Evolution of dental age estimation methods in adults over the years from occlusal wear to more sophisticated recent techniques

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Abstract

Background: Age estimation has been an integral part of forensic science, and age estimation by dental means is by far the most commonly employed method. Dental age estimation in children is more accurate and straightforward as most methods use the chronological stages of odontogenesis that are highly systematic, reducing the chances of dispersed results. In contrast, estimation of age in adults becomes tricky and less accurate with varied approaches since tooth formation is already complete.

Main body: The methods of adult dental age estimation have come a long way from a calculated guess based on crude visual observation of teeth to radiological methods and to more recent sophisticated methods. Technological advances have opened up molecular and genetic methods by utilizing DNA methylation and telomere length to improve the accuracy of age estimation by reducing error chances.

Conclusions: Although dental age estimation methods in children and adolescents have been extensively reviewed, various adult age estimation methods are not reviewed as a whole. The aim of this review is to appraise the evolution of dental age estimation methods in adults over the years from mere visualization of dental attrition to employing more sophisticated means such as radioactive carbon dating and genetics. This comprehensive review also attempts to add an account of the accuracy and suitability of various adult dental age estimation methods.

Keywords: Adult, Age determination by teeth, Dental age, Forensic dentistry, Human identification

Background

Age estimation is one of the prime sub-disciplines of forensic odontology that has a vital role in assisting the medico-legal branch. The main goal is to identify a living or deceased person by presenting a probable age range. It forms an important step in constructing a biological profile from unknown human skeletal remains along with gender and race (Nayak et al. 2014).

Teeth have been used to estimate the age of living individuals since ages which might be required in cases related to child labor, child abuse, refugees, and admission to military and schools. For instance, in Rome, the eruption status of second molars was used to admit volunteers in the military. Also, in some nations, tooth eruption of permanent first molars was used as a criteria for deciding punishments for suspects as there was no punishment against an individual younger than 7 years of age. Further, in the early nineteenth century in England, the law suggested no child under 9 years of age should be employed and a child below 13 years should not work for more than 9 h a day (Stavrianos et al. 2008).

Age estimation by dental means is the most accepted method till date, especially in children as they are highly accurate. Almost all the methods employed for dental age estimation in children and adolescents are based on the tooth development process, which is a well-organized and orchestrated process, a *Nature's blueprint* that is

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least affected by environmental factors and hence is quite accurate.

Contrary to that, tooth formation is already complete in adults, and thus, dental age estimation mainly depends on post-developmental (regressive) changes that are variable. Technological advancements have affected almost every field of science, and forensic odontology is no exception. This implies that a wide array of methods of dental age estimation are available ranging from simple visualization and calculated guess to newer sophisticated molecular and genetic techniques.

This comprehensive review aims to highlight the captivating evolution of adult dental age estimation methods right from mere visualization of dental wear to more improved and advanced techniques. Further, a working classification is also given which portrays the evolution of various methods of adult dental age estimation along with a note on the accuracy and reproducibility of these methods.

Main text
Working classification of methods for dental age estimation in adults
Visual methods

i) *Dental attrition*
ii) *Colour of teeth*

- Naked eye visual method
- Spectrometric method

Histological methods

i) *Combination of different regressive alterations of teeth*

- Gustafson's method (1950)
- Bang and Ramm method (1970)
- Johanson method (1971)
- Maples method (1978)
- Kashyap and Koteshwar method (1990)
- Lamendin method (1992)
- Solheim method (1993)

ii) *Dentinal translucency—as a sole indicator*
iii) *Secondary dentin deposition—as a sole indicator*
iv) *Cementum—as a sole indicator*

- Thickness of cementum
- Annulations or rings of cementum

v) *Fluorescence from dentin and cementum*

vi) *Microscopic measurements by scanning electron microscope (SEM)*

Radiographical methods

i) *Pulp-tooth dimension ratio*

- Kvaal: based on length
- Cameriere: based on area
- Based on volume

ii) *Tooth coronal pulp cavity index—Ikeda method*
iii) *Age calculation using X-ray micro-focus computed tomographical scanning of teeth*
iv) *Age estimation using mental foramen and mandibular canal*

Biochemical methods

i) *Aspartic acid racemisation*

- Helfman and Bada method
- Ritz et al method—(dentinal biopsy)

ii) *Enamel uptake of radioactive carbon 14*
iii) *Miscellaneous biochemical methods*

Genetic and epigenetic methods
(i)   *Human telomere shortening*
(ii) *DNA methylation*

Visual methods
The visual methods are one of the oldest methods based on attrition of occlusal surfaces. With the passage of time, the tooth color also developed as a method of age estimation.

