the assessment of young individuals, such as those up to the age of 15,99 [Franco et al., 2013]), the application of the method should be restricted to children. The inclusion of individuals from 12 to 17,99 years limited the staging technique to the development of roots because crowns were already formed in most of the cases. Only four of the Demirjian's stages (used in Willems' method) use root development (stages E-H). Stage H, more specifically, lacks an upper limit and makes the age estimation process more difficult in older individuals. In short, an individual with all permanent teeth staged H could be 17 or 57 years, for instance. Other techniques with more detailed descriptions (more stages) of the process of dental development (e.g. Moorrees' et al. 1963, staging system) could be used and tested in practice to investigate whether error rates could be reduced from age estimates.

Future studies in the field also should consider the recruitment of multiple examiners to test the existence of systematic errors and the assessment of a higher number of radiographs to reach outcomes that could be more regionally representative. Strategies to reduce the error of the method, such as the artificial neural network (ANN) and the multiple linear regression (MLR) proposed by Rocha et al. (2022), could be used as an approach to improve the age estimates for the specific population addressed in this study.

5. Conclusion

In the population addressed in the study, Willems' method led to age estimation error rates that were considerably higher than outcomes previously reported in the scientific literature. Given the known credibility of the method worldwide and the existing meta-analysis validating the method in Brazil, Willems' method should not be disregarded for dental age estimation in Brazilians but should be studied in detail for the reasons of its performance in the population addressed in this study. Testing and validating the method in populations of other geographic regions of the country and worldwide remain important for future studies.

Acknowledgments

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