



UNIVERSIDADE ESTADUAL DE CAMPINAS
FACULDADE DE ODONTOLOGIA DE PIRACICABA

VANESSA MOREIRA ANDRADE PINTO

**Estimativa de idade e sexo com base no volume pulpar
utilizando tomografia computadorizada de feixe cônico:
desenvolvimento e validação de fórmulas em uma amostra
brasileira.**

**Age and sex estimation based on pulp cavity volume using cone
beam computed tomography: development and validation of
formulas in a Brazilian sample.**

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Age and sex estimation based on pulp cavity volume using cone beam computed tomography: development and validation of formulas in a Brazilian sample.

Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestra em Biologia Buco-Dental, na Área de Odontologia Legal e Deontologia.

Dissertation presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Master in Oral and Dental Biology in Legal Dentistry and Deontology area.

ORIENTADOR: Prof. Dr. Eduardo Daruge Junior.

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“Escolha uma ideia. Faça dessa ideia a sua vida. Pense nela, sonhe com ela, viva pensando nela. Deixe cérebro, músculos, nervos, todas as partes do seu corpo serem preenchidas com essa ideia. Esse é o caminho para o sucesso”.

(Swami Vivekananda)

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RESUMO

A análise da deposição de dentina secundária e consequente diminuição do tamanho da cavidade pulpar constitui um indicador útil para a estimativa da idade, fundamental nas investigações forenses para a criação do perfil biológico do indivíduo. Porém, dentro da literatura consultada, não há estudo que tenha realizado estimativa do sexo com base nesta análise. Com este objetivo, foi mensurado volumetricamente a cavidade pulpar de 232 dentes, incisivos centrais e caninos superiores, a partir de 116 exames de tomografia computadorizada de feixe cônico (TCFC) de indivíduos brasileiros, de ambos os sexos, com idade entre 13 a 70 anos, pertencentes ao Arquivo da Clínica de Radiologia Odontológica da Faculdade de Odontologia da Universidade Federal do Rio de Janeiro. A análise foi realizada por dois examinadores utilizando o software de livre acesso ITK-SNAP versão 3.4.0. Os resultados permitiram desenvolver fórmulas de regressão linear simples e múltiplas para estimativa da idade e do sexo na população brasileira, sendo validadas por medição de outra amostra composta de 72 dentes. O coeficiente de correlação de Pearson entre idade e volume pulpar foi negativo e significativo para os dois tipos de dentes, obtendo o incisivo central coeficientes maiores ($r = -0.8782$; $R^2 = 0.7713$) do que o canino ($r = -0.8738$; $R^2 = 0.7635$). Das fórmulas desenvolvidas, os melhores resultados foram de estimativa etária para o sexo feminino na presença do volume pulpar apenas dos caninos ($R^2 = 0.8367$) e quando se utiliza o volume pulpar dos dois tipos de dentes ($R^2 = 0.8341$). Os piores coeficientes foram encontrados para as equações de estimativa do sexo quando se tem o volume de apenas um dente e a idade é desconhecida ($R^2 = 0.0268$ para incisivos centrais; $R^2 = 0.075$ para caninos). Já a validação observou menor valor de erro médio absoluto para a estimativa etária da parte da amostra feminina, além de alta precisão da estimativa da idade, de modo geral, nos indivíduos com mais de 35 anos. Foi constatada precisão de 94% para estimativa do sexo quando disponível a informação etária na amostra feminina. As fórmulas desenvolvidas e validadas retrataram bons resultados para aplicação na população brasileira.

Palavras-chave: Odontologia legal; Dentina secundária; Determinação da idade pelos dentes; Determinação do sexo pelo esqueleto.

ABSTRACT

Secondary dentin deposition assessment and its consequent decrease in pulp cavity size represent an useful indicator for age estimation, fundamental in forensic investigations for creation of individual's biological profile. However, in consulted literature, there is no study that has carried a sex estimation based on this analysis. With this purpose, was volumetrically measured the pulp cavity of 232 teeth, upper central incisors and canines, from 116 cone beam computed tomography (CBCT) scans of Brazilian individuals, of both sexes, aged between 13 to 70 years, belonging to the Dental Radiology Clinic's archive from Dental School at the Federal University of Rio de Janeiro. This analyses was carried out, independently, by two examiners using ITK-SNAP software version 3.4.0. Based on data obtained, it was possible to develop simple and multiple linear regression formulas for age and sex estimation in Brazilian population, beign validated from analysis of another sample composed of 72 teeth. Pearson's correlation coefficients between age and pulp volume was negative and statistically significant for both teeth types, obtaining the central incisor higher coefficients ($r = -0.8782$; $R^2 = 0.7713$) than canine ($r = -0.8738$; $R^2 = 0.7635$). From developed formulas, the best results were of the age estimation for female sex in presence only of canines pulp volume ($R^2 = 0.8367$) and when using the pulp volume of both teeth types ($R^2 = 0.8341$). Worst results were observed for the sex estimation equations when there was volume of only one tooth and the age was unknown ($R^2 = 0.0268$ for central incisors; $R^2 = 0.075$ for canines). Validation has found the lowest value of mean absolute error for age estimation in female part of the sample, as well as high accuracy in age estimation, in general, in individuals older than 35 years. Was ascertained 94% of accuracy for sex estimation when the age information was available in the female sample. Developed and validated formulas showed good results for application in Brazilian population.

Keywords: Forensic dentistry; Dentin, secondary; Age determination by teeth; Sex determination by skeleton.

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1. INTRODUÇÃO

A estimativa da idade no tocante a crianças constitui um procedimento relativamente simples e preciso, entretanto, em adultos se torna um desafio para a ciência médico-legal (Juneja et al., 2014). Isso se deve ao fato do processo de maturação esquelética e dentária estar completo nos indivíduos adultos, sendo utilizadas técnicas com base em processos degenerativos, as quais não são tanto precisas como aquelas com base no desenvolvimento dentário utilizadas para estimativa etária em crianças e adolescentes (Nemsi et al., 2017).

Nos últimos anos, esta estimativa tem recebido muita atenção, em virtude do aumento global de corpos e ossadas não-identificadas (Cameriere et al., 2012). E com relação a indivíduos vivos, seu principal objetivo para menores de idade é resolver problemas judiciais relacionados, por exemplo, à adoção, imputabilidade e pornografia infantil, e, para adultos, é impreterível em questões civis relacionadas à aposentadoria ou na ausência de documentos válidos de identificação (Cameriere et al., 2012). Ressaltando-se as investigações criminais onde são frequentes os casos em que indivíduos falsificam ou se opõem a falar sua idade atual, numa tentativa de evitar processo no sistema de justiça criminal dos adultos (Karkhanis et al., 2014; Zanetti, 2014).

Na legislação brasileira, como exemplos de códigos que possuem preceitos de acordo com a idade, observam-se:

- o Código Penal Brasileiro (Brasil, 1940): “Art. 27º - Os menores de 18 (dezoito) anos são penalmente inimputáveis, ficando sujeitos às normas estabelecidas na legislação especial”
- o Estatuto da Criança e do Adolescente (Brasil, 1990): “Art. 2º - Considera-se criança, para os efeitos desta Lei, a pessoa até doze anos de idade incompletos, e adolescente aquela entre doze e dezoito anos de idade.”
- o Código Civil Brasileiro (Brasil, 2002):

Art. 3º - São absolutamente incapazes de exercer pessoalmente os atos da vida civil os menores de 16 (dezesseis) anos. (Redação dada pela Lei nº 13.146, de 2015)¹.