Based on dental attrition
Age estimation based only on occlusal wear is not an accurate estimate as the teeth wear depends on many factors such as dietary habits, mastication, number of teeth present, restored or prosthetic teeth, malocclusion, geographic, and environmental factors as well as parafunctional habits like bruxism (Lu et al. 2017). Numerous systems or indices are available for evaluating occlusal wear; however, there is no universally accepted method.

Murphy in 1959 was the first one to study various patterns of occlusal wear and exposure of dentin depending

on the morphology of individual tooth by examining human skeletons (Murphy 1959). Later in 1962, Miles came up with a method to estimate the age by assessing the relative wear of only three permanent molars. He considered some parameters as tooth wear symbols such as (i) unworn or polished enamel, (ii) severely worn enamel surface, (iii) progressive dentin exposure, and (iv) secondary dentin or pulp cavity. He gave 8 grades from A to H where A stood for slightest wear while H stood for the maximum wear (Miles 1962).

Molnar in 1971 not only considered dentin exposure but also included shape and orientation of wear facets (Molnar 1971). Scott in 1979 gave a scoring system for surface wear in molars from score 0 to 10 where 0 represents no information available due to the absence of tooth and 10 represents no enamel present and dentin completely exposed (Scott 1979). Further, Brothwell in 1989 proposed a table representing the expected dentin exposure in permanent three molars in four age classes (Prince 2004). The main aim of such studies seems to be the understanding of occlusal patterns rather than correlating them with age as none of the above researches gave a clear error estimate for the studies conducted.

Kim et al in 2000 presented a method of recording the occlusal wear on a scale of 0–8 and was found to be reliable and accurate. The biggest practical drawback was that the system mandatorily required sound and healthy teeth but oral conditions like dental caries, fractures, and missing teeth are highly common worldwide. In order to overcome this limitation, Yun et al. proposed a modification by including 2 additional points to the already existing 0–8 point scale of Kim. They studied 1092 full-arch maxillary and mandibular diagnostic casts belonging to the age range 21–87 years and estimated error margin of **±5 years.** They considered all teeth except the 3rd molars and gave scores for unsound, restored, and even the missing teeth (Nayak et al. 2014; Yun et al. 2007).

Based on the color of the tooth

It is a well-known fact that the color of the teeth becomes darker and more yellowish with aging. This is believed to be due to the deposition of organic components in enamel and dentin which alters the refractive indices of the same.

The first ones to correlate tooth color with age were De Jonge in 1950 and Brudevold in 1957, but they did not pursue further. It was Biedow in 1963 to suggest that tooth color must be added in Gustafson's parameters instead of root resorption (Rer.nat 2007).

Ten Cate et al. in 1977 used tooth color as a sole parameter and estimated a mean error of less than ±10 years (Rer.nat 2007). Solheim in 1988 chose 1000 teeth, 100 of each type excluding molars from the Caucasian population aging 14–99 years and gave a 5° scale to rank the color of crown and root with the help of dental shade guide and color densitometry, respectively. He concluded that the dental shade guide gave the weakest correlation (Solheim 1988). Lackovic and Wood in 2000 measured the values at four different points on each root using a flat-bed digital color scanner and found the majority of correlation values above 0.9 (r>0.9) suggesting a good correlation of root color and aging (Prince 2004).

Martin-de las Heras in 2002 proposed spectroradiometry as an objective method of dentin color measurements for estimation of age. It produced an associated error of ±13.7 years. They found that dentinal colors white, cream, and yellow were associated with the age group 12–37 years, while dark yellow and brown were associated with the age group 55–64 years (Martin-de-las-Heras et al. 2016).

