Chapter 37

The use of the micro-computed tomography in age estimation

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ABSTRACT
Age estimation is important in Forensic Anthropology because it allows estimating the age of individuals, in addition to performing a significant role in legal proceedings. Teeth as well as the human skeletal measures are reliable structures during the identification process. Micro-computed tomography (micro-CT) is a non-destructive 3D technology to allow the visualization and analysis of microstructural of bones and teeth. The aim of this study was to analyze, through a literature review, the age estimation using micro-CT. For this purpose, articles published in the last 20 years and made available in full in Portuguese, English and Spanish were used. Micro-CT is considered a reliable alternative for age estimation through measurements on human teeth and skeletons. The association of this tool with other techniques provides promising results, besides proposing new methods through quantitative analysis in high-resolution.

Keywords: Forensic Anthropology, Age Determination by Teeth, Age Determination by Skeleton, X-Ray Microtomography, Radiology.

1 INTRODUCTION

Age estimation is an important process in Forensic Anthropology, as it allows to determine the age range of individuals, assist in the immigration and adoption process, as well as play a relevant role in criminal and civil proceedings (Bjørk & Kvaal, 2018; Kuhnen et al., 2021; Veras et al., 2021). It also assumes great prominence in occasions of mass disasters that result in unidentified remains and in cases of living individuals (Baryah, Krishan & Kanchan, 2019). Age estimation is performed through assessments of tooth growth and development on periapical and panoramic radiographs, hand and clavicle radiographs, as well as 3D image-based methods (Schmeling et al., 2008).

Dental age can aid in the estimation of physiological age (Maret, Peters, Dedouit, Telmon, &Sixou, 2011; Asami et al., 2019). Teeth are among the structures of the human body that best resist time and extrinsic influences; moreover, they are extremely important in the identification of decomposed or skeletal stage human remains (Aboshi, Takahashi, & Komuro, 2010; Asami et al., 2019; Kuhnen et al., 2021). In age estimation of children and adolescents, it is recommended to use teeth that are in the developmental period (Bjørk & Kvaal, 2018; Kuhnen et al., 2021). Third molars can be used in age estimation of adolescents and young adults because they have later development (Someda et al., 2009; Bjørk & Kvaal, 2018; Gioster-Ramos et al., 2021; Veras et al. 2021). However, caution is needed in interpreting these results, due to the high variability regarding the timing of development and formation of these teeth (Bjørk & Kvaal, 2018). Also, after the complete development of the dentition, estimating dental age becomes difficult, because changes caused by habits such as bruxism and tooth wear directly influence the human physiological occlusion (Aboshi et al., 2010). Measurements of pulp chamber size, correlations between age and height and width proportions of dental elements, and evaluation of secondary dentin deposition on periapical radiographs, panoramic radiographs, and 3D images are non-destructive methods widely used in estimating dental age (Schmeling et al., 2008; Someda et al., 2009; Asami et al., 2019).

Age estimation is also determined on human skeletal measurements, achieving good results, especially in young individuals (Deguette, Ramond-Roquin, & Rougé-Maillart, 2017; Pham et al., 2021). On the other hand, age estimation in adults is a more challenging method because of skeletal and dental maturity (Deguette et al., 2017). The degeneration of specific bones or even loss of part of some bones such as femur, tibia, and ribs makes the process much more difficult. Therefore, a combination of different resources can be a facilitating means and lead to more accurate results (Pham et al., 2021). Within this context, micro computed tomography (micro-CT) has been shown to be an excellent tool in age estimation (Wade, Nelson, Garvin, & Holdsworth, 2011; Deguette et al., 2017; McGivern et al., 2020; Campioni, Pecci, & Bedini, 2020; Nudel et al., 2020; Pham et al., 2021). Micro-CT is a non-destructive 3D technology that allows, among other applications, the visualization and analysis of microstructural features of bones and teeth (Rutty et al., 2013; Campioni et al., 2020). Due to its non-destructive nature, it ensures permanent records for future investigations, and the dataset obtained can be used for judicial purposes (Rutty et al., 2013). For tooth analysis, a 3D perspective of structures such as enamel, dentin, and the pulp cavity can be obtained (Someda et al., 2009). Volumetric correlations, using micro-CT, between tooth structures and age estimation have been methods developed and well accepted in the literature (Vandevoort et al., 2004; Someda et al., 2009; Agematsu et al., 2010; Asami et al., 2019; Nudel et al., 2020). Thus, tooth age estimation was optimized from the use of micro-CT, since 2D radiographic methods do not characterize the actual structural of teeth (Rutty et al., 2013; Asami et al., 2019; Nudel et al., 2020, Campioni et al., 2020; Gioster-Ramos et al., 2021).