Art. 4º - São incapazes, relativamente a certos atos ou à maneira de os exercer: (Redação dada pela Lei nº 13.146, de 2015)¹ I - os maiores de dezesseis e menores de dezoito anos;

¹Brasil. Lei nº 13.146, de 6 de julho de 2015 [acesso 2019 Jan 6]. Institui a Lei Brasileira de Inclusão da Pessoa com Deficiência (Estatuto da Pessoa com Deficiência). Disponível em: http://www.planalto.gov.br/CCIVIL_03/_Ato2015-2018/2015/Lei/L13146.htm.

Art. 5º - A menoridade cessa aos dezoito anos completos, quando a pessoa fica habilitada à prática de todos os atos da vida civil.”

- a Lei da Previdência Social (Brasil, 2011):

Art. 48º - A aposentadoria por idade será devida ao segurado que, cumprida a carência exigida nesta Lei, completar 65 (sessenta e cinco) anos de idade, se homem, e 60 (sessenta), se mulher. (Redação dada pela Lei nº 9.032, de 1995)²

- o Estatuto do Idoso (Brasil, 2003): “Art. 1º - É instituído o Estatuto do Idoso, destinado a regular os direitos assegurados às pessoas com idade igual ou superior a sessenta anos.”

Para os três primeiros códigos mencionados a faixa etária referente à segunda década de vida dos indivíduos é capaz de ser um fator de distinção na aplicabilidade da lei, e, os demais códigos referidos trazem situações particulares nas quais os indivíduos têm certos direitos quando compreendidos na faixa etária referente à sétima década de vida.

A estimativa do sexo, assim como a estimativa da idade, é um dos aspectos fundamentais para se traçar o perfil biológico do indivíduo, juntamente à estimativa da espécie, estatura e ancestralidade. A determinação do sexo em indivíduos vivos e em cadáveres íntegros geralmente se dá por observação dos aspectos macroscópicos. Porém, em ossadas, corpos mutilados ou em avançado estágio de decomposição pode ser mais difícil dependendo da presença e conservação dos restos humanos, por isso se realiza uma estimativa do sexo.

No ordenamento jurídico brasileiro, como exemplos de normas específicas de acordo com o sexo, no caso, específicas para a população feminina, observam-se:

- a Lei do Feminicídio (Brasil, 2015):

Art. 1º - O art. 121 do Decreto-Lei nº 2.848, de 7 de dezembro de 1940 - Código Penal, passa a vigorar com a seguinte redação: Matar alguém: Feminicídio VI - contra a mulher por razões da condição de sexo feminino; § 2º -A Considera-se que há razões de condição de sexo feminino quando o crime envolve: I - violência doméstica e familiar; II - menosprezo ou discriminação à condição de mulher.

- o Código Penal Brasileiro (Brasil, 1940): “Infanticídio Art. 123º - Matar, sob a influência do estado puerperal, o próprio filho, durante o parto ou logo após.”

²Brasil. Lei nº 9.032, de 28 de abril de 1995 [acesso 2019 Jan 6]. Dispõe sobre o valor do salário mínimo, altera dispositivos das Leis nº 8.212 e nº 8.213, ambas de 24 de julho de 1991, e dá outras providências. Disponível em: http://www.planalto.gov.br/ccivil_03/LEIS/L9032.htm#art3.

O crime do feminicídio é uma qualificadora do crime de homicídio e decorre da desigualdade de poder e força entre os sexos, e o crime de infanticídio procede quando o sujeito ativo do crime é a mulher.

Embora, diversas partes do corpo possam ser usadas para estimativa da idade, algumas podem estar ausentes ou inutilizáveis em virtude da condição precária dos remanescentes corpóreos, em particular nos casos de colisões severas, incêndios, condições de excessiva umidade e inumações arqueológicas (Saxena, 2011; Misirlioglu et al., 2014; Jeevan et al., 2011).

Com isso, a maioria das técnicas reportadas para estimativa etária tem como base os dentes por ser a estrutura mais dura do corpo humano, passam por mudanças fisiológicas relacionadas à idade e mostram grande resistência contra alterações *postmortem* causadas por umidade, altas temperaturas, atividades microbianas e forças mecânicas (Du et al., 2011).

Também é observado auxílio dos dentes na estimativa do sexo, devido ao dimorfismo sexual em caráter morfológico, volumétrico, na cronologia de erupção dos dentes (Vanrell, 2015), e em cunho genético com a pesquisa de cromatina sexual na polpa dentária (Silva, 1997).

A idade de um indivíduo no período da dentição permanente pode ser estimada com um número limitado de indicadores, como níveis de atrição dentária e redução do tamanho da cavidade pulpar. Entretanto, os níveis de atrição são influenciados por fatores ambientais incluindo a oclusão dentária, dieta e hábitos pessoais, não sendo precisa a estimativa etária (Sakuma et al., 2013).

Já a redução gradual do tamanho da cavidade pulpar ocorre devido ao depósito contínuo de dentina secundária após a erupção dentária, sendo esta deposição um critério significativo para estimativa da idade em adultos (Gulsahi et al., 2018). Não sendo observado, na literatura consultada, estudo que tenha realizado a estimativa do sexo com base neste parâmetro.

Essa deposição de dentina secundária pode ser avaliada através de seccionamento do dente para análise microscópica ou através de técnicas radiográficas (Zanetti, 2014), as quais são preferíveis por constituírem método não destrutivo e viável de ser utilizado tanto em indivíduos vivos quanto em cadáveres.

Para superar as limitações das imagens bidimensionais, métodos dentários com a medição da aposição de dentina secundária em imagens tridimensionais foram propostos (Tardivo et al., 2014). E a tomografia computadorizada de feixe cônico representa o exame 3D com amplo uso na prática odontológica, permitindo visualizações dos dentes sem

superposição, distorção ou magnificação, oferecendo uma imagem de alta qualidade e com dose de radiação relativamente baixa (Gulsahi et al., 2018).

Dessa forma, foi elaborado o estudo a seguir visando propor e validar fórmulas nacionais para estimativa da idade e do sexo com base na análise do volume pulpar de caninos superiores e incisivos centrais superiores através de tomografias computadorizadas de feixe cônico, utilizando uma amostra brasileira, de ambos os sexos e ampla faixa etária.

2. ARTIGO: “Age and sex estimation based on pulp cavity volume using cone beam computed tomography: development and validation of formulas in a Brazilian sample.”

Artigo submetido para análise no periódico: “Dentomaxillofacial Radiology” (Anexo 4)

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Abstract

Objectives: To develop and validate formulas for age and sex estimation through pulp cavity volume of teeth using cone beam computed tomography (CBCT) images. **Methods:** The sample was composed of 116 CBCT scans of Brazilian individuals from both sexes, aged 13 to 70 years old. In total, 232 teeth were evaluated, upper central incisors and canines. The evaluation was conducted by two calibrated examiners, using ITK-SNAP software to obtain pulp cavity volumes. Linear regression models were developed for age and sex estimation and its validation was conducted using another sample of 72 teeth. **Results:** Pearson's correlation coefficients between age and pulp volume was negative and significant ($p<0.0001$) for both teeth, with similar values ($r = -0.8782$; $R^2 = 0.7713$ for central incisors, and $r = -0.8738$; $R^2 = 0.7635$ for canines). Best determination coefficients were those of age estimation equations for females, in presence of canines' pulp volume ($R^2 = 0.8367$) and of the two teeth together ($R^2 = 0.8341$). Lower coefficients was observed for sex estimation equations when there was volume of only one tooth and age was unknown ($R^2 = 0.0268$ for central incisors; $R^2 = 0.075$ for canines). Validation observed high accuracy of age estimation in individuals older than 35, as well as 94% accuracy in sex estimation when age was known in female sample. **Conclusions:** Our formulas obtained excellent results, and can be applied in Brazilian population. Best results were observed for age estimation in female sample and for sex estimation when the age of female individuals was known.

