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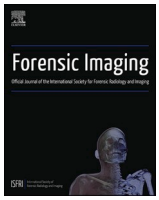

A comparison of Demirjian and Willems age estimation methods in a sample of Brazilian non-adult individuals

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ABSTRACT

Age estimation methods take into account the organism variation during its development or involution. Dental methods can often be considered, because the dental development presents a series of recognizable events with small variation. Each method presents a different methodological proposal, so it is important to consider the type of dentition, the presence or absence of teeth, available information and the method's validation, as dental development may differ among different populations. The present study aimed to assess the agreement between the ages estimated by the methods of Demirjian and Willems and the real age of Brazilian individual. To compose the sample and apply the methods, 220 panoramic radiographs of individuals from 6 to 16 years were selected, being 110 females and 110 males. After applying the methods by three examiners, it was possible to observe that both methods showed high correlation values among males. However, the results for female brought lower values. In addition, the Willems' method was more suitable to the population considered when compared to the Demirjian's method, despite the overestimation of real ages by both methods.

Introduction

Dental forensic reports in living people have increasingly been applied to forensic work in recent years, including studies in countries like Croatia [1], India [2] and China [3], mainly due to the increase of the migration flow. According to the 2020 International Organization for Migration 2020 report (IOM) [4], 3.5% of the world's population was composed by immigrants. There are many undocumented immigrants looking for better life opportunities, especially in developing countries [4].

Knowledge of the age plays an important role in undocumented immigrants' cases, since these individuals need to have their rights secured [5]. Also, in a criminal context, in cases of kidnapping, rape and crimes against children and adolescents, if the accused's age is unknown (e.g. the birth certificate is not available or records are suspect), it should be assessed whether the individual has reached the age of majority [6].

In addition to cases in living people, the age estimation can be used to assist the formation of the biological profile for deceased individuals. There are also skeletal methods, based on the stages of bone formation. However, teeth are structures protected by lips, tongue and cheeks and show an efficient preservation even after years of inhumation [7,8].

When estimating the age of a person, the biological variations that the organism experiences during its development must be considered, and it is possible to estimate the physiological or biological age. The parameters can be based on somatic, sexual, skeletal, and dental maturity or involution [9].

The dental method may often be considered the method of choice, since teeth differ from bones not only in their in their biological and functional properties, but also they are well preserved over the time [10]. In addition, dental development is an useful measure of maturity as it represents a series of recognizable events that occur in a same

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sequence, from an initial event to a constant end point, with little expected variation. Thus, stages of dental development are an useful tool for estimating the age of someone [9].

Regarding the available dental methods, three basic parameters are considered: development, tooth wear and histology. In order to use less invasive and more applicable methods, the use of imaging analysis is recommended for dental assessment for age estimation, most commonly performed through radiographs [9].

The age estimation methods for non-adult individuals are methods that involve deciduous or mixed dentition, taking into account mineralization and dental eruption. But if the individual is considered an adult, it is necessary to use methods based on dental involution, such as dental wear, darkening and decreased dimensions of the pulp chamber [11].

These methods can be used directly through the clinical examination, observing the dental eruption or dental wear, and indirectly, through complementary imaging tests, analyzing the eruption chronology and the degree of mineralization or the dental involution, depending on it is non-adult or adult, living or dead [12].

The method of Demirjian, Goldstein and Tanner [13] uses a non-metric analysis by classifying the seven left mandibular teeth (i.e. from permanent central incisor to permanent second molar) among eight developmental stages. Afterwards, these stages are converted into numeric values, summed, and a final conversion of the sum is made to obtain the estimated age. This method is applicable for both males and females, according to its peculiarities. Based on this method, Willems et al. [14] used the same teeth and developmental stages, but after determining each developmental stage, they simplified the scores so that the sum resulted in the final age without the need for a second table.

