Development of the teeth, cervical vertebrae, hand and wrist combined for the estimation of the biological age

Desenvolvimento dos dentes, vértebras cervicais, mão e punho combinados para estimativa da idade biológica

Desarrollo de dientes, vértebras cervicales, mano y muñeca combinados para estimar la edad biológica

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Marisa de Matos Ferraz Pêgo
ORCID: https://orcid.org/0000-0001-5984-146X
São Leopoldo Mandic College, Brazil
E-mail: marisamfpego@gmail.com
Paola Fernanda Leal Corazza
ORCID: https://orcid.org/0000-0002-8639-8392
APCD College of Dentistry, Brazil
E-mail: paola_corazza@hotmail.com
Fernando Martins Baeder
ORCID: https://orcid.org/0000-0001-7101-5689
Cruzeiro do Sul University, Brazil
E-mail: fernandobaeder@uol.com.br
Daniel Furtado Silva
ORCID: https://orcid.org/0000-0003-3319-2996
Federal University of Paraíba, Brazil
E-mail: furtado.ds@gmail.com
Ana Carolina Lyra de Albuquerque
ORCID: https://orcid.org/0000-0002-6532-5020
Federal University of Paraíba, Brazil
E-mail: lina.lyra@gmail.com
José Luiz Cintra Junqueira
ORCID: https://orcid.org/0000-0001-6788-4021
São Leopoldo Mandic College, Brazil
E-mail: joseluiz@slmandic.edu.br
Francine Kühl Panzarella
ORCID: https://orcid.org/0000-0002-5650-7711
São Leopoldo Mandic College, Brazil
E-mail: francine.panzarella@gmail.com

Abstract
This study aims to perform age estimation using three different parameters from dental and skeletal development. The sample consisted of 98 dental records of patients aged from 10 to 16 years old, containing the chronological age and a set of radiographs (panoramic, lateral cephalometric and carpal radiographs) taken in the same day. The biological age was assessed through the dental development from panoramic radiographs according to the Nicodemo's method. The stages of dental development were registered and imported in CRONOL software (UNESP, São Paulo, Brazil), which provided the estimated dental age. The lateral cephalometric radiographs were analyzed to assess the development of the vertebrae C2, C3 and C4. And carpal radiographs were evaluated according to Fishman's method. Shapiro-Wilk test was used to verify the normality of the chronological and estimated age. T-test for unpaired samples was used to compare the normal data. Chi-square test was used to analyze the age in function of sex. Moderate and strong correlations were found between the chronological and biological (estimated) ages for all the methods. Statistically significant differences between the development of males and females were not observed ($p>0.05$). A linear regression formula was designed to allow age estimates statistically more accurate ($p<0.0001$). The formula reached an accuracy rate of 71.4%. In general, the methods underestimated the age. The combination of methods led to optimal performances for age estimation. The performances were similar between males and females.
Keywords: Age estimation; Biological age; Cervical vertebrae; Hand and wrist; Teeth.

Resumo
Este estudo tem como objetivo realizar a estimativa da idade utilizando três diferentes parâmetros de desenvolvimento dentário e esquelético. A amostra foi composta por 98 documentações de pacientes com idade entre 10 e 16 anos, contendo a idade cronológica e radiografias (panorâmica, cefalométrica lateral e radiografia carpal) realizadas no mesmo dia. A idade biológica foi avaliada através do desenvolvimento dentário a partir de radiografias panorâmicas segundo o método de Nicodemo. As etapas de desenvolvimento dentário foram registradas e importadas no software CRONOL (UNESP, São Paulo, Brasil), fornecendo a idade dentária estimada. As radiografias cefalométricas laterais foram analisadas para avaliar o desenvolvimento das vértebras C2, C3 e C4. E as radiografias do carpo foram avaliadas por meio do método de Fishman. O teste de Shapiro-Wilk foi utilizado para verificar a normalidade da idade cronológica e estimada. O teste t para amostras desemparelhadas foi usado para comparar os dados normais. O teste qui-quadrado foi utilizado para analisar a idade em função do sexo. Correlações moderadas e fortes foram encontradas entre as idades cronológica e biológica (estimada) para todos os métodos. Não foram observadas diferenças estatisticamente significativas no desenvolvimento dos gêneros masculino e feminino (p> 0,05). Uma fórmula de regressão linear foi projetada para permitir estimativas de idade estatisticamente mais precisas (p <0,0001). A fórmula atingiu uma taxa de acerto de 71,4%. Em geral, os métodos subestimaram a idade. A combinação de métodos levou a desempenhos ideais para estimativa de idade. Os desempenhos foram semelhantes entre os gêneros masculino e feminino.
Palavras-chave: Estimativa de idade; Idade biológica; Vértebra cervical; Mão e pulso; Dentes.