Keywords: forensic dentistry; cone-beam CT; secondary dentin; age determination by teeth; sex characteristics.

Introduction

In forensic investigations, age and sex estimation is fundamental for the creation of the individual's biological profile. In cadavers, intact or reduced to pieces, age estimation helps in human identification with the narrowing of potential combinations of identity within the sample available.¹ Similarly, sex estimation contributes in the identification cases, mainly in skeletonization situations. Moreover, when it comes to living individuals, such estimations are relevant in different situations, like in the context of civil law, in cases of refugees and asylum seekers, where there is lack of information concerning identification.¹ Similarly, there are cases of criminal nature, which require the estimation of age for imputation of criminal responsibility, determining the status of adult.¹

Teeth are widely used in forensic and anthropological investigations to determine the sex and age of human remains, as they are the best-preserved parts of the human body, regardless of the cause of death and state of preservation of the body.^{2,3}

After the complete development of the apical part of the root, the eruption of the tooth in the oral cavity and the beginning of the tooth's functioning, there is a physiological deposition of dentin in the pulp cavity, called secondary dentin.⁴ Its deposition by odontoblasts is a continuous process, causing progressive narrowing in the volume of the pulp chamber of all teeth.^{4,5} As it is deposited along the inner surfaces of the tooth, which makes it well-protected from environmental influences, pulp evaluation has the potential to eliminate, at least in part, the effects of external factors.⁶

The assessment of deposition of secondary dentin can be conducted via destructive methods, such as dental sectioning for microscopic study, or via conservative methods, such as imaging exams. Destructive methods are not acceptable for forensic purposes for causing the loss of evidence.^{1,7} In addition, due to ethical issues, non-invasive imaging analyses such as cone beam computed tomography (CBCT) are preferable for application in living individuals.

The use of three-dimensional images demonstrates the actual morphological changes, being best suited for teeth-based age estimation due to their three-dimensional nature, when compared to two-dimensional radiographic images such as periapical radiographs.^{2,4} In dental practice, CBCT offers good image quality, reduced radiation dose in relation to multidetector computed tomography, in addition to allowing a multiplanar and accurate assessment of the teeth's volume.^{2,5}

The morphological changes of the pulp cavity or/and the accumulation of dentin serve as more promising age estimation indicators, being often used.⁸ According to the literature on sex estimation, there is a study that obtained this estimate based on the total volume of the tooth², but no prior study evaluated only the pulp volume.

In Brazil, there is a scarce amount of studies about age estimation based on the pulp volume parameter. Due to the anthropological differences that exist between ancestries, methods must be developed in a specific population, because the application of foreign standards results in a loss of estimation accuracy.⁹

Thus, this study aimed to develop and validate new formulas to estimate age and sex from the pulp volume of upper central incisors and canines, using CBCT images of a Brazilian sample.

Methods and materials

Sample

This project was approved by the local research ethics committee (CAAE 79944817.4.0000.5418).

Software BioEstat version 5.0 (Mamirauá Foundation, Belém, PA, Brazil) was used for the calculation of sample power and size, based on the study by Porto et al¹⁰, which shows minimal difference between the means described for the extreme age groups corresponding to 8.5 for pulp volume, with 5.0 mean standard deviation. Thus, considering a 5% alpha and 95% test power, a sample with 90 patients would be significative.

In total, 116 CBCT scans were selected of patients aged from 13 to 70 years old of both sexes, having at least one upper central incisor and one upper canine. Thus, the final sample was composed by 232 teeth. Given the symmetry of the internal anatomy of the tooth observed by a previous study¹¹, when the tooth on the right side did not fit the criteria, the tooth on the left side of the same patient was used. In this way, the sample was balanced according to the age and sex variables, with a patient for each sex and for each age in the range between 13 and 70 years old, thus enabling an equitable distribution of the sample. The exams had no race/phenotype information available.

The inclusion criteria for the selection of the sample were: exams containing data concerning the patient's date of birth and sex, in addition to the date of completion of the examination, as well as those who had at least one healthy permanent upper central incisor and one healthy permanent upper canine with fully-formed roots and erupted in the oral cavity. Exams including teeth with decay or cavities, root canal therapy, restorations, excessive dental wear reaching the incisal/occlusal third of the tooth's crown, orthodontic or prosthetic devices, pulp calcification, dental fracture, impaction, severe rotation or some other severe type of malocclusion, parodontitis, torn roots, development abnormalities and periapical lesions were excluded. In addition, exams with low quality due to the significant formation of artifacts caused by the presence of materials with high atomic number were also excluded.

The scans were obtained in the period from June 2012 to April 2018 using a KODAK K9500® scanner (CARESTREAM Health, Rochester, USA), with 0.2 mm³ and 0.3 mm³ voxel size, and with the same energy parameters (90kVp and 10mA), as well as variation in the size of the field of view (FOV) as indicated for each patient (15 x 9 cm and 20 x 18 cm).

Image analysis

The proprietary software (CS 3D Imaging®, Carestream Health, Rochester, NY) was used for selecting the exams. After this, the exams were exported in the DICOM format. These images were individually exported to the ITK-SNAP segmentation software version 3.4.0 STABLE (Cognitica, Philadelphia, PA) to measure the selected teeth's pulp volume.

The evaluations were conducted by two examiners, dental surgeons, previously calibrated and with prior knowledge about computed tomography and about the use of the ITK-SNAP software's tools. The evaluations were performed individually, using laptop computers (15.6-inch LCD Full HD monitor) under low light conditions.

The two examiners performed prior calibration under low light conditions, using a sample of 20 teeth, upper central incisors and canines of 6 CBCT scans, which did not compose the study sample. The teeth were evaluated twice in different moments with a 15-day interval between them to obtain the pulp volumes. The average of density range observed in teeth from calibration sample led the setting of the default density range (0 to 1300) for segmentation of the assessment, reassessment and validation sample. Afterwards, the intraclass correlation coefficient (ICC) was used to determine the intra- and inter-examiner agreement in the calibration phase. The intra-examiner agreements ranged from 0.994 to 1.000, while the inter-examiner agreements ranged from 0.994 to 0.998. Therefore, the examiners were able to perform the image analysis.

The pulp volumes were carried out with the software's semi-automatic segmentation mode. Each examiner applied manual refinement, observing the limits of the pulp space in the three plans of the image.

The limits of any extension of the tooth to be examined were marked by the examiners in the multiplanar reconstructions, defining the region of interest (ROI) for segmentation (**Figure 1a**). Next, the default density range was adjusted (0 for the lower threshold and 1300 for the upper threshold), so the 3D model to be built would have only the voxels with grey values within this range (**Figure 1b**). Then, bubbles with radius ranging from 0.5 to 0.9 were added throughout the entire pulp extension of the region of interest delimited in the multiplanar reconstructions (**Figure 1c**), and the segmentation was initiated gradually, allowing a minimum of 200 iterations for the contour evolution of the bubbles before finalizing the 3D segmentation of the area of interest. At the end of this process, the image of the 3D reconstruction of the pulp cavity was reconstructed in the software's 3D window (**Figure 2**). The volumes of the segmented structures were measured in cubic millimeters (mm^3). It is important to report that no smoothing or other effect was used.