Each method presents different methodological issues, applicability and purposes. To decide which method to apply, it is important to consider the type of teeth, presence or absence of teeth, physiological factors of maturation and eruption, materials and information available and the validation of the method [15]. In addition, it is worth remembering that dental development can differ among different populations, either for genetic or environmental reasons [16].

The age estimate is a barrier to the Brazilian population, given the existing miscegenation with high levels of gene mixture, result of immigration of European, African immigrants and the native population, a circumstance that differs according to the region of the country [17]. The present research aimed to evaluate the agreement between the ages estimated by the methods of Demirjian and Willems and the chronological age of Brazilian non-adult individuals.

Material and methods

This research was approved by the institutional Research Ethics Committee (CAAE 06635118.2.0000.5419). 924 radiographs from the institutional image databank were analyzed, but just 220 were selected to this study to standardize the same sample number by sex and age, covering individuals from 6 to 16 years old, being 110 females and 110 males. The radiographs were produced on the same machine and protocol: Veraviewepocs (Morita, Kyoto, Japan) - operating at 67 kV, 5 mA, and exposure time of 8.1.

Panoramic radiographs that showed good image resolution and the presence of the lower central incisor to the lower second molar were selected. If there was an absence of a tooth on one side, it was replaced by the contralateral one. Radiographs that showed developmental dental changes, bilateral absence or bone lesions or fractures were not included in the sample.

Random identification codes were assigned to each radiograph, and age and sex were registered. All radiographs were evaluated by three examiners (a specialist in Oral and Maxillofacial Radiology, a specialist in Forensic Odontology, and a last-year dental undergraduate student). Additionally, 10 random radiographs (5 males and 5 females) of the total sample were reevaluated after 3 weeks to calculate intra-observer agreement.

Application of the methods

The methods of Demirjian and Willems were chosen since they have clearly defined stages of development and with few intermediate stages, facilitating the classification of the development of each dental element [18,19].

Training and calibration of the classification used in the methods was carried out by performing age estimation on 20 random radiographs by the examiners (Fig. 1), independently, with consecutive comparison and discussion of the results.

Demirjian, Goldstein and Tanner's method [13] (method 1) uses eight developmental stages assigned to the seven lower right teeth (i.e. from the central incisor to the left lower second molar - Fig. 2). A score is attributed to each tooth, corresponding to its stage, and subsequently, the scores of the seven teeth are added, obtaining a value called "Maturity Score". Finally, using a second table, this value is converted to the individual's estimated age.

Willems et al's method [14] (method 2) consists of a review of the previous method, using the same stages of development of the seven lower left teeth (A-H). However, a score already expressed in years is attributed to each tooth specifically according to their respective stage. At the end, the seven teeth score is added, obtaining the estimated age.

The application of both methods was done preferably in the lower left hemiarch because it is easier to view the lower teeth when compared to the upper teeth [15]. The left side was chosen only for the purpose of standardization between the examiners [13]. However, in the study of Bagic et al. [1] there was a replacement for the corresponding tooth on the right side in the absence of any dental element in the lower left hemiarch and even with the replacement, there was no discrepancy in the results, so this also was used in the present study.

Statistical analysis

To perform the statistical tests, the following software were used: R Core Team (R: A language and environment for statistical computing, R Foundation for Statistical Computing; Vienna, Austria, 2016) and SAS Statistical Software (version 9.3; SAS Institute, Inc Cary; NC). Initially, intra- and inter-rater analysis was performed using the Intraclass Correlation Coefficient (ICC) with its respective 95% Confidence Interval, in order to verify reproducibility. Agreement analysis was also carried out using the Intraclass Correlation Coefficient (ICC) to verify whether the Demirjian's and Willems' methods are capable of producing equal or satisfactorily similar values for the "age" variable. To calculate the agreement and magnitude of the differences between real and estimated ages, a linear mixed effects regression was performed.

