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Dental Age Estimation in Argentine Adults Based on Lower Premolars' Pulp/tooth Area Using Digital Orthopantomographs

Estimacion de la Edad Dental en Adultos Argentinos Mediante Medición de Área Pulpa/Diente en Premolares Mandibulares, Utilizando Ortopantomografias Digitales

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SUMMARY: The objective of this study was to assess the efficacy and applicability of a dental age estimation method in adults utilizing digital orthopantomographs (OPGs) to measure pulp/tooth area ratios in the lower premolars. The investigation was conducted on a sample of individuals residing in the city of Córdoba, Argentina. A descriptive, observational, cross-sectional study was performed using 300 digital OPGs. The pulp/tooth area of the left lower first premolar and its contralateral analog were evaluated following the protocols developed by Juneja and Cameriere. Analysis of all dimensional ratios demonstrated highly significant values when correlating both premolars with age. The most significant ratios were P, b, and AR. Regarding sex, the a, c, and AR ratios were significant, indicating a direct correlation between them. A more pronounced tendency was observed in females for ratios a and c, whereas in males the tendency was for AR. Regression analysis was conducted in accordance with the proposed model. All factors, except parameter a, demonstrated a significant effect as a predictor of individuals' age, with the AR factor being particularly notable. Subsequently, the average b and c values for the second premolar were calculated. This was followed by the calculation of the average P ratio of both premolars and finally, consideration of the sex factor. The method is reliable and accurate within the age range of 19 to 24 years but tends to overestimate ages younger than 19 and underestimate ages older than 24 years. Males exhibited lower levels of underestimation and overestimation than females. Regression analyses facilitated the development of a customized equation specific to the aforementioned study population.

KEY WORDS: Dental age estimation; Orthopantomography; Pulp/tooth area.

INTRODUCTION

Forensic odontology is defined as the application of dental science to the field of law. It encompasses various domains including the identification of human remains, bite mark comparison, interpretation of oral injuries, and dental malpractice evaluation (Committee on Identifying the Needs of the Forensic Science Community *et al*., 2009). This discipline involves the handling, examination, evaluation, and presentation of dental evidence in civil or criminal procedures, all of legal interest (Avon, 2004; González *et al*., 2005; Ciocca Gómez, 2010).

Identification determines "the traits or qualities that distinguish a person from others and make them unique" (Villanueva Cañadas, 2019). The structure, features, and resilience of teeth render them suitable for identifying both surviving and deceased individuals. Although the comparison of *ante-mortem* (AM) and *post-mortem* (PM) data forms the basis of reliable dental identification, in cases where AM information is lacking, dental age estimation (DAE) and its correlation with an individual's chronological age play a significant role as a forensic sub-discipline (Pretty

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& Sweet, 2001). In children, this procedure is relatively straightforward and accurate because human dentition follows a reliable and predictable developmental sequence (Flores *et al*., 2010).

In adults, DAE presents a significant challenge, both anthropologically and forensically (Cameriere *et al*., 2009; Jain & Rai, 2009). Various methods have been proposed for this purpose, including the use of periapical radiographs, with radiological methods gaining prominence (Cameriere *et al*., 2007; De Luca *et al*., 2011). However, given the fragile nature of dental structures in cases of extreme trauma, the use of orthopantomography (OPG) has been recommended to avoid artifact generation (Rakesh & Juneja, 2014).

The reduction of the pulp chamber space caused by the continuous secretion of dentin matrix by odontoblasts (Cameriere *et al*., 2012) is directly correlated with an individual's age. This occurs because, for physiological and pathological reasons, the dentin-pulp complex produces secondary dentin throughout an individual's lifespan. This secondary dentin is progressively deposited in the pulp chamber, leading to gradual obliteration. While measuring the reduction in pulp chamber space is a significant biological indicator for DAE in adults, few studies have been conducted on this topic, and these have only focused on specific populations (Fabbri *et al*., 2015; Silva Azevedo *et al*., 2015).

The precision and reliability of the methodology employed are critical factors concerning the potential underestimation or overestimation of chronological age. The objective of this investigation was to assess the efficacy and applicability of a DAE method in adults based on the measurement of pulp/toot area ratios in lower premolars through OPGs in a sample of individuals residing in the city of Córdoba, Argentina.