Figure 1 Steps of the process of segmentation of a right upper central incisor using CBCT imaging. **(a)** Selection of the region of interest (ROI) in the axial, sagittal and coronal planes. **(b)** After adjustment of the default density range. **(c)** Addition of bubbles throughout the entire pulp extension in the axial, sagittal and coronal planes.

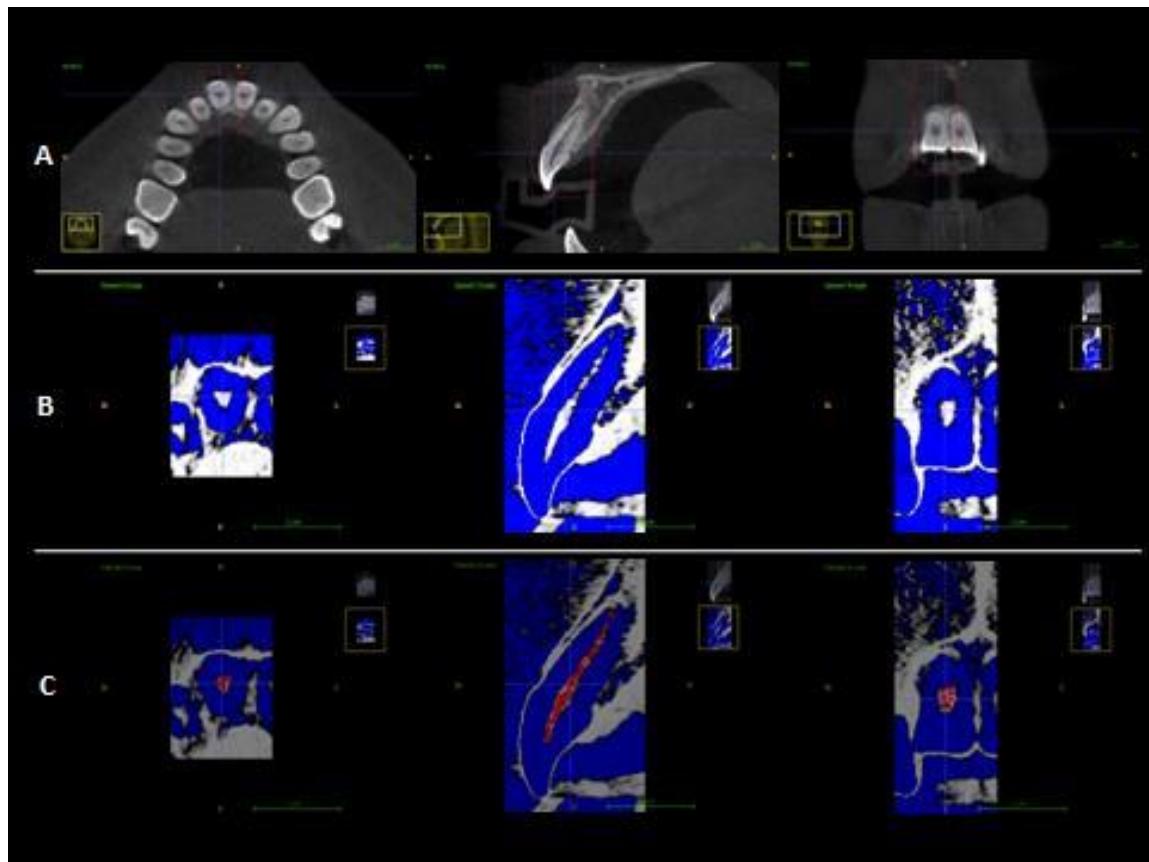
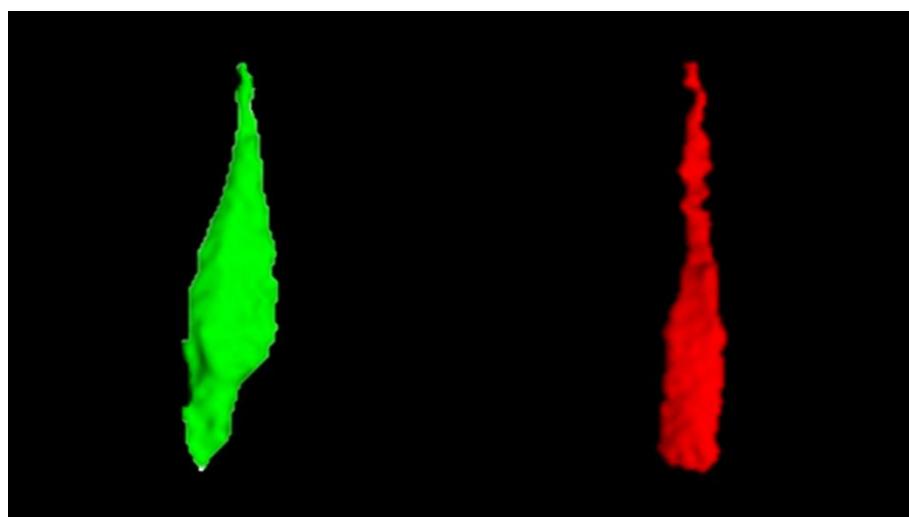


Figure 2 Image of the 3D reconstruction of the pulp of an upper right canine (in green) and of an upper right central incisor (in red)



Reproducibility

Thirty days after the completion of the assessments, 20 teeth were randomly selected from the main sample and re-evaluated for obtaining the reproducibility.

Statistical analysis

The analyses were carried out using the BioEstat software version 5.3 (Instituto de Desenvolvimento Sustentável Mamirauá, Tefé, MA, Brazil).

ICC determined the intra- and inter-examiner agreement.

Single and multiple linear regression formulas were developed to estimate age and sex using pulp volume as the dependent variable, when an upper canine, an upper central incisor or both types of teeth were present in the dental arch.

Pearson's correlation coefficient was used to assess the correlation between chronological age and pulp volume.

Validation

For validation of the developed formulas, another sample was selected by applying the same previously established inclusion and exclusion criteria. This sample was made up of 36 CBCT scans, 18 of males and 18 of females, with 3 individuals for each age group, thus totaling 72 teeth. The pulp volumes obtained in this sample were used in the formulas developed with the main sample analysis and age and sex were estimated.

The age found through formulas and chronological age were compared by paired Student *t* test was conducted and validation charts were drawn in Excel.

Results

The ICC for intra-examiner agreements were 0.9998 for examiner 1 and 0.9825 for examiner 2. The ICC for inter-examiner agreement was 0.9997. Both ICC values revealed excellent reproducibility of the volumetric measurements; because of that, the mean value of the pulp volumes obtained by the examiners were used to perform the linear regression and Pearson's analysis.

Pearson's correlation coefficient between chronological age and pulp volume was negative and statistically significant ($p<0.0001$) for both types of teeth (**Table 1**), with high correlation values having been observed. The upper central incisor had a slightly higher coefficient ($r = -0.8782$; 95% CI = -0.91 to -0.83; $R^2 = 0.7713$) than the upper canine ($r = -0.8738$; CI 95% = -0.91 to -0.82; $R^2 = 0.7635$). Correlation graphics showing correlation

between chronological age against the pulp cavity volume of upper central incisors, and between chronological age against the pulp cavity volume of upper canines, are shown in **Figure 3** and **Figure 4**, respectively.