Resumen
Este estudio tiene como objetivo estimar la edad utilizando tres parámetros diferentes de desarrollo dental y esquelético. La muestra estuvo conformada por 98 documentaciones de pacientes de 10 a 16 años, que contenían la edad cronológica y un conjunto de radiografías (panorámica, cefalométrica lateral y radiografía carpiana) realizadas el mismo día. La edad biológica se evaluó a través del desarrollo dental mediante radiografías panorámicas según el método Nicodemo. Las etapas del desarrollo dental fueron registradas e importadas en el software CRONOL (UNESP, São Paulo, Brasil), que proporcionó la edad dental estimada. Se analizaron radiografías cefalométricas laterales para evaluar el desarrollo de las vértebras C2, C3 y C4. Y las radiografías del carpo se evaluaron según el método Fishman. Se utilizó la prueba de Shapiro-Wilk para verificar la normalidad de la edad estimada y cronológica. Se utilizó la prueba t para muestras no apareadas para comparar datos normales. Se utilizó la prueba de chi-cuadrado para analizar la edad según el sexo. Se encontraron correlaciones moderadas y fuertes entre las edades cronológicas y biológicas (estimadas) para todos los métodos. No hubo diferencias estadísticamente significativas en el desarrollo de los géneros masculino y femenino (p> 0.05). Se diseñó una fórmula de regresión lineal para permitir estimaciones de edad estadísticamente más precisas (p <0,0001). La fórmula alcanzó una tasa de aciertos del 71,4%. En general, los métodos subestimaron la edad. La combinación de métodos condujo a resultados ideales para la estimación de la edad. Las actuaciones fueron similares entre hombres y mujeres.
Palabras clave: Estimación de edad; Edad biológica; Vertebra cervical; Mano y muñeca; Dientes.

1. Introduction

The health sciences benefited considerably from the discovery of the X-rays – in 1895. In parallel, the forensic sciences grew gradually and used Radiology as the key tool for overcoming limitations and solving crimes under the needs of justice. Forensic radiology emerged in the interface of Forensics and Radiology to support other fields in sciences, especially Medicine and Dentistry. In these fields, age estimation figures as an essential tool to support the society under contemporary problems. In specific, it contributes in cases that involve persons with missing documents, suspects and victims of crimes, asylum seekers and illegal immigrants (Cameriere et al., 2007; Carvalho et al., 2010; Pinchi et al., 2016; Pineau, 2020).

The assessment of dental and skeletal parameters consists of a reliable procedure to perform age estimation of the living and deceased. In general, the methods developed for the assessment of these parameters are founded on the association between age and the stages of tooth and bone mineralization. For that reason, these methods are applicable for children and adolescents (Pineau, 2020; Molina et al., 2021). The current trend on age estimation research recommends the combination of parameters from the same person in order to reach optimal performances (Pinchi et al., 2016; Tobel et al., 2020). On the other hand, the scientific literature is still scarce (Manhães Júnior 2006; Carvalho et al., 2010) of studies that combined dental and bone parameters for age estimation. The main reason behind the lack in the literature relies on the difficulty to register dental and skeletal parameters from the same person in the same day. Orthodontics is the field of Dentistry dedicated to the treatment of malocclusions and to the management of functional and aesthetics disorders. Usually, dental and skeletal radiographs are taken from orthodontic patients for treatment planning and follow-up. From a forensic scope, these radiographs are source of multiple age information that may be combined to improve the accuracy in age estimation.

The present study aims to perform age estimation using three different parameters that include dental and skeletal development registered in panoramic, lateral cephalometric and carpal radiographs. Next, the biological age estimated through teeth and bones will be compared to the chronological age for the investigation of performance (accuracy). Finally, a formula combining the three parameters will be designed from linear regressions models and will be tested in the sample. The age estimation outcomes obtained with the formula will be compared to the chronological for a second assessment of performance. All the analyses will be performed with the total sample and stratified by sex.