Results

Intra- and inter-observer analysis

The high values of Intraclass Correlation Coefficient (ICC) in the intra- and inter-observer analysis (Tables 1 and 2) demonstrated that the examiners were calibrated, and also confirmed that the Demirjian's (method 1) and Willems' (method 2) methods are reproducible, since the ICC values were close to 1, proving high agreement between the estimated ages.

Agreement between real and estimated ages using ICC

To be able to obtain equal or satisfactorily similar values for the variable "Age", the Intraclass Correlation Coefficient (ICC) was calculated with 95% Confidence Interval for each method, as shown in Table 3.

The agreement between the estimated ages and the real ages was

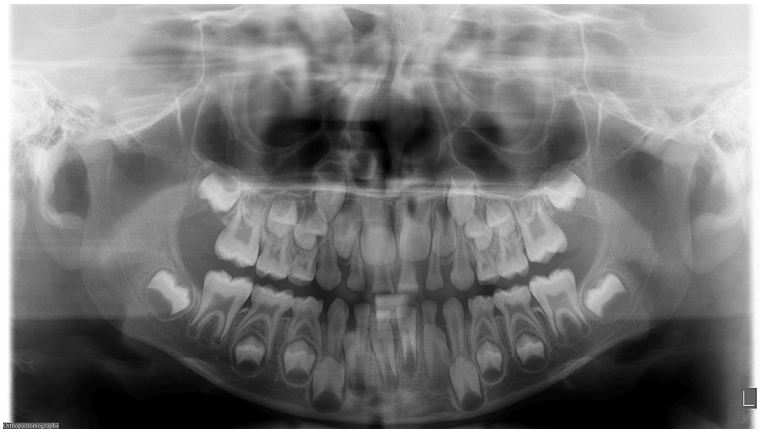

Fig. 1. Example of panoramic radiography used by the authors.
Source: image from the institutional image databank.

higher for males when compared to females, since the minimum value for males was 0.87 and the maximum was 0.93. For women, the minimum value was 0.59 and maximum 0.69. In addition, the Willems' method (method 2) presented more agreement than the Demirjian's method (method 1).

Linear regression with mixed effects

Linear regression with mixed effects was performed, aiming to compare the real and the estimated ages with a random effect per individual. Thus, were calculated the *p-value* of the estimated differences and their respective 95% confidence intervals, because the CI does not include the zero value bring evidence of differences between the compared groups. The CI that includes the zero value does not provide evidence of difference between the real ages and the estimated ages, in addition, their limits show the magnitude of the respective difference. Tables 4 and 5 compare the real age with the estimated age of each method; Table 5 does not provide the separation by examiner.

In general, and without segmentation by examiner, both methods overestimate the ages, since the estimated difference is calculated by subtracting the real age and the age estimated by the methods, and resulted in negative values for both method 1 and method 2.

Regarding the *p-value*, was possible to observe that in method 1, for females and males, this value was lower than 0.05, bringing evidence of statistical differences between real and estimated ages, which is also confirmed by the confidence interval, which does not cover zero. For method 2 for males, the *p-value* is greater than 0.05 and the confidence interval covers zero, with no statistical differences between the estimated ages and the real ages.

Discussion

The miscegenation has a great influence on the accuracy of age estimation methods. Since Brazil has a history of immigration from different regions of the world, it is necessary to verify the applicability of age estimation methods in different locations in the country [20].

It is important that all the examiners are calibrated to follow the evaluation in the same way at different times and also in the same way as the other examiners [21]. In view of this, the Intraclass Correlation Coefficients values (ICC) of the intra- and inter-observer analysis demonstrate that both methods are reproducible and that the examiners were calibrated, as approaches of the maximum value 1: minimum of 0.86 and maximum of 0.99 for intra-observer analysis and minimum of 0.93 and maximum of 0.98 for inter-observer analysis.

These results are similar to the study by Chaudhry et al. [2], with a Kappa coefficient of 0.83 for inter-observer analysis and 0.86 for intra-observer analysis. Similarly, in the analysis by Hegde et al. [22], the Kappa coefficients indicated a value of 0.81 for inter and 0.90 for intra-observer analysis, demonstrating a high correlation and calibration value.