Although methods based on visualization are relatively easy and non-invasive, they lack accuracy and precision. They are not quite reliable as the subjective variations are high leading to inaccurate error estimates.

Histological methods

It was Gustafson, who for the first time considered the histological method for dental age estimation in adults. The histological methods were the first methods where the regressive age changes could be scientifically measured. He published his seminal paper on adult dental age estimation in 1950, and since then, it has been the most studied method (Lucy and Pollard 1995). However, due to the accessibility of the entire data in the original articles, the mean error estimate by Gustafson was quite contested. Despite such criticism, the fact that Gustafson pioneered the adult age estimation cannot be overemphasized. Moreover, most of the histological methods in use now are in fact modifications of Gustafson's method; even the radiological methods depend on the secondary dentin deposition which is one of the parameters of Gustafson's method.

Gustafson's method

Gustafson considered six parameters for age estimation namely, attrition (A), secondary dentin apposition (S), periodontitis (P), cementum apposition (C), root resorption (R), and root transparency (T). Equation of the regression line given by Gustafson as "Age = 11.43 + 4.56x", where x is the total point count and the mean error was ±3.63 years. An important factor extensively studied is the reproducibility of Gustafson's original error estimate for the technique. Maples and Rice were the first ones to study Gustafson's data and recalculate error estimates. They identified a few problems with the statistical analysis of original data and failed to substantiate

Gustafson's original error estimate of ±3.63 years. Their calculation produced an associated error of ±7.03 years, which was almost twice the error estimate given by Gustafson. Upon recalculation of Gustafson's data, Maples and Rice gave the following formula: Age = 4.26x + 13.45 (r = 0.912), where x is the total point count (Lucy and Pollard 1995).

Maples and Rice noted that Gustafson's regression line was calculated from a whole sample of 41 teeth, which were derived from only 19 teeth. Some of the same teeth were apparently used in a test of the accuracy of that regression line. This turned out to be a major drawback as it is not a justified test of the model (Lucy and Pollard 1995).

Pollard and Lucy later revisited both Gustafson's and Maples and Rice's methods. They noted that the linear regression equation of Gustafson was based only on 8 samples and not 19 as said by Maples and Rice. They even pointed out that both the methods used linear regression methods whereas it is not the preferred method of statistics to be followed, as the methods take multiple parameters into account. They recalculated the statistics with a multiple regression model and obtained an error estimate of ±15.9 years (Lucy and Pollard 1995).

Several modifications have been made to Gustafson's method by removal of one or more parameters, mainly periodontal attachment and root resorption. Yet, most of the modifications failed to give a more accurate error estimate. Bang and Ramm's method is based on a single parameter—*dentin translucency* and obtained an error estimate of ±4.7 years (Bang and Ramm 1971). Amongst the modified methods, Kashyap and Koteshwar method is considered accurate and most reproducible method; hence most accepted (Kashyap and Koteshwar Rao 1990). Table 1 shows all the modifications of Gustafson's method (Bang and Ramm 1971; Kashyap and Koteshwar Rao 1990; Gustafson 1950; Dalitz 1962; Johanson 1971; Maples and Rice 1979; Lamendin et al. 1992; Solheim 1993).

Several modifications pointed a few parameters that can be used independently for age estimation such as the following:

Dentin translucency
Dentin translucency, as a sole parameter, was first considered by Bang and Ramm in 1970 (Saxena and Tiwari 2010). They studied 1013 teeth from 201 patients and concluded that accurate results were obtained from teeth sections until 75 years of age with a mean error estimate of ±4.7 years (Bang and Ramm 1971). Drusini in 1991 evaluated dentin transparency in roots using computerized densitometric analysis but failed to demonstrate any

credits as compared to earlier methods because results deviated by more than 5 years (Drusini et al. 1991).