2. Methodology

This is a cross-sectional observational research with a correlational approach and statistical comparative procedure (Pereira et al., 2018). The present research was conducted after the approval of the local Committee of Ethics in Research under the protocol #1.303.139.

Dental records (n=121) were selected from the Department of Radiology of a private university in Brazil. The inclusion criteria established consisted of dental records of Caucasian patients containing their date of birth and sex; they should belong to patients aged between 10 and 16 years old; and they should contain a set of panoramic, lateral cephalometric and carpal radiographs of the patient. The exclusion criteria consisted of patients that underwent previous orthodontic treatment or the extraction of permanent teeth; trauma in the face, cervical vertebrae or hands; and medical history of systemic diseases that influence on dental and bone development (Vieira et al., 2009; Araújo et al., 2010). After inclusion and exclusion criteria, 98 dental records (48 females and 50 males) remained eligible. All the radiographs stored in the dental records were analyzed by a single trained examiner blind for the age and sex of the patients.

The biological age was initially assessed in panoramic radiographs extracted from the dental records. In these radiographs the developmental stages of the permanent teeth were classified according to the method of Nicodemo, Miranda & Rangel (1974). The teeth chosen for classification according to this method were the canines, first and second premolars and first and second molars (Moraes, Aragão & Heck 1998). The stages considered for each tooth were imported in CRONOL software package (UNESP, São Paulo, Brazil), available in: http://ict.unesp.br/#!/departamentos-de-ensino/diagnostico-e-cirurgia/disc-radiologia/. The software estimates the mean age of the patient taking into account the stages of all the teeth analyzed previously. In order to make the outcomes also compatible with the clinical routine in Dentistry, the developmental stage of the mandibular left second molar was also classified. The stage was implemented in Scpan software package (UNESP, São Paulo, Brazil), available in: http://ict.unesp.br/#!/departamentos-de-ensino/diagnostico-e-cirurgia/disc-radiologia/. The software reports the relation between the dental stage and the pubertal growth spurt (Moraes et al., 2008).

The cervical vertebrae C2, C3 and C4 were assessed in the lateral cephalometric radiographs and were classified according to the method of Hassel & Farman (1995). This method is founded on the morphology of the cervical vertebrae, which is classified in 6 developmental stages. The stages correspond to developmental initiation, acceleration, transition, deceleration, maturation and finalization.

The development of the hand and wrist was assessed in carpal radiographs according to the method of Fishman (1982), from which 4 stages were used to classify the development of the first (thumb), third and fifth fingers and the radius. The developmental stages were observed in the epiphyses and diaphyses of the hand and wrist bones, as well in the mineralization of the sesamoid.

2.1 Statistical analysis

Shapiro-wilk test was used to assess the normality of chronological and estimated dental ages, while the homocedasticity was assessed with F test. Intraclass correlation coefficient was used to assess the agreement between the chronological and dental ages. Mann-Whitney test was used to assess the agreement between the chronological age and the estimated ages from cervical and carpal bones.

Chi-square test was used to investigate the influence of sex in the developmental stages of the teeth, cervical vertebrae and hand and wrist bones.

Pearson's and Spearman's correlation coefficient were used to associate the chronological and estimated ages. Forward linear regression analysis was used to investigate the level of dependence between the chronological age and the other variables considered in the present study. Mann-Whitney test was used to investigate the relationship between the pubertal growth spurt and the chronological age.

The statistical tests were performed with GraphPad Prism 6.0 (GraphPad Software Inc., La Jolla, CA, USA), BioEstat 5.0 (Instituto de Desenvolvimento Sustentável Mamirauá, Tefé, AM, Brazil) and SPSS 2.0 (IBM Corp., Armonk, NY, USA) software packages considering a significance level of 5%.

3. Results

Weak concordance between the chronological and estimated age was observed both for the total sample (ICC = 0.263) and for the sample stratified on sex (males: ICC = 0.206; females: ICC = 0.310). The absolute errors between the dental and chronological age ranged between +30 (overestimation) and -53 (underestimation) months. In general, underestimations were observed with the method of Nicodemo et al. (1974). Statistically significant differences were not observed between males and females (p>0.05) (Table 1).