Table 1 Correlation coefficients between age and pulp volume

Tooth	Pearson correlation	IC 95%	R ²	t value	p value
Upper central incisor	-0.8782	-0.91 a -0.83	0.7713	-19.609	<0.0001
Upper canine	-0.8738	-0.91 a -0.82	0.7635	-19.1829	<0.0001

Figure 3 Correlation graphic showing correlation between chronological age against the pulp volume of upper central incisors

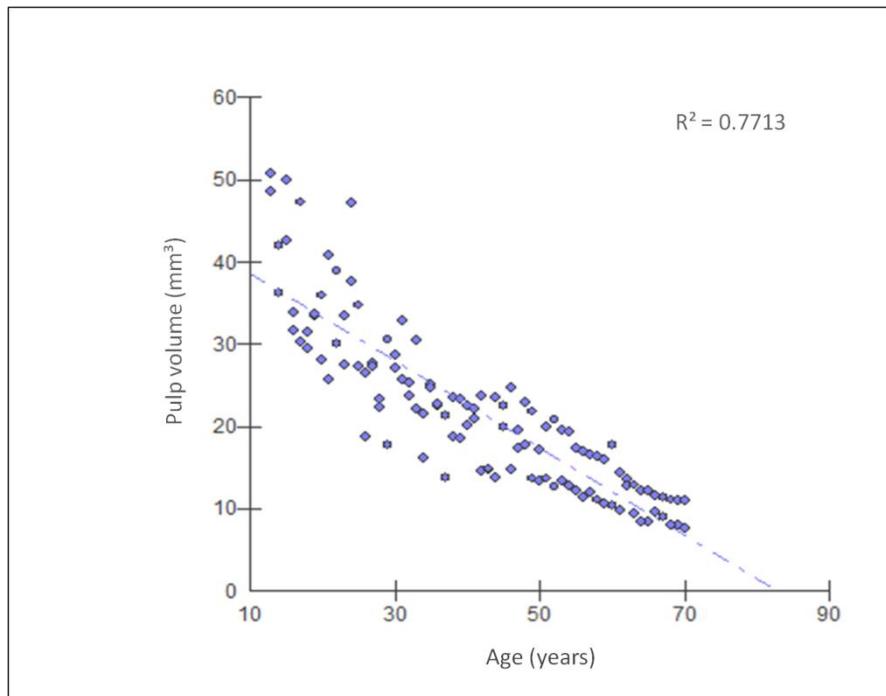
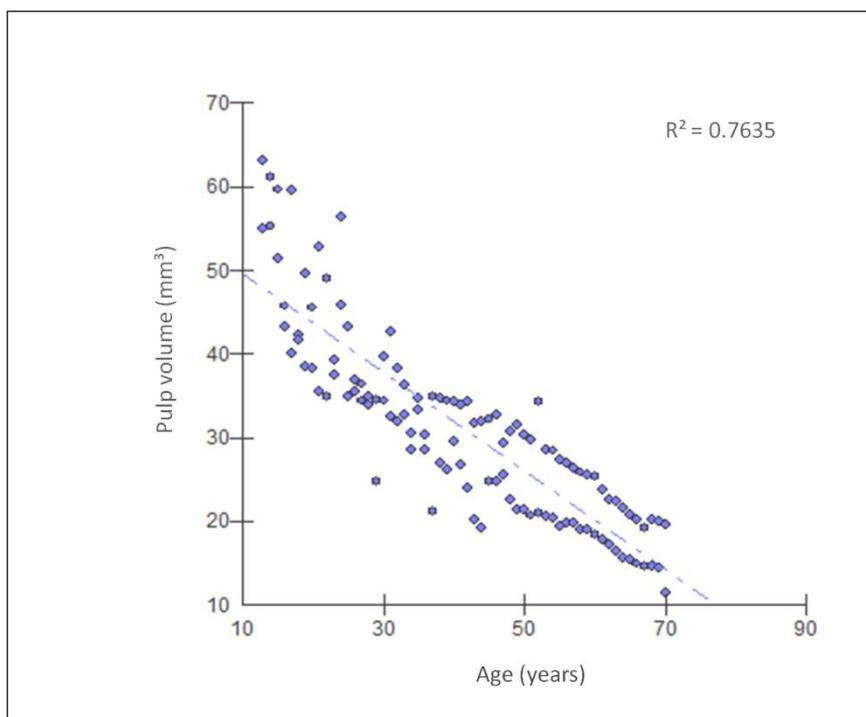


Figure 4 Correlation graphic showing correlation between chronological age against the pulp volume of upper canines



Pearson's correlation coefficient between sex and pulp volume was not used, since sex did not constitute a parametric variable. It was also not employed when the two types of teeth were present in the dental arch, because the coefficient only allows the correlation between two variables.

Single and multiple linear regression equations were developed for the sample population. In **Table 2**, equations when only one type of tooth is present may be observed. Five equations were developed for each type of tooth, three for age estimation and two for sex estimation. Of the equations for age estimation, the first was used when there was certainty that the individual belonged to the female sex, and pulp volume was known; the second was used when there was certainty that the individual belonged to the male sex, and pulp volume was known; and the third was used in the absence of information about sex, and when only pulp volume was known. Of the equations for sex estimation, the first was used when both age and pulp volume were known; and the second was used in the absence of information about age, and when only pulp volume was known. It was established that when using the equations to estimate sex, if the value found was close to 1, it would be regarded as a female tooth, and if it was close to 2, as a male tooth.

Table 2 Equations when there is only one type of tooth

Tooth			Regression linear equation	R ² adjusted	p values
Upper central incisor	For age estimation	Female	Age = 70.4276 + (-1.449 x central incisor volume)	0.7938	< 0.0001
	Male		Age = 78.8388 + (-1.5712 x central incisor volume)	0.8007	< 0.0001
	Unknown sex		Age = 73.2547 + (-1.4524 x central incisor volume)	0.7693	< 0.0001
Upper canine	For sex estimation	Known age	Sex = -0.2802 + (0.0405 x central incisor volume) + (0.0215 x age)	0.1391	0.0002
	Unknown	age	Sex = 1.2973 + (0.0093 x central incisor volume)	0.0268	central incisor < 0.0001 age 0.0001
	Female		Age = 80.0709 + (-1.3924 x canine volume)	0.8367	0.0435
Upper canine	Male		Age = 90.2228 + (-1.4232 x canine volume)	0.8228	< 0.0001
	Unknown sex		Age = 81.4635 + (-1.2905 x canine volume)	0.7614	< 0.0001
	For sex estimation	Known age	Sex = -1.4839 + (0.0537 x canine volume) + (0.0318 x age)	0.3396	< 0.0001
	Unknown	age	Sex = 1.1064 + (0.0127 x canine volume)	0.075	canine < 0.0001 age < 0.0001
					0.0017

The best determination coefficients were demonstrated for the age estimation equations when using the pulp volume data of the upper canines in the presence of information about sex, and when using the upper central incisors, the results were slightly lower. However, in the absence of information about sex, a lower determination coefficient may be noted, being slightly higher for the upper central incisors than for the upper canines.

The sex estimation equations obtained lower results. When age was known, the determination coefficient of the regression equation was greater than when age was unknown; also, the coefficient was higher for the upper canines than for the upper central incisors.

The multiple linear regressive equations of **Table 3** were developed for when both types of teeth are present in the dental arch.