When the ICC values of the intra- and inter-observer analyzes of the two methods are compared, there were no major differences in reproducibility and calibration between the two methods. This similarity occurs because the method of Willems et al. [14] was based on the study by Demirjian, Goldstein and Tanner (1973) [13], which uses few intermediate stages, facilitating the classification of the stage of each dental element for both methods [18,23].

When the correlation coefficients are compared, the Willems' method presented higher values for each examiner and sex when compared to the Demirjian's method. When the values of the Linear Regression of Mixed Effects are analyzed, the Willems' method showed a lower overestimation of ages (-0.57 for females and -0.24 for males), since the Demirjian's method resulted in an overestimation of -1.05 for females and -0.57 for males.

These results were also reported by Apaydin and Yasar [24], which the Willems' method showed minor differences with the real ages, being the method with the highest accuracy among the evaluated methods. This situation is associated with the fact that the Willems' method was based on the Demirjian's method, seeking to simplify the application and reduce the overestimation of real ages [14].

Likewise, the meta-analysis by Esan et al. [16] demonstrates that the Willems' method provides more accurate estimation of chronological age in different populations, emphasizing that both methods significantly overestimated chronological age. However, in the study by Yang et al. [3], the Willems' method presented a slightly higher error prediction than the Demirjian's method: 0.86 and 0.85, respectively.

It is important mentioning that only the Willems' method, in the male's sample, presented a *p-value* greater than 0.05 and a confidence interval that covers zero, which means, it did not present evidence of statistical differences between the estimated ages and the real ages. This can be explained because the males presented a greater agreement than females did. Besides that, the Willems' method presented a lower overestimation for being a development of the Demirjian's method [13], resulting in values closer to the real age.

Regarding the differences between the sexes, the males presented higher values of ICC when compared to the females, with a minimum of 0.87 and a maximum of 0.93 for males and a minimum of 0.59 and maximum 0.69 for females.

In the study by Frítola et al. [25], which also used a Brazilian sample, the ICC values were slightly higher for females, being 0.930 for females and 0.913 for males. However, the results showed a greater overestimation of chronological age for females when compared to males.

