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CONTENT

Hari Wibowo et al. / Prehistoric populations from Gua Bedug in the context of early-mid holocene of Java, Indonesia	1
Abdulla Al-Shorman et al. / Strontium isotope analysis of human dental enamel from a mass burial at Udhruh fortress, Southern Jordan: a paleomobility study	16
Arofi Kurniawan et al. / The applicability of Demirjian's and Nolla's dental age estimation methods for children in Surabaya, Indonesia	25
Prajakta Khelkar et al. / Palatal rugae pattern and tongue print as a potential tool for gender identification in forensic odontology: a cross-sectional study	33
Beta Novia Rizky et al. / Knowledge and awareness of medicolegal aspects among dental practitioners in rural, urban, and suburban areas of Indonesia: a cross-sectional study	42
Ananda Nandita Dewana et al. / Comprehensive review: update in age estimation of forensic odontology	48
Aminah Zahrah et al. / Microbiological analysis in forensic identification and machine learning: a review	55
Nisrina Saputri / Analyzing orocraniofacial structures for sex estimation using advanced imaging technologies in forensic odontology: a review	64
Georgi Tomov et al. / Bilateral odontogenic maxillary sinusitis due to advanced tooth wear in a female individual from late antiquity Philippopolis (Bulgaria)	70
Laura Vranješ et al. / Microdontia and hypodontia in two female skeletons from the Rovinj – St. Euphemia site	76
Sayem A. Mulla / Forensic odontology for edentulous cases – a diagnostic bereft.....	83

Reviewers of this issue:

Aspalilah Alias, David Bulbeck, Aman Chowdhry, Andrea Cucina, Jannick Detobel, Lorenzo Franceschetti, Laura Gonzalez-Garrido, Rakesh Gorea, Tamas Hajdu, Hebalbrahim Lashin, Matthew James Lee, Senad Muhasilović, Masniari Novita, Oskar Nowak, Amir Abdul Rahim, Rabi'ah Al-Adawiyah binti Rahmat, Kasia Sarna Bos, Ricardo H.A. Silva, Ana Maria Silva, Parul Sinha, Nurtami Soedarsono, Marlin Tolla, Leticia Vilela Santos, Selma Zukic.

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Comprehensive review: update in age estimation of forensic odontology*

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Abstract

Age estimation is a fundamental aspect of forensic odontology, yet it presents numerous challenges that impact the accuracy and reliability of human identification, especially in legal and disaster scenarios. Although teeth are highly durable and resistant to environmental factors, the diversity of available methods-including clinical, radiographic, and biological approaches-often leads to inconsistent results due to a lack of standardization and population-specific variability. Many commonly used techniques, such as Demirjian scoring and root formation analysis, may yield different outcomes when applied to individuals of varying ethnic backgrounds, ages, or health conditions. While recent advances in 3D imaging and artificial intelligence show promise for improving accuracy, the current lack of universally accepted protocols and the need for well-designed validation studies remain significant obstacles. By integrating these diverse methodologies, forensic odontology can significantly improve the reliability of age estimations, providing critical information for legal and medical applications in the identification of both living and deceased individuals. This review emphasizes the importance of a multidisciplinary approach in advancing forensic odontology and enhancing age estimation techniques.

Keywords: age estimation; forensic odontology; methodological challenges

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Introduction

Forensic odontology plays a vital role in the identification of individuals, particularly in cases of mass disasters, missing persons, and criminal investigations. Age estimation of decomposed or skeletonized human remain is one of the pillars of establishing a biological profile, thus playing a vital role in aiding the identification process or gaining insight into a population's demographic, morbidity, and mortality pattern (1). Age estimation is essential to be able to identify the biological profile of unidentified human remains and helps in narrowing down the identification of individuals from the list of potential candidates (2). Just like fingerprints, the teeth of each individual are unique and are also the strongest structures present in the human body (3). Teeth are preferred for age estimation as they have high durability and resistance to heat, chemicals, putrefaction, and other factors (4). In odontology forensic, age estimates are crucial for determining the unique traits of the deceased in cases involving missing and unidentified individuals (5). The phases of tooth mineralization, root formation, root maturity, tooth eruption, and root apex maturation are each shown by dental maturity and can be examined and utilized as a means of estimating age (6). This review aims to provide a comprehensive overview of the update in odontology forensic can be accomplished clinically through tooth eruption, radiograph examination as an instrumental examination that helps in mass disaster, and emerging biological techniques such as DNA methylation, telomere length, secondary dentin deposit, and cementum incremental line (5). In addition, various advanced technologies are also used in forensic odontology in age estimation such as 3D imaging (CBCT, CT, and MRI) that can observe tooth development and wear, tooth formation ratio, and tooth maturation. Artificial intelligence (AI) has also been widely developed especially in virtual autopsy in identifying a person based on age (7,8). The necessity to advance the science of forensic odontology and improve the accuracy and dependability of age estimation in the forensic environment is the driving force behind this issue.