Thus, the objective of this study was to analyze, through a literature review, the performance of age estimation using micro-CT.

2 METHODOLOGY

For the development of this study an extensive search was conducted in the following databases: PubMed, Scopus, Web of Science, Latin American and Caribbean Literature on Health Sciences (LILACS), Virtual Health Library (VHL) and Google Scholar from April to July 2020. The descriptors used to search the articles were ¨micro-CT¨, ¨age estimation¨, ¨teeth¨, ¨bone mineral density¨, ¨bone microarchitecture¨, ¨forensic anthropology¨, ¨forensic odontology¨.

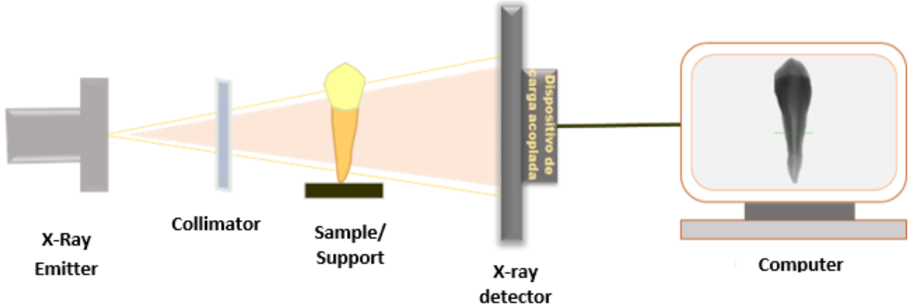
Within the inclusion criteria, we considered articles published in the last 20 years, available in full and in Portuguese, English, and Spanish. Papers that did not fit the research objective or were not available in full were excluded.

3 RESULTS AND DISCUSSION

Computerized Microtomography:

Computed microtomography is a reproducible and non-destructive technology, allowing quantitative and qualitative analysis of the same sample at high resolution (Campioni et al., 2020). It was discovered in the early 1980s, however the first device was made commercially available in 1994 (Bjørk & Kvaal, 2018). The microtomographic image is obtained from the emission of X-rays generated by the microfocal emitter, subsequently this radiation passes through the collimator and passes through the specimen. The radiation is attenuated according to the composition and density of the material or type of sample. This attenuation is measured by the X-ray detector containing a charge-coupled device (CCD), which provides the transformation of the radiation into visible light (Boerckel, Mason, McDermott, & Alsberg, 2014). In this way, the light struck by the CCD emits an electronic signal that allows the scanning and sending of the image to the computer (Figure 1) (Boerckel et al., 2014). Micro-CT has demonstrated its potential in several areas of research (Boerckel et al., 2014; Sousa-Neto et al., 2018; Bjørk & Kvaal, 2018; Campioni et al., 2020; Oliveira et al., 2021), and even in forensic practice (Rutty et al, 2013).In addition, scanning without intact cleaning of the samples, permanent recording of the images, return of the remains, and making high-quality virtual replicas are pointed out as advantages of micro-CT (Rutty et al., 2013; Campioni et al., 2020). On the other hand, this technology presents certain limitations, such as high cost of the device, scanning time, reduced sample size, specific software and algorithms with the need for prior preparation before use (Vandevoort et al., 2004; Maret et al., 2011; Campioni et al., 2020), as well as restrictions when used *in-vivo* samples (Asami et al., 2019).

Figure 1. Representative image of microtomographic imaging.