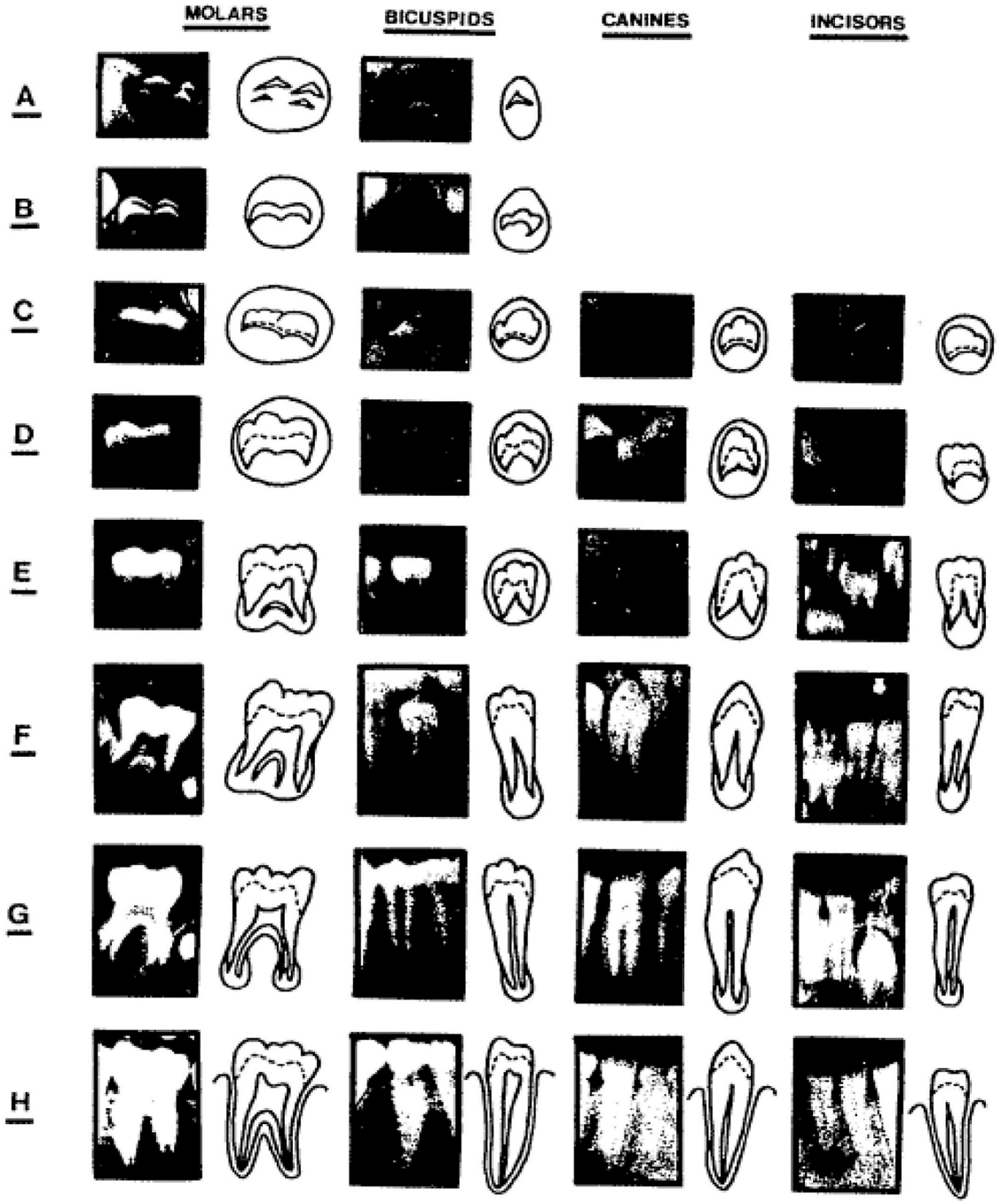

Fig. 2. Stages of dental development according to Demirjian, Goldstein and Tanner (1973).
Source: Demirjian, Goldstein and Tanner (1973).

Table 1
Intraclass Correlation Coefficients (ICC) of intra-observer analysis.

Examiner	ICC Methodology 1	ICC Methodology 2
1	0.87 (0.18;0.97)	0.96 (0.81;0.99)
2	0.99 (0.96;1)	0.99 (0.98;1)
3	0.86 (0.39;0.97)	0.98 (0.91;0.99)

This is because the estimated ages for males, despite being more distant from the real ages, are better distributed around them, resulting in a lower overestimation.

In contrast, Franco et al. [26] also used a Brazilian sample, but it is possible to observe a greater overestimation for males than for females

Table 2
Intraclass Correlation Coefficients (ICC) of the inter-observer analysis.

SEX	Examiner	ICC Methodology 1	ICC Methodology 2
F	1-2	0.97 (0.77;0.99)	0.97 (0.83;0.99)
	2-3	0.98 (0.91;0.99)	0.97 (0.95;0.98)
	3-1	0.93 (0.26;0.98)	0.93 (0.6;0.98)
M	1-2	0.96 (0.75;0.99)	0.98 (0.87;0.99)
	2-3	0.98 (0.96;0.99)	0.98 (0.97;0.99)
	3-1	0.94 (0.44;0.98)	0.96 (0.72;0.99)

Table 3
Intraclass Correlation Coefficients (ICC) between real and estimated ages of females and males according to each examiner and method.