Age estimation role in forensic odontology

The first step in the identification process is to create a biological profile by determining gender, ancestry, age, and height, and to provide a general description of the unknown person (1). Age estimation is a crucial aspect of forensic odontology, serving the purpose of identifying

unknown deceased individuals (3). Dental age estimation is a field of forensic odontology filled with a multitude of methods mostly developed for application in children and adolescents (9). The task of age estimation plays a pivotal role in forensic sciences and civil investigations, aiding in the reconstruction of biological profiles for missing-person cases, confirming the age of younger criminals, and assisting in situations where personal documents are unavailable (10). Age estimation in children and adolescents is important for variety of legal procedures such as child labor, employment, status of majority, rape, adoption, eligibility for marriage, and when the birth certificate is not available. Therefore, the correct method and criteria for age estimation should be selected depending on the age range (5). Teeth and human skeletal remains can be utilized for age estimation (3). For adult victims, estimating age at death is particularly difficult and less accurate than for younger victims, because most methods are based on the gross analysis of morphological features of bones and teeth that have already completed their development and are subject to intrinsic factors over the course of an individual's life and exposure to extrinsic factors (11). Teeth, the strongest structures in the human body, are protected by the soft and hard tissues of the face, making them resistant to environmental and traumatic conditional or external factors such as decay and extreme temperatures and thus can be used as biological indicators in age estimation (11). Tooth eruption, tooth calcification, attrition, periodontal disease, secondary dentin deposition, root translucency, cementum apposition, and root resorption are the dental changes most commonly analyzed in age estimation methods (3). Moreover, for dental age estimation, it is very important to consider population-specific differences with genetic predisposition in geographical areas (3).

Method of age estimation in dental examination

Several approaches are available for age estimation, including clinical, radiographic, and biological examinations of the teeth. The visual technique is based on the eruption order of teeth and morphological indicators of aging, such as attrition, deposition of secondary dentin, and color changes (4). There are two commonly used visual methods, dental attrition (an estimate of tooth wear that depends on various factors such as diet, chewing, number of existing teeth, restored teeth or dentures, malocclusion, geography, and bruxism) and the color of the

teeth (a darker and yellowish change in the color of the teeth with age) using the naked eye visual method or spectrometric method (12). The method of tooth growth and development starting from crown formation, root formation, and apex closure can be seen through the stages of tooth growth and development through panoramic radiography (13). Panoramic radiography is one of the most frequently used projections to obtain images of all teeth and surrounding tissues with low dose, less invasive, and low cost effectiveness (10,14). Radiographs can be used at prenatal age because at this age we can see and assess the stage of formation and development of unerupted primary teeth (15). Radiographic methods in estimating age are the Schour and Massler, Adisty Atlas, Gleiser and Hunt modified, Demirjian scoring, Al-Qahtani method, Camerier, and crown and root formation stages with the modified Moores method (14–16). Some biochemical markers used in age estimation include radiocarbon dating of tooth enamel, measurement of telomere length, and determination of the number of mitochondrial DNA mutations (2). However, not all are used in forensic odontology due to low accuracy or limited application. What is commonly used is DNA methylation because it has important roles such as embryonic development, cellular differentiation, and gene expression regulation (2). Cytosine methylation of CpG islands upstream of specific genes is developmentally regulated in a tissue-specific manner. DNA methylation analysis was the best approach in age estimation. In addition, hypermethylation or hypomethylation has a linear correlation with age in some CpGs, so it can be used for age estimation using some DNA biomarkers (17).

Root formation and age estimation

The ability to estimate age is not only good for legal proceedings and medical diagnosis, but also provides insight into the intricacies of population demographics (18). There are numerous methods for estimating age, such as teeth and skeletons. Teeth are important oral structures that are often used for individual identification in cases of child labor, child abuse, and violence, refugees and military schools, and death cases (6). Teeth and its supporting tissues are superior to skeletal material in age estimation due to their high degree of individuality, robustness, and non-destructibility (14). Dental maturity shows the stages of tooth mineralization, root formation, maturity of the root, tooth eruption, and root apex maturation which can be analysed

and used as one of the age estimation methods (6). Root formation is a critical indicator in dental maturation and chronological age. Dental maturity is effectively accurate because as the teeth and skeletal development progresses, the teeth undergo morphological development that has a high accuracy value to chronological age (13). Furthermore, root development proves effective in age estimation because dental maturation is not affected by nutritional deficiencies compared to other age estimation parameters such as skeletal (19). Permanent tooth root development follows a predictable pattern that correlates with age so it can be assessed in both children and adults.