MATERIAL AND METHOD

This descriptive, observational, cross-sectional study was conducted utilizing 300 digital OPGs (scale 1:1) from the archives of a private diagnostic imaging institute. It was performed during the periods 2015-2018. All images were previously obtained for diagnostic purposes unrelated to this study. The OPGs were repurposed for this investigation and were selected through non-probabilistic convenience sampling. The inclusion criteria specified excellent quality and absence of distortions in OPGs from adult individuals residing in Córdoba, Argentina, over 18 years of age, with both right and left lower premolars. The exclusion criteria encompassed premolars with dental caries, periodontal disease, periapical lesions, fractures, physiological or intentional wear, developmental anomalies, misaligned or rotated premolars, and orthodontic or prosthetic appliances. Digital OPGs were anonymized, and each case was assigned an identification number, sex, year of birth, and date of radiographic acquisition recorded on a computer.

Ethical considerations: This study adhered to protocols for human subject OPG collection in accordance with the standards set forth by the Academic Committee for Health Sciences Research (CAIS-FOUNC in original Spanish) Resolution RR1966/22. The research was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki. Medical history data were neither considered nor recorded in the process of selecting OPGs.

Radiographic analysis methodology: Radiographic analysis was conducted by two team members (IR and MG), who underwent prior calibration. A maximum of 10 radiographs were obtained per session for assessment. Radiographic images of the lower premolars were analyzed utilizing ImageJ 1.53a software (Wayne Rasband, National

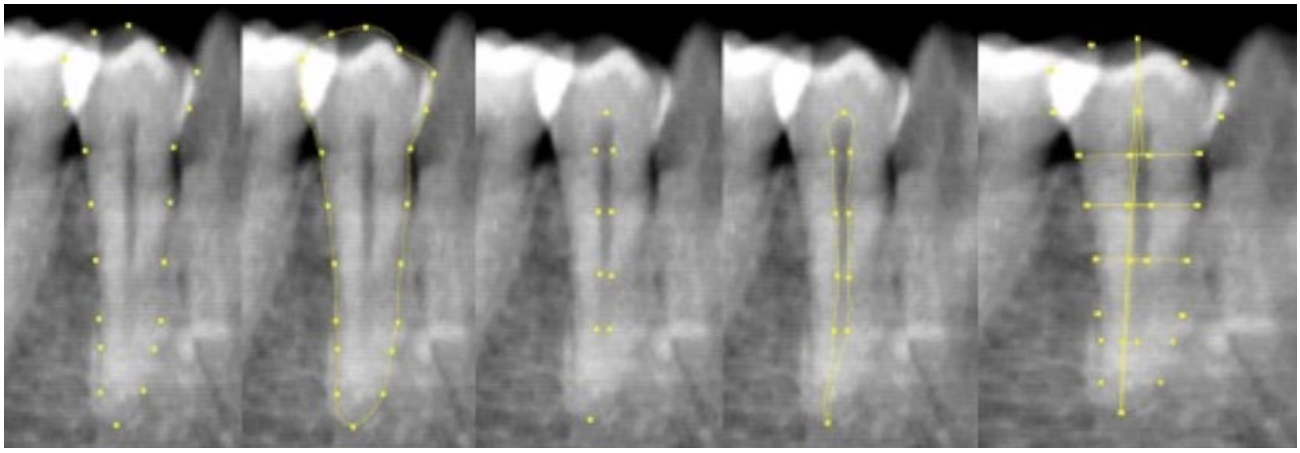

Fig. 1. Protocol for taking measurements and areas.

Institutes of Health, USA). The dental and pulp areas of the left lower first premolar (tooth 45 according to the FDI notation system) and its contralateral analog (tooth 35 according to FDI) were evaluated by placing 20 points along the entire external margin from incisal to apical and 10 points along the entire external margin following the protocol published by Juneja *et al*., (2014). Linear measurements and the dental and pulp areas were assessed according to the parameters established by Cameriere *et al*. (2012) (Fig. 1). The following morphological variables were recorded: pulp length/root length ratio (ratio P), pulp length/tooth length ratio (ratio r), pulp width/root width at the cementoenamel junction level (ratio a), pulp width/root width at the middle part level (ratio c), pulp width/root width at the midpoint between the cementoenamel junction and the root's middle part (ratio b), and pulp area/total dental area ratio (ratio AR).