Lamendin in 1992 applied two parameters—dentin transparency and periodontal height and observed a mean error of ±8.4 years. The periodontal attachment level was criticized as a parameter as it is highly variable and affected by many factors such as irritation, poor dental hygiene, and effects of systemic diseases and drugs (Lamendin et al. 1992). Further, Kamann in 1998 evaluated the width of dentinal tubules on cryosections and found that the diameter of dentinal tubules decreased from 3–4 to 2 μm with increasing age (Rer.nat 2007).

Acharya in 2010 attempted to quantify translucency using digital aids where the analog signal is converted to digital and subsequent image processing is done using customized software programs. They concluded that the digital method could better estimate age with a mean error of ±5 years in 60% of cases as against 40% for the conventional method. They also commented that both methods had an equal tendency to either over- or underestimate age, and no apparent difference could be observed in their ability to reliably estimate age in younger or older age groups (Acharya 2010).

Secondary dentin deposition
The continuous formation of secondary dentin is thought to be a biological response and an indicator of aging. Bodecker in 1925 was the first one to show the correlation between deposition of secondary dentin and chronological age. Then, in 1950, Gustafson considered it as one of the parameters (Gazge et al. 2018).

Cementum
Age estimation based on cementum can be done in two ways: cementum apposition and cemental annulations. Zander and Hurzeler discovered a linear relationship between the growth of cementum and chronological age for the first time. They studied 231 single-rooted teeth and concluded that the cemental thickness tripled over the study age of 11 to 76 years (Zander and Hurzeler 1958). Pinchi et al. in 2007 considered thickness of radicular cementum as a means of dental age estimation. They studied 127 teeth in age ranging from 16 to 90 years and estimated age up to 95% reliability with an error estimation of ± 1.49 years. The main drawback in this method is to determine the area from which the thickness has to be measured as the thickness of cementum is not uniform on all the surfaces (Pinchi et al. 2007).

The determination of the age of the tree is carried out by a very well accepted method—counting the annulations of the bark of a tree. Similarly, the annulations in cementum were thought to be an ideal parameter for dental age estimation. Stott et al. in 1982 published

Table 1 Details of Gustafson's method of age estimation and its modifications

Author	Year and country	Sample size (n)	Formula[a]	Error estimate[b]	Specifications
Gustafson (Gustafson 1950)	1950 Sweden	n=146 teeth	**Age: 11.43 + 4.56X** where X equalled overall score	Standard error = ±3.4 years Correlation r = 0.91	First histological method
Dalitz (Dalitz 1962)	1962 Australia	n= 146 teeth (4 teeth per person)	**Estimated age= 8.691+ 5.146A+ 5.338P+ 1.866S+ 8.411T**	r = 0.88	Root resorption and cementum apposition can be disregarded
Bang & Ramm (Bang and Ramm 1971)	1970 Norway	n= 1013 teeth (only dentin trans-lucency was considered)	**Age= $B_0 + (B_1{}^a X) + (B_2{}^a X^2)$**	Mean error = ±4.7 years r = 0.61 to 0.83	Only intact roots measured and histologic sections might not reflect real limit of transparent dentin.
Johanson (Johanson 1971)	1971	n= 162 teeth	**Age= 11.02+ (5.14 x A) + (2.3 x S) + (4.14 x P) + (3.71 x C) + (5.57 x R) + (8.98 x T)**	±5.16 years r= 0.92	Most appreciated method Ground section to be 0.25 mm
Maples (Maples and Rice 1979)	1978 USA	n= 19 teeth	**Root resorption had weakest correlation while best were secondary dentin and root transparency.**	±7.03 years r = 0.90	Simpler method
Kashyap & Koteshwar (Kashyap and Koteshwar Rao 1990)	1990 India	n= 25 teeth	**Age= (A)+ (D)+ (T)+ (CE)/ 4**	Average error = ±1.59 years r = 0.998	More accurate, reliable, and reproducible method.
Lamendin (Lamendin et al. 1992)	1992 Orleans	n= 306 single rooted teeth	-	Mean error = ±8.4 years	Periodontal attachment level was criticized as a parameter.
Solheim (Solheim 1993)	1993 Norway	n= 1000 teeth of Caucasian population	-	r= 0.78 to r= 0.91 **or** r= 0.76 to r=0.89	Added 3 more parameters: Surface roughness, color of teeth, and gender of individual.