Table 1. Mean and standard deviation of the chronological and dental ages and their difference distributed based on sex.

Variable	F+M (n=98)	F (n = 48)	M (n = 50)
CA	153.3 (±21.0)	149.8 (±20.9)	156.7 (±20.8)
DA	136.6 (±10.6)	135.5 (±11.2)	137.6 (±10.0)
Difference between CA and DA	16.7 (±16.3)	14.3 (±16.7)	19.1 (±15.7)
Error between CA and DA	From +30 to +53	From +30 to -50	From +9 to -53

CA: chronological age; DA: dental age; F: females; M: males; Age expressed in months.

Source: Authors.

Statistically significant differences (p<0.05) were observed for the prevalence of developmental stages of teeth, cervical vertebrae and hand and wrist bones in function of sex. The dental stage representing the end of the pubertal growth spurt was more prevalent in males, while in females the ascending curve was the most prevalent stage (p=0.0004). The ascending curve was less prevalent in males (p=0.0238). Statistically significant differences were not observed between the stages of cervical vertebrae development (grouped between 1 and 3; and grouped between 4 and 6). Yet in the development of the hand and wrist, stage >9 was the most prevalent (p=0.0076) in females compared to males (Table 2).

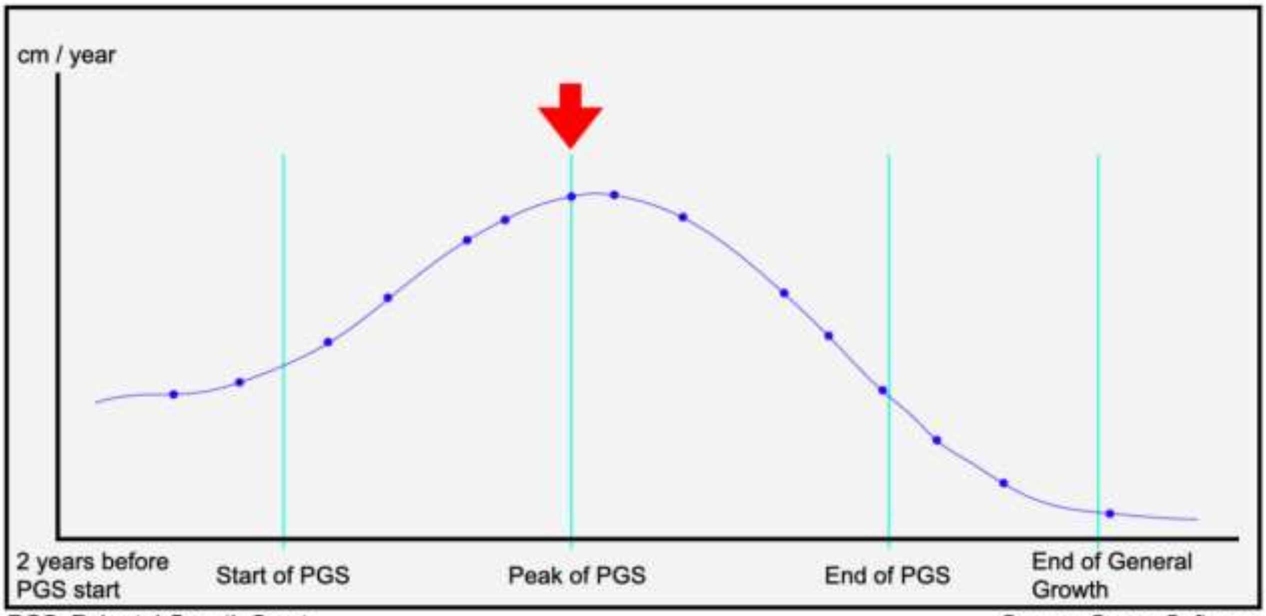
Table 2. Absolute (n) and relative (%) frequency of the sample distributed based on sex according to the different methods used for age estimation.