Table 3 Equations for using when both types of teeth are present

Teeth		Multiple regression linear equation	R ² adjusted	R ² upper canine	R ² upper central incisor	R ² upper canine and central incisor	p values
Upper canine and central incisor	Female	Age = 79.4605 + (-1.2857 x canine volume) + (-0.1175 x central incisor volume)	0.8341	83.96%	0.03%	83.99%	< 0.0001 canine 0.0003 central incisor 0.7451
	Male	Age = 87.3157 + (-0.9881 x canine volume) + (-0.5045 x central incisor volume)	0.8255	82.59%	0.57%	83.16%	< 0.0001 canine 0.0041 central incisor 0.1774
	Unknown sex	Age = 77.4387 + (-0.5677 x canine volume) + (-0.8397 x central incisor volume)	0.7779	77.13%	1.05%	78.18%	< 0.0001 canine 0.0215 central incisor 0.0026
For sex estimation	Known age	Sex = -1.3783 + (0.0282 x age) + (0.0827 x canine volume) + (-0.039 x central incisor volume)	0.3756				< 0.0001 canine < 0.0001 central incisor 0.0071
	Unknown age	Sex = 0.806 + (0.0667 x canine volume) + (-0.0627 x central incisor volume)	0.183	8.30%	11.42%	19.72%	< 0.0001 canine < 0.0001 central incisor = 0.0001

When using the pulp volume values of the two types of teeth, the age estimation equations obtained a high determination coefficient, which was slightly higher for women compared to men. On the other hand, the age estimation equation in the absence of information about sex obtained a lower coefficient. It was observed that when inserting the upper canine pulp volume covariate, the regressive model explained the total age variation significantly, with a discrete increase in the determination coefficients when adding the upper central incisor pulp volume covariate.

The sex estimation equations with the pulp value of the two types of teeth obtained inferior results, which were a little better when age was known. It was observed that the addition of the upper incisor pulp volume covariate, posteriorly to the upper canine data, increased the equation's determination coefficient more significantly.

Results of the validation

Table 4 shows the mean absolute error (MAE) and *p* values when estimated and chronological ages were compared.

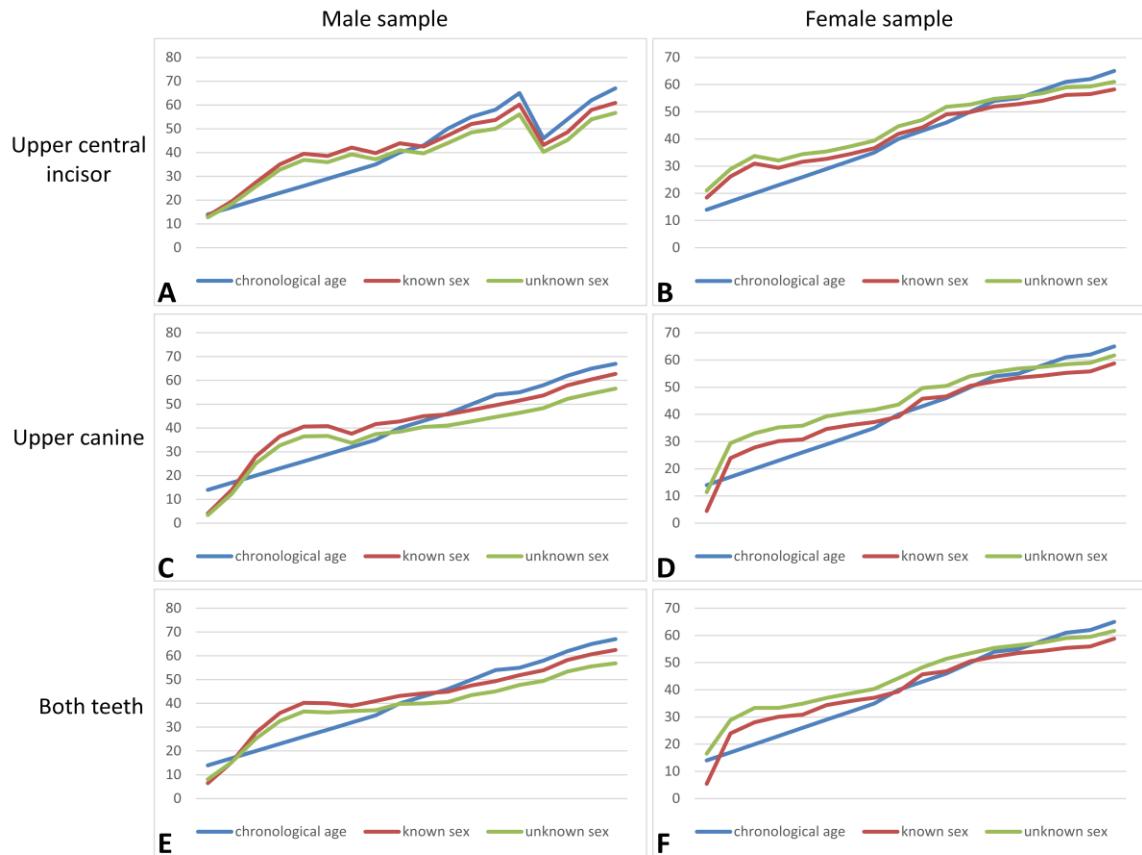
Table 4 Value of *p* and mean absolute error for the age estimate, comparing the chronological age and the estimated age of the validation sample using the developed formulas

Sex sample	Information available	Tooth type	<i>p</i> value	Mean Absolute Error
Male sample	With sex info	Upper central incisor	0.4623	5.45297
		Upper canine	0.6744	5.86164
		Both teeth	0.5641	5.58014
	Without sex info	Upper central incisor	0.3251	6.22535
		Upper canine	0.0678	7.05212
		Both teeth	0.1479	6.39402
Female sample	With sex info	Upper central incisor	0.4241	4.21398
		Upper canine	0.9287	4.34364
		Both teeth	1.0	4.25219
	Without sex info	Upper central incisor	0.0066	5.25634
		Upper canine	0.0075	5.96617
		Both teeth	0.0028	5.36004

It was observed that the best age estimation results were found when the information concerning sex was known.

The validation enabled the creation of the graphs of **Figure 5**, allowing a better view of the comparison between real or chronological age and estimated age in situations where sex was known and in situations when it was not.

Figure 5 Comparison of chronological age (blue line) and estimated age in situations when sex is known (red line) and in situations when it is not known (green line). **(a)** Using only the upper central incisor of the male validation sample. **(b)** Using only the upper central incisor of the female validation sample. **(c)** Using only the upper canine of the male validation sample. **(d)** Using only the upper canine of the female validation sample. **(e)** Using the two types of teeth, upper central incisor and upper canine of the male validation sample. **(f)** Using the two types of teeth, upper central incisor and upper canine of the female validation sample.



In the situations of **Figures 5a** and **5b**, it may be observed that until the age of approximately 20 years old, there was a similarity between the chronological and estimated age, up to 20 until 35 years old the age was overestimated, while from approximately 35 years old up to 70 years old there was again the similarity between the chronological and estimated age. In the situations of **Figures 5c** and **5e** it was found that up to around 20 years old, age was underestimated. In the range between 20 years old and approximately 35 years old, age

was overestimated, and after this age, there was similarity between the chronological and estimated age. **Figure 5d** shows that in individuals up to about 15 years old, when sex was known, the underestimation of age was greater than when sex was not known. After this age until approximately 35 years old, and in the range between 35 and 70 years old, the graph behaved in the same way as the previous ones. And finally, in **Figure 5f** it may be verified that in individuals up to about 15 years old, age was underestimated when sex was known, with overestimation in individuals up to approximately 35 years old and similarity in those up to 70 years old. When sex was not known, age was overestimated in individuals up to about 35 years old, and it was similar to chronological age in individuals up to 70 years old.

The same pulp volume values were inserted in the sex estimation formulas, and the accuracy results are observed in **Table 5**. The formulas developed by the linear regression established that results close to 1 mean the patient is female while results close to 2 mean the patient is male. Therefore, it was used results higher than 1.47, rounded up to 1.5 indicated male and lower than 1.47 indicated female. Afterwards, the estimated sex was compared to actual patient's sex and a percentage of accuracy was calculated.