For Bang and Ramm method—B_0, B_1, and B_2; regression coefficients of Student's test; X variable

For Kashyap and Koteshwar method—A attrition, P periodontitis, D secondary dentin, T translucency of root, CE cementum apposition

[a] Abbreviations: A attrition, P periodontitis, S secondary dentin apposition, C cementum apposition, R root resorption, T root transparency

[b] Note that the method of the error estimate in all the studies is not uniform

the first study using tooth cemental annulations. Their method estimated the age of the first individual (known age = 57 years) to be 58±1 years using premolars and 57.5±0.5 years using molars. The age estimated for the second individual (known age = 67 years) was estimated between 63 and 70 years and that for the third individual (known age = 76 years) to be between 73 and 78 years. They observed that in multi-rooted teeth, as the inter-radicular distance decreased, cementum became thicker. Hence, it became almost impossible to achieve representative areas of annulation in such cases. (Stott et al. 1982). Lipsinic et al. in 1986 sectioned 31 teeth of known age and observed cemental incremental lines. They concluded that direct age predictions based on cemental incremental lines underestimated the age of older specimens (Lipsinic et al. 1986). This idea of counting cemental annulations was first done on ground sections of the teeth observed under light microscopy. With advances in microscopic techniques, polarized and phase-contrast microscopy were applied along with digital image analysis software. However, there was no significant improvement in the accuracy of determining age.

Wittwer-Backofen in 1990 made an attempt to correlate the cemental annulations with age and found the error of only 2.5 years or less. However, this result obtained was after the elimination of 16% of the sample as the cementum pattern was irregular. Not every part of the cementum band within a section is suitable for counting. Areas with low image contrast, the inclusion of artifacts, root fractures, or narrowing in root bifurcation in the cementum band may be excluded from further analysis as they could lead to counting mistakes as in poorly preserved skeletal materials (Wittwer-Backofen et al. 2004).

Kvaal and Solheim in 1989 evaluated the fluorescence of dentin and cementum in human premolars and showed that fluorescence from cementum was stronger than that of dentin. Also, teeth from human remains gave stronger intensity of fluorescence than teeth from living patients. They showed a correlation ranging with age from 0.77 to 0.87, but it is not well accepted as they used it as an arbitrary scale (Solheim 1990). Griffin et al. in 2008 proposed that infusion of decomposition products from RBCs such as porphyrins causes changes in dentin and cementum which can be detected by fluorescence (Nayak et al. 2014).

Gustafson's method was originally performed on the European population. Its application in various populations has been tried out but its reproducibility is questionable. Chandler et al. applied Gustafson's method on the Western Cape population and concluded that it was not accurate as the average error estimate was obtained to be ±13.7 years and ±11.6 years by two examiners. Extensive research of parameters by numerous

modifications concluded that dentin translucency reproduced the best correlation while periodontal attachment gave the least correlation (Chandler and Phillips 2018).

Micrometric measurements by scanning electron microscope

The diameter of dentinal tubules reduces with age due to the deposition of peritubular dentin, which is a mineralized deposit formed centripetally in the dentinal tubules. Kosa et al. in 1900 first used scanning electron microscopy (SEM) to observe aging changes as shrinkage of the pulp tissue and presence of a predentin layer microscopically. They determined weight concentrations of calcium and phosphorus and further calculated Ca/P weight ratio with the help of electron probe microanalysis in the dentin of single-rooted teeth of 25 individuals and obtained a higher correlation with age (r=0.9712) (Kosa et al. 1990). Kvaal et al. in 1994 investigated the amount of peritubular dentin and extent of obliteration of tubules and correlated with age. They also attempted to determine whether this parameter was worth being used as a parameter for age estimation (Nayak et al. 2014).

Kedici et al. in 2000 used a SEM micrometric scaler to obtain 20 measurements of different variables in incisor teeth. They obtained a mathematical formula based on multiple regression analysis which gave statistically acceptable results. Their results showed a better correlation with gender differences (Kedici et al. 2000).