Methods	Stages	F(n=48)	M (n=50)	*p*
DA	ascending curve	10 (20.8%)	2 (4%)	< 0.05
	peak	21 (43.8%)	9 (18%)	
	descending curve	17 (35.4%)	21 (42%)	
	end of PGS	-	18 (36%)	
CVA	1 - 3	18 (37.6%)	26 (52%)	0.2499
	4 - 6	30 (62.6%)	24 (48%)	
HWA	1-3	2 (4.2%)	7 (14%)	< 0.05
	2-6	8(16.7)	17 (34%)	
	7-9	14(29.2%)	14(28%)	
	>9	24 (50%)	12 (24%)	

DA: dental age; CVA: cervical vertebrae age; HWA: hand and wrist age; F: females; M: males; PGS: pubertal growth spurt ; p: p-value out of Chi-square test considering a significance level of 0.05.
Source: Authors.

In order to investigate the performance of the method for estimating the chronological age was assessed and compared with the age estimates of each method (Table 3). Moderate (rS ou rP between 0.4 and 0.6) or strong (rS ou rP or 0.6 and 0) correlations were found between all the involved variables ($p<0.0001$). The stratification based on sex did not alter the outcomes. The association between the chronological age and the remaining variables is expressed in Figure 1.

Figure 1. Correlation between the chronological and dental (A), hand and wrist (B) and cervical vertebrae (C) ages.



PGS: Pubertal Growth Spurt
Source: Scpan Software

Table 3. Correlation between chronological and estimated ages distributed based on sex.

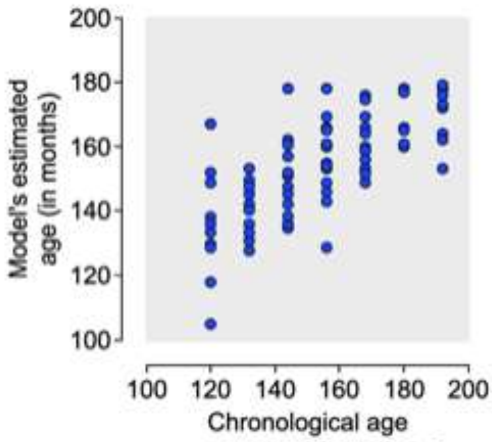
Sex	Method	Correlation outcomes		
		CA	DA	HWA
Females + Males	DA*	0.64		
	HWA**	0.55	0.6	
	CVA**	0.53	0.62	0.75
Females	DA	0.6		
	HWA	0.7	0.63	
	CVA	0.6	0.51	0.7
Males	DA	0.68		
	HWA	0.6	0.71	
	CVA	0.53	0.75	0.74

CA: chronological age; DA: dental age; CVA: cervical vertebrae age; HWA: hand and wrist age; *Pearson's correlation; ** Spearman's correlation.

Source: Authors.

The forward linear regression analysis resulted in a linear model (Table 4), which reliability (Table 5) and performance were optimal (Table 6). The best model combined the following information: "Dental age" ($p=0.0003$), "Hand and wrist classification" ($p=0.0001$) and sex (females=0, males=1, $p=0.0019$). The development of the cervical vertebrae did not contribute significantly to the model ($p=0.2840$). The final formula is described as follows: Chronological age = 27.26 + (0.72 × Nicodemo's dental age) + (2.93 × Fishman's hand and wrist stage) + (11.09 × Sex). Nearly 50% of the age is explained by the variables and the accuracy rate reaches 71.4%. In other words, the formula has better performance for estimating the age compared to a random chance of age estimation ($p<0.0001$) (Figure 2).

Figure 2. Correlation between the chronological age and the age estimated by the new regression model.



Source: Scpan Software

Table 4. Linear regression model obtained with the variables dental age, hand and wrist classification, cervical vertebrae classification and sex for the estimation of age.

						CI 95% of B	
	B	**SE**	**Beta**	**T-test**	***p***	**Min.**	**Max.**
Constant	27.26	22.93	-	1.19	0.24	-18.3	72.8
DA	0.7236	0.2081	0.36	3.68	0.0003	0.33	1.11
HWA	2.93	0.73	0.42	4.0	0.0001	1.48	4.39
Sex	11.09	3.48	0.27	3.18	0.0019	4.17	18.0

DA: Dental age; HWA: hand and wrist classification; SE: standard error; CI: confidence interval; Min.: minimum; Max.: maximum.
Source: Authors.

Table 5. Reliability of the regression model.