Sex	Examiner	ICC Methodology 1	ICC Methodology 2
F	1	0.66 (0.54;0.76)	0.69 (0.58;0.78)
	2	0.64 (0.46;0.76)	0.66 (0.54;0.76)
	3	0.59 (0.33;0.75)	0.6 (0.45;0.71)
M	1	0.89 (0.84;0.92)	0.93 (0.9;0.95)
	2	0.92 (0.79;0.96)	0.93 (0.9;0.95)
	3	0.87 (0.64;0.94)	0.9 (0.84;0.94)

Table 4
Comparison between real and estimated age of each method in each examiner.

Sample	Comparation		Estimated difference	*p-value*	CI 95% Inferior limit	Superior limit
F	Real age	M1AV1	-0.56	0.0004	-0.87	-0.25
	Real age	M1AV2	-1.10	0.0001	-1.41	-0.79
	Real age	M1AV3	-1.50	0.0001	-1.81	-1.19
	Real age	M2AV1	-0.13	0.4265	-0.43	0.18
	Real age	M2AV2	-0.63	0.0001	-0.94	-0.32
	Real age	M2AV3	-0.94	0.0001	-1.25	-0.63
M	Real age	M1AV1	-0.11	0.5001	-0.42	0.20
	Real age	M1AV2	-0.67	0.0001	-0.98	-0.36
	Real age	M1AV3	-0.92	0.0001	-1.23	-0.61
	Real age	M2AV1	0.08	0.6080	-0.23	0.39
	Real age	M2AV2	-0.30	0.0544	-0.61	0.01
	Real age	M2AV3	-0.49	0.0019	-0.80	-0.18

Table 5
Comparison between real and estimated age of each method considering information from all examiners.

Sample	Comparation		Estimated difference	*p-value*	CI 95% Inferior limit	Superior limit
F	Real age	M1	-1.05	0.0001	-1.41	-0.69
	Real age	M2	-0.57	0.0023	-0.93	-0.20
M	Real age	M1	-0.57	0.0023	-0.93	-0.20
	Real age	M2	-0.24	0.2001	-0.60	0.13

when using the Willems method. In the Brazilian study by Vieira et al. [27], which used the Demirjian's method, it was demonstrated that the male also had a higher overestimation (average between 1.33 and 1.5) when compared to the female (average of 1 year).

Also different from the ICC values of Frítola et al. [25], Akkaya et al. [28] reported that the performance of these methods was superior when applied to males when compared to females, since there was an overestimation of 0.07 for males and 0.15 for females.

In the present study, the Linear Regression of Mixed Effects showed an overestimation in relation to the real age for males and females. The negative values of the differences between estimated ages and real ages demonstrate that there was a greater overestimation for females (-1.05 for the Demirjian method and -0.57 for Willems) when compared to males (-0.57 for Demirjian and -0.24 for Willems).

These divergences between the sexes can be explained by the difference in development chronology, especially with regard to the stages of dental development. These are affected first by females and then by males, almost entirely, a fact that directly interferes with the age estimation process [21,29].

In addition, the differences presented with previous studies are the result of ethnic differences between the populations and samples analyzed, establishing the importance of applying different methods so that it is possible to find the one with the best performance [15].

Even in the same country, it is possible to observe differences between the studies, especially in a country with large territory such as Brazil. This becomes clear when the studies of Frítola et al. [25], Franco et al. [26] and Vieira et al. [27] are compared with the present study, in which different accuracy and overestimation were found for each sex.

Conclusion

This research compared the Demirjian age estimation method to the Willems age estimation method on a sample of Brazilian non-adults. Despite the overestimation of real ages by both methods, the Willems' method was more suitable to the population considered when compared to the Demirjian's method. Both methods showed high correlation values for males that differ from the female's sample, which showed smaller correlation between the estimated ages and the real ages. Understanding that the results of several studies differ, further studies are needed before using these age estimation methods and the sample should be increased and diversified, considering the miscegenation of the Brazilian population.

Declaration of Competing Interest

None.

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