Table 5 Sex estimation of the validation sample using the developed formulas

Sex sample	Information available	Tooth type	Accuracy
Male sample	With age info	Upper central incisor	78%
		Upper canine	78%
		Both teeth	78%
	Without age info	Upper central incisor	72%
		Upper canine	72%
		Both teeth	83%
Female sample	With age info	Upper central incisor	94%
		Upper canine	94%
		Both teeth	94%
	Without age info	Upper central incisor	50%
		Upper canine	50%
		Both teeth	67%

As was found in the age estimation formulas' validation, the results of the sex estimation formulas' validation were generally better when some other biological profile information was available, in this case, the information regarding age. The sex estimation formulas showed high accuracy during validation, which was higher for the female sample, in all three situations, using the upper central incisors, the upper canines and both teeth. There

was also good accuracy when using the pulp volume data of the two types of teeth in the absence of information regarding age.

Discussion

In our study, the correlation between chronological age and pulp volume was negative and statistically significant ($p<0.0001$) for both types of teeth, a marginally higher value having been found for the upper central incisor ($r = -0.8782$ and $R^2 = 0.7713$) when compared to the upper canine ($r = -0.8738$ and $R^2 = 0.7635$). High correlation values for these types of teeth were also found in another study¹¹, in which after assessing the relationship between the pulp volume/tooth ratio of anterior upper and lower teeth and age, was observed a stronger correlation for upper central incisors and canines compared to other teeth, commenting that this result can be explained by the smaller internal anatomical variation of these teeth.

A prior study¹², which analyzed the pulp volume/tooth ratio based on CBCT images of several types of teeth, also found a higher Pearson's correlation coefficient for the upper central incisors ($R^2 = 0.532$) than for the upper canines ($R^2 = 0.153$). However, it should be noted that our correlation values refer to a Brazilian population and are more expressive as several previous studies.¹³⁻¹⁵

In the current study, the age estimation equation using the upper canines had slightly higher correlation for women than for men (adjusted $R^2 = 0.8367$ for women; adjusted $R^2 = 0.8228$ for men). The same happened when using the age estimate equations for the two types of teeth (adjusted $R^2 = 0.8341$ for women; adjusted $R^2 = 0.8255$ for men). As for the age estimation equation using the upper central incisors, the opposite occurs (adjusted $R^2 = 0.7938$ for women; adjusted $R^2 = 0.8007$ for men).

This greater correlation for men as compared to women was also confirmed in other studies^{13,16}. The former analyzed the upper central incisors, having found high determination coefficients ($R^2 = 0.851$ for men and 0.776 for women), and the latest found more modest values, having analyzed the upper canines ($R^2 = 0.273$ for men and 0.180 for women).

In the current study, estimating age when sex was unknown using only one type of tooth resulted in lower determination coefficients (adjusted $R^2 = 0.769$ for the upper central incisors and $R^2 = 0.7614$ for the upper canines). The same happened when using the two types of teeth, R^2 having also been lower when sex was unknown (adjusted $R^2 = 0.7779$).

In this study, it was observed that in isolation, when sex is known, the canines have better results when compared to the central incisors. However, when sex is unknown, the

central incisors are slightly better. The use of two teeth did not significantly increase the values of the formulas' determination coefficient.

In relation to the sex estimation formulas, these obtained lower results, which were lower when using the volume of only one tooth if age was unknown (adjusted $R^2 = 0.0268$ for the upper central incisors; $R^2 = 0.075$ for the upper canines). When age was known, the sex estimation equations using only one type of teeth obtained slightly higher determination coefficients (adjusted $R^2 = 0.1391$ for the upper central incisors; adjusted $R^2 = 0.3396$ for the upper canines). As for the sex estimation equations using the pulp value of the two types of teeth, these also obtained low results, which were slightly better when the age covariate was added to the equation (adjusted $R^2 = 0.3756$) than when age was unknown (adjusted $R^2 = 0.183$).

The validation found better age estimation results when the information concerning sex was known, with emphasis on the high values of p in the female part of the sample, using only the upper canines ($p = 0.9287$) and using the two types of teeth ($p = 1.0$).

The lowest mean absolute error values were found for the female part of the sample, when information concerning sex was known and when using only the central incisors (MAE = 4.21398) and the two types of teeth (MAE = 4.25219), while the highest mean absolute error values were found for the male part of the sample, when information concerning sex was unknown and when using only the upper canines (MAE = 7.05212) and the two types of teeth (MAE = 6.39402). In a previous study¹⁴, a lower mean absolute error value was found for the upper canines, considering the total sample, that is, both sexes (MAE = 3.876).

The results of our validation demonstrated that in individuals up to the age of 35 years old in general, age is overestimated, and after this age there is excellent harmony between chronological age and the age estimated with the formulas proposed, both when there is information concerning sex and when there is not. This observation applies to situations when only the pulp volume of the upper central incisors is known, when only the pulp volume of the upper canines is known, and when the pulp volume of the two types of teeth is known.

In relation to the validation of the sex estimation formulas, good results were found, the best having been generally obtained when the information regarding age was available, with emphasis on the high accuracy of the formula when applied to the female part of the sample if age was known, which corresponded to 94% when using the pulp volume of the upper central incisors only, when using the pulp volume of the upper canines only, or when using the two types of teeth. In the male sample, 83% accuracy was found when using the two types of teeth in the absence of information regarding age, the second highest of the total

sample. Finally, the third highest accuracy, 78%, is present also in the male sample, in relation to age, in situations where the pulp volume of the central incisor, of the canine or both together were used.

In relation to the ITK-SNAP software used, it was validated by its developers, being a segmentation solution for clinical research in neuroimaging and beyond¹⁷. In addition, it also has the advantage of being a free software, which allows it to be widely used by forensic anthropology and medical professionals. This software enables three-dimensional segmentation, being already used in researches on age estimation via dental analysis^{4,18}, with proven efficiency and reliability.^{17,19}

When using three-dimensional imaging methods, several authors have studied the correlation between secondary dentin formation and age by means the analysis of the tooth volume/pulp volume ratio. However, in our study, we used the pulp volume data only, in agreement with the findings and recommendations of several previous studies.^{4,18,20,21} Tooth volume can be reduced by the attrition of the enamel and its calculation is less accurate due to the image's contrast, hindering the distinction between cortical alveoli, the space corresponding to the periodontal ligament and cementum.

As limitation of this study, there was the need of using CBCT scans with two voxel sizes. The main sample was comprised of 105 tests with 0.2 mm³ voxel; however, to compose a sample that was equitable distributed between the sexes and between the actual ages, 11 CBCT tests with 0.3 mm³ voxel were also used, all the tests having been carried out by the same scanner and with the same energy parameters. However, the study by Adisen et al¹⁶, which aimed to investigate the effect of voxel resolution on age estimation, found there were no significant differences between chronological age and the age estimated using different voxel groups, with 0.2 and 0.4 mm³.

It is suggested that future studies conduct age and sex estimation based solely on the pulp volume of other types of teeth and in other populations. Additionally, it is recommended, preferably, that the formulas developed to estimate age and gender from the analysis of the pulp volume of the upper central incisors and upper canines is used in conjunction with others with proven effectiveness, that use other bodily structures, if available, or even in conjunction with other methods that use teeth, such as for sex estimation analysis from the pelvic bones^{22,23}, and age estimation analysis from the bones of the hand and wrist²⁴, and dental development.^{25,26}

Conclusions

The formulas developed to estimate age and gender from the analysis of the pulp volume of the upper central incisors and upper canines provided good results, and can be applied to the Brazilian population. It was found that, in general, in individuals above 35 years old, excellent results may be obtained with the age estimation formulas, as well as high accuracy (94%) in sex estimation for the female sample, when age is known. In addition, the validation allowed to observe a lower mean absolute error in age estimation when sex is known.