Statistical Analysis: The data were entered into a Microsoft Excel spreadsheet. Correlation coefficients were assessed between age and the predictive variables. A linear multiple regression model was developed to estimate age based on morphological variables and participants' sex utilizing the stepwise selection method. The mean values of morphological variables between men and women were compared using univariate ANOVA, and intra- and inter-observer reproducibility were evaluated using the Intraclass Correlation Coefficient (ICC). Statistical analyses were conducted using the IBM SPSS Statistics software (version 28.0). Statistical significance was established at $P < 0.05$.

RESULTS

A total of 300 OPGs (140 females and 160 males) were studied, each showing both lower premolars (Table I). No statistically significant differences were observed in the determination of intra- and inter-observer errors, with ICC >0.99 according to Lin (1989). Individual exploratory statistical analyses were conducted for each dimensional parameter or ratio P, r, a, b, c, and AR, followed by the evaluation of generalized linear regression models through the combination of different variables and their interactions to obtain the optimal model and/or the most effective predictor for estimating the dental age of individuals. The analysis of all dimensional ratios revealed significant values when correlating both premolars (35/45) and age. The ratios that demonstrated the highest significance were ratios P, b, and AR. Regarding sex, the ratios that exhibited the most significant values were a, c, and AR, indicating a direct correlation with a more pronounced tendency in females for a and c and in males for AR (Table II).

After testing various generalized linear regression models, the model with the best fit and the lowest RMSE was determined. This includes the following variables:

Response variable: Chronological age
Predictors: Sex; average AR; average P; average a; average (b-45 with c-45)
Excluded: r; b-35; c-35 (r was excluded because its effect size on age was not significant in any of the models tested).
Table III presents the regression analysis conducted, considering the proposed model. All factors except parameter a demonstrated a statistically significant effect as predictors

Table I. Number of OPGs evaluated according to age and sex.

Age	*Frequency*	*Female*	*Male*
18	31	18	13
19	42	16	26
20	49	23	26
21	57	29	28
22	54	21	33
23	67	33	34
Total	**300**	**140**	**160**

Table III. Proposed regression model: The factors were ordered in descending order of the magnitude of the effect in relation to the variation in age.

Origin off the effect (Factor / Covariate)	Chi-square	p value
AR average	43,26	5, E-11
Average bc_45	28,92	8, E-08
P average	14,91	1, E-04
Sex	8,86	0,003
a average	3,25	0,071

Table II. Pearson correlation coefficient between 35/45 and age, as well as univariate ANOVA (F) for sex.

Ratio	**Pearson coefficient (35/45)**	**p value**	**Pearson coefficient (age)**	**p value**	**ANOVA (F) (sex)**	**p value**
P	0,485	6E-19	0,482	7E-19	0,15	0,698
r	0,499	3E-20	0,239	3E-05	0,04	0,849
a	0,385	4E-12	0,320	1E-08	4,71	0,031
b	0,452	2E-16	0,558	6E-26	3,40	0.066
c	0,255	8E-06	0,501	2E-20	8,12	0,005
AR	0,668	4E-40	0,673	7E-41	21,74	4,7E-06

of individuals' age, with the AR factor exhibiting the most pronounced influence. Subsequently, the mean values of b and c corresponding to premolar 45 were positioned, followed by the average P ratio of both premolars and finally the sex factor. The mean for ratio a was included in the model due to the effect's significance level approaching 0.05, and its exclusion resulted in a marginal decrease in the model's R2.

General regression equation including the sex variable (1 for females and 0 for males):

Age = 0.401 **AR average** - 7.609 **P average** + 3.504 **a average** - 8.623 **average bc_45** + 0.397 **sex** + 27.367