R	**R^2**	**Adjusted R^2**	**SE**
0.714	0.51	0.49	14.9

R: Determination coefficient; SE: standard error expressed in months.
Source: Authors.

Table 6. ANOVA outcome from the obtained regression model.

	SS	DF	MS	F	*P*
Regression model	21786.7	3	7262.2	32.5	<0.0001
Scatter	20990.1	94	223.3		
Total	42776.8	97			

SS: sum of the squares; DF: degrees of freedom; MS: mean square;
Source: Authors.

The analysis founded on the estimation of the pubertal growth spurt using the mandibular left second molar indicated that the chronological age (p<0.0001), and the dental (p<0.0001), hand and wrist (p=0.0006) and cervical vertebrae (p<0.0001) development were more advanced in the period between the descending curve and the end of the growth spurt.

4. Discussion

Several methods were designed for the analysis of hand and wrist development in carpal radiographs (Greulich & Pyle, 1959; Tanner & Whitehouse, 1959; Eklöf & Ringertz, 1967; Grave & Brown, 1976; Martins & Sakima, 1977; Camacho-Basallo et al., 2017; Lecca-Molares & Carruitero, 2017). The present research followed previous studies (Duarte et al., 2008; Mohammed et al., 2014) that used Fishman's method (1982). This reliable method (Mohammed et al., 2014) consists of a simple procedure to detect bone development within 6 anatomic regions of the hand and wrist.

In Orthodontics, most of the studies that investigated the development of the cervical vertebrae in relation to the pubertal growth spurt (Caldas, Ambrosano & Haiter Neto, 2010) used the method proposed by Lamparski (1972). In order to simplify the method, Hassel & Farman (1995) modified the analysis of the second and fourth vertebrae. This method was applied in the present study because it is considered reliable for a large part of the sample (Moscatiello et al., 2008).

The sample size addressed in the present study (n=98) is similar to other studies in the field (Santos et al., 2005; Damian et al., 2006; Moraes et al., 2007; Lara et al., 2008). Moreover, the present study has the advantage of examining the age information retrieved from the teeth, cervical vertebrae and hand and wrist. In specific, the reason behind the sample size relies in the sampling process to collect three different types of radiographs taken in the same day from the same patient, as well as the study of Camacho-Basallo et al. (2017). Additionally, the age range addressed in the present study was restricted to cover the period of pubertal growth spurt, as well as Pineau (2020) study, which is an important moment for therapeutic approaches in the dental routine, for example, the right time to choose an orthopedic or surgical treatment.

Mandibular canines, premolars and molars were used in the present study following the scientific literature (Graziosi et al., 1999; Eto & Mazzieiro 2005; Lecca-Molares & Carruitero, 2017; Camacho-Basallo et al., 2017). More specifically, in the investigated age range most of these teeth have incomplete root formation – enabling age estimation through dental staging methods. A deeper investigation was conducted with the mandibular left second molar to associate the development of this tooth in relation to the pubertal growth spurt. This tooth was chosen for this analysis because it is considered a proper indicator of the skeletal maturation and generally completes root development at the end of puberty (Bagherpour, Pousti & Adelianfar, 2014; Kumar et al., 2012; Khan & Ijaz 2011; Sachan, Sharma & Tandon, 2011). Recent studies (Lecca-Molares & Carruitero, 2017; Camacho-Basallo et al., 2017) found a strong correlation between the mandibular second molar calcifications stages and maturation index, mainly pubertal growth peak (cervical vertebrae) and females respectively (Lecca-Molares & Carruitero, 2017; Camacho-Basallo et al., 2017). Nevertheless, Perinetti et al. (2012) reported that the dental calcification was only usefull for diagnosing pre-pubertal growth phase.

Statistically significant differences between the chronological age and the age estimated through the dental development (Nicodemo et al., 1974) were not detected for both the males and females sampled in the present study. Similarly to the previous scientific literature (Graziosi et al., 1999; Silva et al., 2005), the estimated age was lower than the chronological age (underestimations) in the total sample and in the sample stratified by sex. Opposite outcomes (overestimations) were found by Moraes et al. (2007) and Topolski et al. (2014). More specifically, a difference of 14.3 months between the estimated and chronological ages was observed in females, while in males the difference reached 19.1 months. According to Moraes et al. (2007), these outcomes are not common, but do not necessarily indicate an abnormal development of the individuals sampled in the present study, since skeletal growth has periods of acceleration and maturation, and may not be directly associated with chronological age.