Radiograph evaluation for age estimation

Radiographic evaluation is a cornerstone of age estimation in forensic odontology. Radiographs have a role in determining the cause of death, predicting age, gender, and even race and are considered accurate data and can be used as valid evidence in the court system (15). Radiographic images are needed to assist in researching age estimates based on tooth development with techniques, such as periapical and panoramic (15,20). The advantages of radiographic evaluation in age estimation are that it can be used at the prenatal age which assesses the stage of formation and development of unerupted primary teeth, not destructive in nature and can be used for age estimation in living and deceased individuals (15,21). Panoramic images are used more often than dental images such as periapicals because they are difficult to perform in children and can produce considerable distortion (22). Staging the crown and root formation of developing teeth is one of the most used techniques to register the tooth developmental status in forensic science and practice (23). Most staging techniques were developed on bidimensional radiographic for age estimation by dental development using radiographic methods include Schour and Massler, Adisty Atlas, Gleiser and Hunt modified, Demirjian scoring, Al-Qahtani method, Camerier, and crown and root formation stages with the modified Moores method (24). Schour and Massler explained that dental age estimation is divided into 21 stages of development and involves primary teeth and maxillary and mandibular dentition which are then categorized as the stages of morphological change in teeth and dentition into infancy and early childhood, late childhood, and adolescence (24,25). The Adisty Atlas is the newest age estimation method

in Indonesia that assesses the stages of root resorption, calcification and eruption of permanent teeth radiographically (24). Based on development and maturation, Gleiser and Hunt modified the grading of first molars into ten stages with 3 stages of crown formation and 7 stages of root formation (20). Demirjian scoring is one of the most commonly used methods using radiographic examination that describes the developmental stages of seven permanent teeth on the left side of the mandible that are classified into eight stages and scored, and the age of the teeth is calculated from the total score (22,25). Al-Qahtani based tooth development and eruption rates on diagrams looking at the second and third molars and requires radiographic image to assist in examining age because it has a broad picture covering all teeth (24,26). Cameriere method uses measurements of the exposed root apices looking at the development of the seven left mandibular permanent teeth for age estimation (15). Modified moorrees by Al-Qahtani divides the 4 stages of root resorption of single and multiple teeth into H (roots intact), Res 1/4 (one-fourth of apical root resorption), Res 1/2 (half apical root resorption), and Res 3/4 (three-fourth of apical root resorption) (24). Various scoring systems have been developed to standardize the assessment of root formation stages, facilitating comparisons across studies and improving the reliability of age estimates.

DNA methylation and biological methods in age estimation

Over the years, identification with various molecular markers for age estimation has been used in the forensic field. DNA methylation is one of the age estimation methods with molecular markers that is often used in individual identification due to its age-specific pattern (2,17). DNA methylation-based age is known as “epigenetic age” or “epigenetic clock”, the binding of a methyl group onto the cytosine ring at the 5' position of a CpG sequence (27–29). DNA methylation is a minisequencing analysis that is flexible, requires a short time to analyze, and easily implemented in forensic laboratories without the need for specific equipment (30). DNA methylation reliably provides age estimation with a wide range of samples from blood; non-blood tissues such as heart, lungs, liver, bile; other body fluids such as saliva and semen; teeth and their parts such as cementum, dentin and pulp; or even samples containing very little DNA substance such as bloodstains, cigarette butts or toothbrushes (17,31–33). However, blood and

teeth have good capabilities for DNA methylation (17). There are several for estimating age by using biomolecular analysis are mitochondrial and DNA deletions (mtDNA deletions), telomere shortening, circular excision of T-cell receptors (sjTRECs), advanced glycation end products (AGEs), and racemization of aspartic acid (17,31). The diagnostic limitations of each method, apart from DNA methylation, are: MtDNA deletions are only effective for age range <20 years and >70 years; telomere shortening and sjTRECs has low accuracy rate, >10 years; AGEs are only effective for ages >45 years; aspartic acid racemization is only effective if dentin is taken as the sample (17). The accuracy of DNA Methylation depends on the DNA markers used, including ELOVL2, C1orf132, EDARADD, PDE4C, TRIM59, KCNAB3, to NPTX2 which correlates with age and FHL2 (17,33,34). Age estimation using biomolecular approach can also be combined with other methods, for example, the radiograph method to increase accuracy (17).