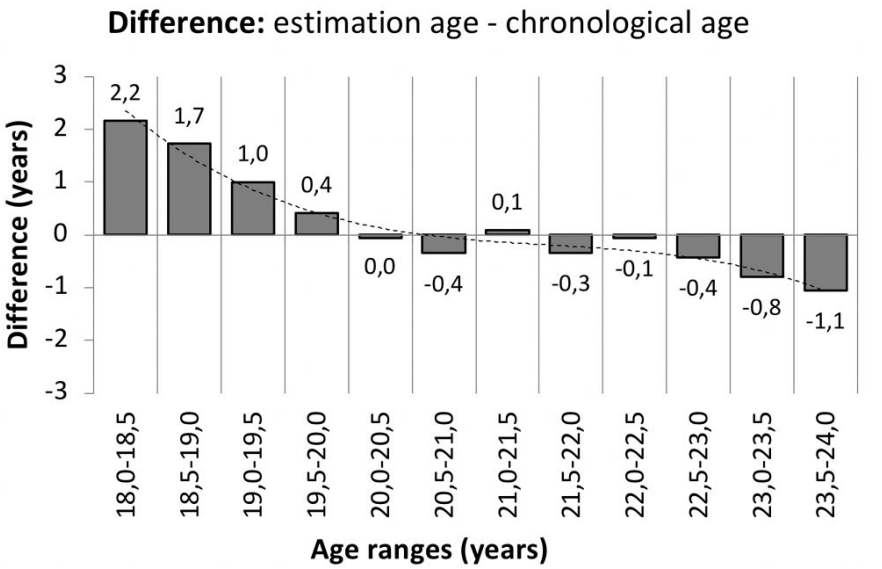

Fig. 2. Differences between the predicted age and the age of the individuals according to age range.

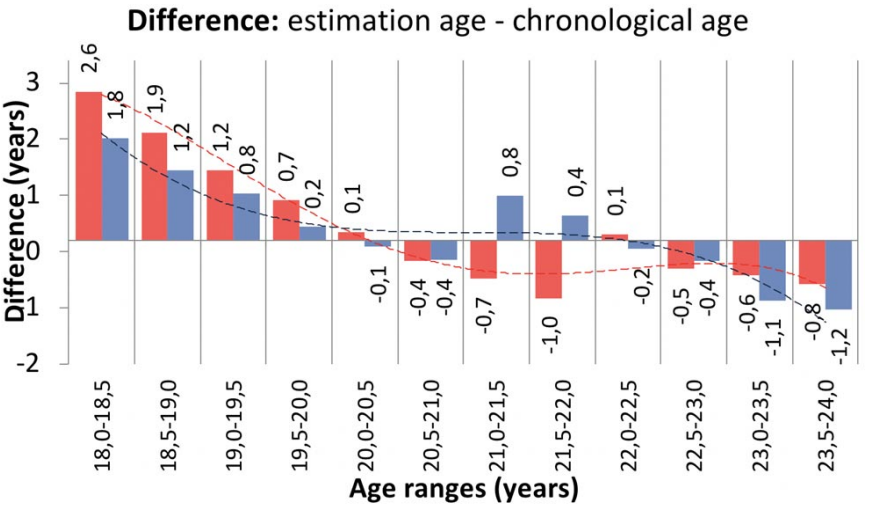

Fig. 3. Differences between the predicted age and the age of the individuals according to age range and sex.

Upon application of the proposed method without sex-based differentiation, an overestimation of age was observed for individuals under 20 years of age, with the magnitude of overestimation increasing as the individual's age decreased, resulting in a mean overestimation exceeding 2 years for the age range of 18 to 18.5 years. Predictive accuracy was deemed satisfactory between 20 and 22.5 years; within this intermediate age range, the relationship between chronological and estimated age exhibited proportionality. Beyond 22.5 years, the trend reversed, leading to an underestimation with a mean difference of 1.1 years by 23.5-24 years (Fig. 2).

When sex-based differentiation was applied, the observed pattern was analogous to the previous findings, with the exception that in the intermediate age range (20–22.5 years), the curves representing both sexes intersected, although the mean differences rarely exceeded one year (Fig. 3).

DISCUSSION

The utilization of DAE methods has recently increased due to population movements and the associated legal requirements (Focardi *et al.*, 2014). Dental age and its correlation with chronological age generate discrepancies among various methods that employ different diagnostic tools and analyze different teeth for this purpose (Liversidge *et al.*, 2003). These methods vary in precision and reliability in underestimating or overestimating dental age compared with chronological age, which renders the selection of a method crucial for accuracy (Verma *et al.*, 2019). Radiographs are frequently the most utilized elements for dental age estimation, with OPGs being particularly significant, as they allow for measurements of all required teeth in a single radiographic image (Karaarslan *et al.*, 2010).

The method employed in this study was based on measuring different proportions at various anatomical sites of the tooth, including the ratios between the pulp and dental areas (Anastácio *et al.*, 2018). Although this

method has been replicated in numerous regions globally (Vadla *et al*., 2020; Deepika *et al*., 2022; Kihara *et al*., 2022; Melo *et al*., 2022), there is a lack of available data on this method in Argentina, specifically in the city of Córdoba, the second most populous city in the country.