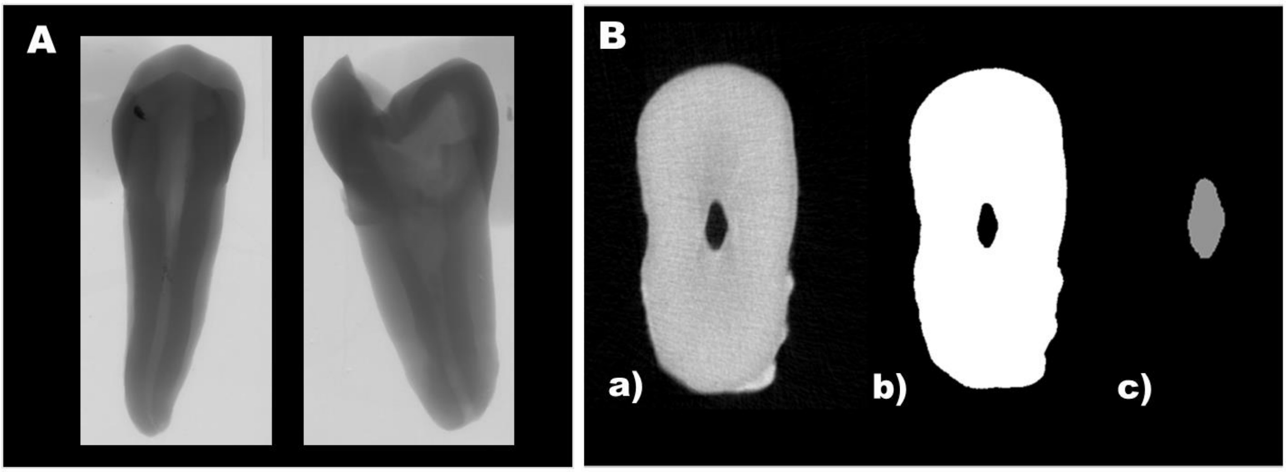
Source: Own elaboration.

Age estimation by measurements on teeth:

Teeth are structures that are resistant to time and different environmental conditions (Bjørk & Kvaal, 2018; Kuhnen et al., 2021), and they also exhibit a unique aging process (Vandevoort et al., 2004). Age-related changes are mainly observed in the dentinopulpal complex compared to that in enamel (Vandevoort et al., 2004; Someda et al., 2009; Nudel et al., 2020). Radiographic methods are commonly used in estimating dental age (DemiDrjian, Goldstein, & Tanner, 1973; Mincer, Harris, & Berryman, 1993; Kvaal, Kolveit, Tomsen, & Solheim, 1995; Cameriere, Ferrante, & Cingolani, 2004; Gioster-Ramos et al., 2021). However, two-dimensional images do not represent the three-dimensional morphology of teeth (Bjørk & Kvaal, 2018; Gioster-Ramos et al., 2021). Thus, the employment of methodologies using 3D images is encouraged (Maret et al., 2011; Rutty et al., 2013; Bjørk & Kvaal, 2018).

The first studies by Vandevoort et al. (2004) and Aboshi, Takahashi, Komuro and Fukase (2005) demonstrated the potential of micro-CT for pulp-tooth volume-based age estimation using single-unit teeth. Sample analyses with software for segmentation and measurement of tooth volume, as depicted in Figure 2, were employed in the study by Vandevoort et al. (2004), while pulp-tooth volume ratio calculations in different regions were used by Aboshi et al. (2005). However, the smaller number of samples was considered a limitation in both studies. Thus, Someda et al. (2009), using 155 mandibular central incisors, showed greater correlation between age and pulp-tooth volume. In addition, they observed a greater relationship between age and decreased pulp/tooth volume in women than in men.

Figure 2. Representative image of pulp/tooth volume analyses employing maxillary premolars. A. Reconstruction of the images after scanning, B. Quantitative analyses of the images: a) Cross section of the tooth, b) Segmentation of the images, c) Analysis of the area of interest (pulp).



Source: Personal files of the authors.

Enamel is considered the hardest structure of the tooth, and under physiological conditions it shows no age-related changes. However, pathological conditions or parafunctional habits can cause irreversible enamel loss (Vandevoort et al., 2004). Methods employing micro-CT recommend the exclusion of enamel, as this structure has no direct relationship with age (Someda et al., 2009). However, when associated with aging methods, such as enamel racemization, it can provide benefits in age group estimation (Griffin et al., 2008).

The dentinopulpal complex is susceptible to age-associated changes (Vandevoort et al., 2004). Secondary dentin deposition and dentin translucency are reliable parameters in age estimation, especially in adults (Arora, Talwar, Sahni & Rattan, 2016; Nudel et al., 2020). Furthermore, the high organic matrix content in secondary dentin enables reliable quantitative analyses when high-resolution methodologies are applied (Agematsu et al., 2010; Nudel et al., 2020). The study by Nudel et al. (2020) evaluated the growth pattern of secondary dentin in 77 mandibular premolars using micro-CT. The secondary/primary dentin volume and secondary dentin thickness were compared with the pulp/dentin volume of the whole tooth, considered to be the gold standard. The authors demonstrated that secondary dentin measurements had an 82% success rate for age estimation, compared to the gold standard reference method of 54% within ±10 years.