All the histological methods apart from modifications of Gustafson's methods are summarised in Table 2 (Drusini et al. 1991; Zander and Hurzeler 1958; Pinchi et al. 2007; Stott et al. 1982; Wittwer-Backofen et al. 2004; Solheim 1990; Kosa et al. 1990). Histological methods although accurate, need an extraction, and sectioning of teeth. Hence, it is an invasive procedure, practically difficult in living beings yet the most preferred method in deceased.

Radiographical methods

It is an established fact that the deposition of secondary dentin is indicative of aging; which occurs at the cost of the pulp space. The shrinkage of the pulp chamber owing to secondary dentin deposition as age advances can be measured radiographically. As the digital radiography evolved, its application in dental age estimation also progressed from two-dimensional pulp-tooth ratio measurements on periapical radiographs to a three-dimensional volume assessment of pulp using cone beam computed tomography (CBCT). Recently, even a 3D volumetric digital tooth reconstruction is made possible which can be quite effective.

Radiography is relatively rapid, easily reproducible, and non-invasive and thus is a preferred method of age estimation in living individuals. Furthermore, the storage

Table 2 Details of other histological methods of age estimation

Author	Year and country	Sample size (n) and study population	Methodology	Formula	Error estimate[a]
Drusini et al. (Drusini et al. 1991)	1991 Italy	n= 152 single-rooted teeth were collected.	Root dentin transparency was measured under bright light. Measurement was done from the apex of the tooth to the most coronal extent of the transparency. Measurements were done both by Vernier caliper and densitometric IBAS system.	-	The errors range from ±5 to more than ±20 years. The highest percentages of success within ±5 years were obtained for premolars.
Zander et al. (Zander and Hurzeler 1958)	1958 New York	n=233 single-rooted teeth were collected from age range of 11 to 76 years.	Teeth were decalcified and sectioned horizontally from apex to cementoenamel junction.	Thickness of cementum= [a x 0.71] ÷ [b x 0.032]	Average cemental thickness below 20 years= 0.076 mm Average cemental thickness from 51 to 76 years= 0.215 mm. Thickness of cementum tripled over years.
Pinchi et al. (Pinchi et al. 2007)	2007 Italy	n=127 teeth from age group of 16–90 years.	Teeth were decalcified and sections were obtained. Measurements obtained were 1/3rd of root length on lingual side, 1/3rd f root length on vestibular side, thickness at apex.	$y=[6 \times 10^{-7}x^3] - [0.0009x^2] + [0.3743x] + 12.14$	Age estimated was 58.6 years ± 1.49 years with 95% reliability.
Stott et al. (Stott et al. 1982)	1982 Texas	n=10 teeth were prepared for processing and they were obtained from 3 dissection cadavers.	Teeth were sectioned using low speed saw and diamond blade and sections of 100–150μ were obtained. Sections were stained with Alizarin red stain and cemental annulations were studied.	-	Cadaver 1: known age=57 years. Estimated age=58±1 years. Cadaver 2: Known age=67 years. Estimate age = 63–70 years. Cadaver 3: Known age=76 years. Estimated age= 73–78 years.
Wittwer-Backofen (Wittwer-Backofen et al. 2004)	1990	-	Teeth were sectioned and sections were observed under transmitting light microscopy. Representative images were captured and processed with image analysis software.	-	A confidence interval of 2.5 years was obtained but can exceed these error ranges if teeth are post-mortally affected.
Solheim et al. (Solheim 1990)	1990 Norway	n=1000 teeth, 100 of each type excluding molars in Caucasian population.	Half-sectioned teeth was stained with carbol fuschin and cementum was studied with stereomicroscope.	-	Strongest correlation was found in range, i.e., r=0.54 for maxillary incisors and r=0.75 for mandibular second premolars.
Kosa et al. (Kosa et al. 1990)	1990 Hungary	Single-rooted teeth of 25 individuals were selected.	Weight concentrations of calcium and phosphorus and Ca/P weight ratio was calculated with the help of electron probe microanalysis in dentin. TESLA BS-300 SEM (scanning electron microscope) was used coupled with X-ray detector and X-ray analyzer.	**Y= 37.17x + 139.37 X is the age Y is the Ca/P weight ratio**	Weight concentration of phosphorus was having closer correlation with age (r=0.9712).