Upon analysis of all studied ratios, a moderate degree of symmetry was observed between the values of teeth 34 and 45, albeit with high dispersion, indicating a greater discrepancy between premolars. The proximity of points to the axis of symmetry is inversely proportional to the difference between the proportions of both premolars; conversely, greater distance from the axis corresponds to a larger difference in ratios when analyzing teeth 35 and 45 independently. This phenomenon results in increased uncertainty in estimating an individual's age. Consequently, it was determined that utilizing the average of the relationships of both lower premolars was preferable to minimize the degree of value dispersion while retaining the information provided by the two premolars. For certain parameters, premolar 45 demonstrated superior predictive capacity compared to the average of both. Similar findings were reported by Anastácio *et al*. (2018). They also acquired significant values in all dimensional ratios when both premolars were correlated with age. The ratios that exhibited the highest significance were P, b, and AR. Regarding the latter dimension, the pulp area/dental area ratio decreased with age, which is consistent with the findings of Aguilera-Muñoz *et al*. (2019) and Paewinsky *et al*. (2005). Based on our analysis, we conclude that AR-45 demonstrates a higher predictive power (R2=0.522) than AR-35 (R2=0.195) and the average AR (R2=0.452). The remaining dimensional ratios exhibited low predictive power for estimating age.

With regard to sex, the ratios that demonstrated significant values were a, c, and AR, and it was determined that the correlation was direct with a more pronounced tendency in females for a and c and in males for AR. Some researchers, such as Cameriere *et al*. (2012) and Dehghani *et al*. (2018), have presented differing findings on this topic. However, both failed to establish statistically significant differences between males and females.

The quality of the radiographic image and sample size are frequently the most significant limitations of this method (Zúñiga, 2017; Aguilera-Muñoz *et al*., 2019). In our investigation, the images were of excellent quality, and standardizing the brightness levels, sharpness, and other scanning parameters mitigated issues during measurements. However, in a preliminary test with data from individuals over 24 years old (excluded from this study due to insufficient sample size), we observed very limited predictive power for DAE. Pulp pathology and regular masticatory forces often render it unfeasible to visualize the pulp chamber accurately due to the continuous dentin apposition (Garizoain, 2022). Therefore, expanding the sample size and further improving the intrinsic conditions of the radiographic images could enhance the accuracy of age estimation within these ranges.

CONCLUSIONS

The methodology demonstrates reliability and accuracy within the age range of 19 to 24 years; however, it exhibits a tendency to overestimate in individuals younger than 19 years and underestimate in those older than 24 years. Male subjects exhibited lower levels of underestimation and overestimation compared to female subjects. Regression analyses facilitated the generation of an equation specifically adjusted for the study population.

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RESUMEN: El objetivo de este estudio fue evaluar la eficacia y aplicabilidad de un método de estimación de la edad dental en adultos, utilizando ortopantomografías digitales (OPGs) para medir la relación de áreas pulpa/diente de premolares inferiores. La investigación se llevó a cabo sobre una muestra de individuos residentes en la ciudad de Córdoba, Argentina. Se realizó un estudio observacional, transversal descriptivo utilizando 300 OPG digitales. Se evaluaron las áreas pulpa/diente de los primeros premolares inferiores izquierdos y sus análogos contralaterales siguiendo los protocolos desarrollados por Juneja y Cameriere. El análisis de todas las razones dimensionales mostró valores altamente significativos al correlacionar ambos premolares con la edad. Las razones más significativas fueron P, b y AR. Con respecto al sexo, las razones a, c y AR fueron significativas, indicando una correlación directa entre ellas. Se observó una tendencia más pronunciada en mujeres para las razones a y c, mientras que en hombres la tendencia fue para AR. El análisis de regresión se realizó de acuerdo con el modelo propuesto. Todos los factores, excepto el parámetro a, demostraron un efecto significativo como predictores de la edad de los individuos, destacando especialmente el factor AR. Posteriormente, se calcularon los valores promedio de b y c para el segundo premolar. A continuación, se calculó el cociente P promedio de ambos premolares y, finalmente, se consideró el factor sexo. El método es fiable y preciso en el rango de edad de 19 a 24 años, pero tiende a sobreestimar las edades menores de 19 años y a subestimar las mayores de 24. Los hombres mostraron menores niveles de subestimación y sobreestimación que las mujeres. Los análisis de regresión facilitaron el desarrollo de una ecuación personalizada específica para la población de estudio mencionada.

PALABRAS CLAVE: Estimación de edad dental; Ortopantomografías; Área pulpa/diente.

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