Disclosure statement

No potential conflict of interest was reported by the authors.

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3. CONCLUSÃO

Foi possível neste estudo, desenvolver e validar fórmulas regressivas nacionais para estimativa da idade e do sexo com base no volume pulpar de incisivos centrais e caninos superiores. Concluindo-se que as fórmulas conseguiram excelentes resultados de estimativa da idade, sobretudo quando utilizada na população acima de 35 anos, e de estimativa do sexo, sobretudo quando utilizada na população feminina com disponibilidade da informação etária fornecendo 94% de precisão.

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ANEXOS

ANEXO 1

PARECER DO COMITÊ DE ÉTICA EM PESQUISA DA FOP UNICAMP



COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS



CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Estimativa de idade e sexo com base no volume da cavidade pulpar utilizando tomografia computadorizada de feixe cônico: uma amostra brasileira**", CAAE **79944817.4.0000.5418**, dos pesquisadores **Vanessa Moreira Andrade Pinto, Rocharles Cavalcante Fontenele e Eduardo Daruge Júnior**, satisfaz as exigências das resoluções específicas sobre ética em pesquisa com seres humanos do Conselho Nacional de Saúde – Ministério da Saúde e foi aprovado por este comitê em 21/12/2017 (protocolo original) e em 04/05/2018 (emenda ao protocolo).

The Research Ethics Committee of the Piracicaba Dental School of the University of Campinas (FOP-UNICAMP) certifies that research project "**Age and sex estimation based on pulp cavity volume using cone beam computed tomography: a Brazilian sample**", CAAE **79944817.4.0000.5418**, of the researcher's **Vanessa Moreira Andrade Pinto, Rocharles Cavalcante Fontenele and Eduardo Daruge Júnior**, meets the requirements of the specific resolutions on ethics in research with human beings of the National Health Council - Ministry of Health, and was approved by this committee on 21st of December of 2017 (original proposal) and on 4th of May of 2018 (amendment).

Fernanda Miori Pascon

Profa. Fernanda Miori Pascon

Vice Coordenador
CEP/FOP/UNICAMP

Jacks Jorge Junior

Prof. Jacks Jorge Junior

Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo e a lista de autores aparecem como fornecidos pelos pesquisadores, sem qualquer edição.
Notice: The title and the list of researchers of the project appears as provided by the authors, without editing.

ANEXO 2**DECLARAÇÃO DE AUTORIZAÇÃO PARA USO DE ARQUIVOS, REGISTROS E SIMILARES**

	UNIVERSIDADE FEDERAL DO RIO DE JANEIRO FACULDADE DE ODONTOLOGIA	
Declaração de Autorização Para Uso de Arquivos, Registros e Similares		
<p>Ao Comitê de Ética em Pesquisa da FOP/UNICAMP</p> <p>Declaro que o acesso e o uso do arquivo de tomografias computadorizadas de feixe cônicoo da Clinica de Radiologia Odontológica da Faculdade de Odontologia da Universidade Federal do Rio de Janeiro, pertencente ao Departamento de Patologia e Diagnóstico Oral, realizadas de junho de 2012 a abril de 2018 totalizando 2.757 (dois mil, setecentos e cinqüenta e sete) exames de imagem, está autorizado aos pesquisadores Vanessa Moreira Pinto, Eduardo Daruge Júnior e Rochelles Cavalcante Fontenele com a finalidade de realização da pesquisa intitulada “Estimativa de idade e sexo com base no volume da cavidade pulpar utilizando tomografia computadorizada de feixe cônicoo: uma amostra brasileira.”. Os pesquisadores terão acesso somente ao computador do tomógrafo, que contém o registro das tomografias realizadas contendo referência apenas quanto ao nome dos pacientes, data do nascimento e sexo do paciente, assim como a data da realização do exame. Os pesquisadores não realizarão novas tomografias. Estou ciente de que os nomes dos pacientes não serão catalogados pelos pesquisadores, sendo substituídos por números, impedindo a identificação do paciente nas tabelas de resultados geradas pelo estudo. Os dados de endereço e contato telefônico dos participantes encontram-se em outro computador, no software de Gerenciamento da Clínica, e seu acesso não será disponibilizado aos pesquisadores, visto que o software possui também informações financeiras e internas deste Setor de Radiologia. Logo, a localização e contato dos pacientes pelos pesquisadores está inviabilizada, uma vez que esses dados não poderão ser acessados pelos pesquisadores.</p> <p>De acordo e ciente</p> <p>Rio de Janeiro, 18 de abril de 2018</p> <hr/> <p>Fábio Ribeiro Guedes - CPF: 025.285.777-10 Professor Adjunto do Departamento de Patologia e Diagnóstico Oral Responsável pelo arquivo da Clínica de Radiologia Odontológica da Faculdade de Odontologia da UFRJ - Departamento de Patologia e Diagnóstico Oral</p> <hr/> <p>Faculdade de Odontologia – Universidade Federal do Rio de Janeiro Av. Carlos Chagas Filho 373 – Prédio do CCS – Bloco K – 2º andar – Sala 56, Ilha da Cidade Universitária – CEP: 21.941-902 – Rio de Janeiro – RJ – Brasil Telefone: (21) 3938-6438</p>		

ANEXO 3

VERIFICAÇÃO DE ORIGINALIDADE E PREVENÇÃO DE PLÁGIO

Estimativa de idade e sexo com base no volume pulpar utilizando tomografia computadorizada de feixe cônico: desenvolvimento e validação de fórmulas em uma amostra brasileira.

RELATÓRIO DE ORIGINALIDADE



FONTES PRIMÁRIAS

1 Mehmet Zahit Adisen, Aydan Keles, Serap Yorubulut, Rana Nalcaci. "Age estimation by measuring maxillary canine pulp/tooth volume ratio on cone beam CT images with two different voxel sizes", Australian Journal of Forensic Sciences, 2018 <small>Publicação</small>	2 % 2%
2 www.birpublications.org <small>Fonte da Internet</small>	1 % 1%
3 Emanuele Sironi, Franco Taroni, Claudio Baldinotti, Cosimo Nardi, Gian-Aristide Norelli, Matteo Gallidabino, Vilma Pinchi. "Age estimation by assessment of pulp chamber volume: a Bayesian network for the evaluation of dental evidence", International Journal of Legal Medicine, 2017 <small>Publicação</small>	1 % 1%
4 monolitos.zip.net <small>Fonte da Internet</small>	1 % 1%
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6 Lei Wang, Ju-peng Li, Zhi-pu Ge, Gang Li. "CBCT image based segmentation method for tooth pulp cavity region extraction", Dentomaxillofacial Radiology, 2018 <small>Publicação</small>	1 % 1%

ANEXO 4**COMPROVANTE DE SUBMISSÃO DO ARTIGO NO PERIÓDICO
“DENTOMAXILLOFACIAL RADIOLOGY”**

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Para: IMLAP-Vanessa Moreira Andrade

Ações

quinta-feira, 7 de fevereiro de 2019 6:18

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Dear Vanessa Andrade,

The submission entitled "Age and sex estimation based on pulp cavity volume using cone beam computed tomography: development and validation of formulas in a Brazilian sample.", for which you are listed as an author, has been assigned the following manuscript number: DMFR-D-19-00053.

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