[a] Note that the method of error estimate in all the studies is not uniform

and revisiting of radiographs are less cumbersome as compared to ground sections.

Pulp-tooth dimension ratio

Morse in 1993 was the first to use the intraoral periapical (IOPA) radiographs and derived six parameters: (A) coronal length, (B) apical length, (C) root canal length, (D) cervical width, (E) mid-root width, and (F) apical width. They observed that shrinkage of root canal occurred with aging—vertical root canal shrinkage at the rate of 0.32 mm/year and horizontal root canal shrinkage at the rate of 0.39 mm/year (Morse et al. 1994).

Kvaal and Solheim in 1994 presented a combined radiological and morphological method but that still required the extraction of teeth (Solheim and Kvaal 1993). As a modification, Kvaal et al. in 1995 conducted a study based on radiological measurements only. The method used length and width of the tooth and dental pulp on IOPA radiographs (Kvaal et al. 1995). A standard error of ±9.5 years was obtained by using the following equation:

$$Age = 129.8 - (316.4 \text{ x } M) (6.8 \text{ x } [W - L])$$

Parameters used were as follows:

- Pulp-root length–(R)
- Pulp-tooth length–(P)
- Tooth-root length–(T)
- Pulp-root width at the cement-enamel junction (CEJ)–(A)
- Pulp-root width at mid-root level–(C)
- Pulp-root width at the midpoint between levels A and C–(B)
- Mean value of all ratios excluding T–(M)
- Mean value of width ratio B and C–(W)
- Mean value of length ratio P and R–(L) (Kvaal et al. 1995)

Bosman in 2005 used orthopantomogram (OPG) instead of IOPA and applied Kvaal's method which gave a high predictive value of age determination only till 18 years. He obtained a standard error estimate from 8 to 11.5 years (Bosmans et al. 2005). The only advantage of using OPG is visualizing all teeth in a single image, and the fact that an individual has to face comparatively less ionizing radiation as compared to full mouth IOPAs.

Then Cameriere in 2004 and 2007 determined pulp-tooth ratio specifically on canines in both IOPA and OPG, respectively, and compared the results. He found that the error estimate on IOPA (error estimate of ±4.06 years) was better than that on OPG (error estimate of ±5.35 years) (Cameriere et al. 2004a; Cameriere et al. 2007). Furthermore, the same method is performed on cone beam computed tomography (CBCT) by various

authors such as Yang et al. who obtained a poor correlation of −0.54 between age estimated and chronological age and the square root of mean square error was 8.3 years (Yang et al. 2006). Asif et al. conducted a software-based study by using CBCT and measuring pulp tooth ratio and concluded that mean absolute error estimated between 5 and 9 years and that error values were higher in older age groups of 45–65 years (Asif et al. 2018; Asif et al. 2019).

Tooth-coronal pulp cavity index

In 1985, Ikeda et al. developed a new index, called the tooth-coronal index or tooth coronal pulp cavity index (TCI), which is based on two linear measurements on dental radiographs of extracted human teeth, crown height (CH), and coronal pulp cavity height (Ikeda et al. 1985). Drusini in 1991 first applied densitometric analysis and computed tooth coronal index but found errors ranging from ±5 years to ±20 years. Then, he in 1993 again used OPG to measure the length of the tooth crown and coronal pulp cavity but could not correlate with better accuracy.

$$TCI = (CPCH \text{ X } 100)/CL$$

CPCH= coronal pulp cavity height or length, CL= coronal length (Drusini 2008)

X-ray microfocus scanning

Vandevoort et al. in 2004 scanned 43 extracted teeth from 25 individuals with an X-ray microfocus scanning computed tomography unit (μCT) with 25-μm spatial resolution. Ages estimation was calculated based on the ratio of pulp volume and tooth volume, and it gave a very strong concordance correlation. The major limitations include the expensive equipment and time-consuming procedure, but it still can be a promising method (Vandevoort et al. 2004).