Telomeres in DNA are understood to function as markers of age within living cells. Telomeres, the end segment of nucleotides on chromosomes, shed a portion of their tail at every somatic cell division results in a reduction in length. This shortening is widely studied as a cellular timekeeper in research on aging, cellular death, eternal life, cell-related aging, cancer, and multiple conditions associated with senescence problems (35). Secondary dentine is a thin layer of dentine adjacent to the pulp that signifies the dentine created following the completion of the root (36). Secondary dentin deposits are one of the characteristics of degenerative teeth that can be used for age estimation because odontoblasts continue to produce dentin continuously which reduces the pulp cavity during life as we aged (37). Secondary dentin deposit can be used for estimate age at death but require additional steps, such as radiograph and tooth sectioning for microscopic analysis (38). Secondary dentin does not form uniformly, but serves to protect the pulp exposure in older teeth, in response to abrasion, caries and increasing age, obliteration of dentinal tubules with mineralized substance leading to the formation of dentin or sclerotic dentin (36). Cementochronology is reliable biological method in age estimation with analyze the cementum incremental line thickness or count because dental cementum contains mineralized tissue deposited all through individual's lifetime that surround the dental root (11). Cementochronology methods are frequently

used as a single indicator which are insensitive to environmental change that refers to an alternation of light and dark bands due to variation in collagens relative mineralization and is formed continuously throughout life (39). Cementochronology is a very effective way for age estimation because it is based on the annual growth of the cementum layer of the root, which forms growth rings that can be counted at 200x magnification (1).

Update in age estimation

Age estimation in children can be assessed through dental development, in contrast to adults who rely on post-developmental (regressive) changes in variables that can be measured using technological advances (12). Advanced technological developments have allowed the introduction of new methods and improved accuracy and reliability of age estimation (12). Artificial Intelligence-enabled dental age assessment allows for more accurate and effective age assessment, improving clinical and forensic practice (40). AI-powered dental age estimation improves clinical and forensic practice by enabling more accurate and effective age assessment. This involves examining dental images such as x-rays and panoramic views to determine age by evaluating tooth development and wear (40). One of the first technologies is an imaging process with an Active Appearance Model that works automatically to localize the third molar tooth as a parameter based on shape and size which is then input into a neural network for age estimation (41). Three-dimensional imaging is becoming increasingly important in forensic odontology. Reconstruction of CBCT and CT scans allows researchers to analyse the pulp-to-tooth volume ratio, which could be an interesting tool to estimate the age after root maturation of third molars (8). A non-invasive imaging method for performing virtual autopsy using CT, MRI, and radiology in odontology cases, combined with 3D body documentation as an aid to examine the corpse and to evaluate the cause and manner of death and other relevant forensic findings (7). Dental pulp, alveolar bone level, and interdental space are used in age estimation using technological advancement with artificial intelligence (AI) based applications (12). In recent years, regressive dental changes have also evolved by utilizing more technological advancements, such as cone-beam computed tomography (CBCT) or machine learning (42).

Conclusion

In conclusion, age estimation is a vital component of forensic odontology, crucial for identifying both living and deceased individuals. It holds particular significance in legal contexts involving children and adolescents, such as determining eligibility for employment, marriage, adoption, and other civil matters when official documentation is unavailable. A variety of methods-including clinical, radiographic, and biological dental examinations-are employed to estimate age accurately. Root formation serves as a key indicator of dental maturation and chronological age, with panoramic radiography being a widely used, cost-effective, and minimally invasive tool to assess tooth development stages such as crown formation, root growth, and apex closure. Additionally, biological markers like DNA methylation, telomere length, secondary dentin deposition, and cementochronology further enhance age estimation accuracy. Recent technological advances, including 3D imaging modalities (CBCT, CT, MRI) and artificial intelligence, offer promising tools to refine these assessments. Integrating clinical, radiographic, biological, and advanced technological approaches holds great potential to improve the precision and reliability of age estimation in forensic odontology.

Declaration of Interest

None

Author Contributions

AND and EIA both contributed to the conceptualization, original draft, discussion of the study results and final manuscript. EIA also supervised the study.

Statement on the use of artificial intelligence in manuscript preparation

Artificial intelligence was not used in the preparation of this manuscript.

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