In Table 1, the developmental stages of the teeth, cervical vertebrae and hand and wrist are distributed in function of sex. Specific differences were observed in the distribution of the stages according to the age estimation method (based on dental or skeletal parameters). Considering specifically the dental development, the most prevalent phase of the pubertal growth spurt was the ascending curve in females, while in males was the descending curve. In these phases, the mandibular second molar presents complete root development (with open or closed apices). This phenomenon corroborates the study of Moraes et al. (1998) and indicates a developmental delay in females compared to males.

A few numbers of individuals represented each of the stages of cervical vertebrae development. For that reason, the individuals were grouped based on a range of stages. Statistically significant differences were not observed between these groups. A similar approach was used for the developmental stages of the hand and wrist. However, statistically significant differences were observed in females for the group with stages above 9. From a biological point of view, this outcome confirms the normal development of females, which remain above stage 9 for a longer period than males. Clinically, this outcome points towards the early development of females, in which an insignificant pubertal growth is expected.

Many studies reported correlation between chronological age and skeletal maturation (Camacho-Basallo et al., 2017; Alkhal, Wong & Rabie, 2008; Uysal et al., 2006), when considering the development of vertebral with hand and wrist stages this association is more reliable in women than in men (Lamparski 1972; San Román et al., 2002; Uysal et al., 2006; Camacho-Basallo et al., 2017). The study of Camacho-Basallo et al. (2017) highlighted the strong correlation between second molars and females.

In order to test the methods based on their performance, the present study compared the chronological and estimated ages. Moderate and strong correlations between ages were observed (Table 3). It indicates that all the methods investigated in the present study may be used for age estimation based on dental and skeletal (cervical vertebrae with hand and wrist) parameters. Similar outcomes were observed by Soegiharto, Cunningham and Moles (2008), Manhães Júnior (2006), Suma et al., (2011), Kumar et al. (2012) and Maló et al. (2014). Moreover, these methods performed accurate in females and males. This finding suggests that the relation between chronological and estimated ages is not affected by sex.

Like in this study, Lecca-Morales and Carruitero (2017) assessed the relationship with dental calcification and pubertal growth peak stages and reported a high correlation between the mandibular second molar calcification and hand and wrist maturation in both sex. However, the correlation of each phase of the pubertal growth spurt (ascending, peak and descending) has not been tested. Therefore, the three parameters (teeth, cervical vertebrae and hand and wrist) were combined in a linear regression analysis. Out of the three parameters, the development of the teeth and hand-wrist played a major part and contributed to a new regression formula. The formula performed within an accuracy rate of 71.4% to estimate the age using the developmental information from the teeth and hand and wrist. The accuracy rate observed with the formula reflected the scientific literature (Carvalho et al., 2010; Camariere et al., 2015), which suggests that optimal outcomes are achieved with the combination of dental and skeletal parameters.

It is important to note that studies in the scientific literature (Carneiro et al., 2010; Gundim et al., 2014; Karaday et al., 2014) claim differences in dental mineralization timing among different populations. In order to overcome this potential bias, the method of Nicodemo et al. (1974) was chosen – especially because these authors sampled Brazilian individuals as the present study also did. This method was previously applied in Brazilians and it is currently considered practical and reliable (Carvalho et al., 2010; Pessamiglio, 2011; Franco et al., 2020).

Methodological concerns on the limitations inherent to the combination between teeth and bones may justified mainly by the sample size and sample age range. On the other hand, few studies combining age parameters are found in the scientific literature. More specifically, the present study is the only combining the method of Nicodemo et al. (1974) and Fishman et al. (1995) in the same formula. Despite the proper association with age, both methods may not be defined as ideal or perfect. In this context, ideal is to combine age information to achieve optimal age estimations.

5. Conclusion

The three methods used in the present study tend to underestimate the age of females and males. The combination of dental and skeletal parameters in a linear regression formula contributed to accurate age estimations. Age estimations stratified by sex did not result different between females and males.

Additional studies remain necessary to assess the performance of the new formula within other populations and its reproducibility.

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