Morphological changes of teeth can influence the accuracy of age estimation (Aboshi et al., 2010; Agematsu et al., 2010; Asami et al., 2019). Lower central incisors have less morphological diversity than other permanent teeth, showing greater reliability in age range estimation (Someda et al., 2009; Agematsu et al., 2010). Upper and lower premolars are considered in age estimation since morphological changes and occlusal patterns are minimal (Asami et al., 2019). Aboshi et al. (2010) reported higher correlation between age and pulp volume when lower premolars were used. On the other hand, the study developed by Asami et al. (2019) observed higher accuracy in age estimation in maxillary second premolars compared to maxillary and mandibular first premolars.

Three-dimensional images provide a proper interpretation of tooth structures (Campioni et al., 2020). The volume of the dentin and pulp chamber, as well as the crown and root, is considered essential for age estimation (Asami et al., 2019). After obtaining these data, linear regression analyses are applied to estimate the age range where volume is considered the independent variable and age the dependent variable (Valsecchi, Irurita Olivares, & Mesejo, 2019; Asami et al., 2019). In addition, the coefficient of estimate (R2) and the standard deviation of the data are employed to verify the accuracy of the equations in estimating age range. Several studies using this methodology have demonstrated a higher accuracy in correlating age with pulp/tooth volume (Vandevoort et al., 2004; Aboshi et al., 2005; Someda et al., 2009; Aboshi et al., 2010; Agematsu et al., 2010; Asami et al., 2019).

Age estimation by measurements on human skeletons:

The human skeleton presents mechanical and morphological characteristics that change over time (McGivern et al., 2020). Different methodologies are employed for age estimation using human skeletons (Buckberry & Chamberlain, 2002; Wade et al., 2011; Denguette et al., 2017), but differences between young and adult individuals must be considered (Pham et al., 2021). Dental development and skeletal growth are analyzed in age estimation in young people (Franklin, 2010), achieving good results. However, age range estimation in adults becomes challenging as degenerative changes affect the strength, internal structure, physical and mechanical of bones such as ribs, cranial sutures, femur or tibia (McGivern et al., 2020; Pham et al., 2021). The clavicle is considered the bone structure commonly employed for age range estimation in adults due to its extended degree of maturation (Milenkovic, Djukic K, Djonic, Milovanovic, & Djuric M, 2013; Wittschieber et al., 2016; Rudolf et al., 2018).

Evaluations of trabecular architecture in the femur, humerus, clavicle and pubis are performed for age range estimation using radiographic techniques (Szilvassy & Kritscher, 1996; Macchiarelli & Bonioli, 1994). This method is considered practical and affordable, but has low contrast resolution (Wade et al., 2011). Histomorphometry of bone tissue is another methodology that allows for the analysis of trabecular bone volume in relation to the age of the individual. Moreover, this method is considered the gold standard for quantitative and qualitative analyses of metabolic bone diseases (Deguette et al., 2017). However, in order to complement or improve these methodologies, micro-CT has been incorporated into forensic research.

Micro-CT provides objective and quantitative analyses in age at death estimation when human skeletons are used (Wade et al., 2011; McGivern et al., 2020). Previous studies have shown application of micro-CT in age estimation using the medial third of the clavicle (McGivern et al., 2020), iliac bone fragment (Deguette et al., 2017), rib end and pubis (Rutty et al., 2013). Trabecular bone volume, as well as measurements of the depth or distance between ribs, by means of specific software, can provide adaptations of the traditional age range for age estimation (Rutty et al., 2013; Deguette et al., 2017). Thus, the authors pointed to micro-CT as a promising alternative, as well as providing data that can serve for future research.

4 FINAL CONSIDERATIONS

Micro-CT is pointed out as a reliable alternative that can optimize age estimation through measurements on human teeth and skeletons. It is considered an alternative to create or complement methodologies through quantitative analysis in high resolution. However, further studies are essential for a better understanding of this technology in forensic practice.

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