Schuer in 2005 evaluated "prosthetically restored," "filled dental root and periodontal bone loss" on OPG and correlated with aging. The prosthetically restored teeth gave the highest correlation with aging, but it was not accepted as the parameters chosen were highly variable (Nayak et al. 2014). Table 3 gives the details of all the major radiological methods (Morse et al. 1994; Kvaal et al. 1995; Bosmans et al. 2005; Cameriere et al. 2004a; Cameriere et al. 2007; Drusini 2008; Cameriere et al. 2004b; Jagannathan et al. 2011).

A systematic review by Marroquin et al. is based exclusively on the radiological methods, and it was demonstrated that Kvaal's method was applied in 12 countries, Cameriere's method was applied in 11 countries, and volume-based studies were carried out in 16 countries. It was revealed that when the same method was applied

Table 3 Details of radiological methods of age estimation

Author	Year and country	Sample size (*n*) and study population	Methodology	Formula	Error estimate[a]
Morse (Morse et al. 1994)	1993 Pennsylvania	n= 500 and only mandibular anterior teeth were evaluated.	Root canal shrinkage was calculated vertically and horizontally comprising of length and width.	-	The first study to suggest use of radiology in age estimation.
Kvaal (Kvaal et al. 1995)	1995 Norway	n= 100 IOPA was used	Molars were not included. Length and width ratios were calculated for pulp and teeth.	$Age = 129.8 - (316.4 \times M)\ (6.8 \times [W\text{-}LI])$	Standard error= ±9.8 years
Cameriere (Cameriere et al. 2004b)	2004 Bologna, Italy	n= 312 Italian population with 135 males and 177 females. OPGs were used.	OPGs of second molars were processed using Adobe Photoshop 7.0. Twenty points from each tooth edge and ten points for the pulp edge were identified and used to evaluate tooth and pulp areas.	$logit(p) = b_0 + b_1 RA + b_2 Tm + b_3\ sex.$ RA= ratio pulp/tooth area Tm= third molar development	A cutoff value of RA= 0.088 if Tm= 0 and RA= 0.097 if Tm= 1. Sensitivity= 0.91 Specificity= 0.945.
Cameriere (Cameriere et al. 2004a)	2004 Italy	n= 100 Italian population (Caucasians) with 46 males and 54 females. OPGs were used.	Single rooted maxillary canines were used and tooth and pulp areas were computed using AutoCAD software.	$Age = 86.53 - 457.15\ AR - 22.98\ c$ AR= pulp tooth area ratio c = pulp/root width at midroot level	Median of the absolute value of residual error= <4 years Standard error= ±5.35 years.
Bosman (Bosmans et al. 2005)	2005 Belgium	n= 197 OPGs with age range of 19 to 75 years.	Molars were excluded.	-	Standard error= ±8.6 to ±9.5 years depending on various teeth used.
Cameriere (Cameriere et al. 2007)	2007 Bologna, Italy	n= 200 IOPAs of age range from 20–79 years, with 114 males and 86 females.	Maxillary and mandibular canines were used and tooth and pulp areas were computed using AutoCAD software.	$Age = 114.624 - 431.183x_1 - 456.692x_2 + 1798.377x_1x_2$ x_1= pulp areas of upper canines x_2= pulp areas of lower canines $Age = 99.937 - 532.775x_1$ (when only the upper canine is included) $Age = 89.456 - 461.873x_2$ (when only the lower canine is included)	Residual standard error= ±4.06 years Mean prediction error (ME) = ±3.36 years. Residual standard error= ±5.44 years ME= ±4.38 years. Residual standard error= ±5.45 years ME= ±4.46 years
Jagannathan (Jagannathan et al. 2011)	2008 India	n= 188 (140 cases and 48 controls) from age 10–70 years. CBCT was used to measure pulp and tooth volumes.	CBCT scanner (3D accuitomo, Japan) was used with slide thickness of 250 µm. Multiplanar volume reconstruction was performed with Advantage Windows workstation (USA).	$Age = 57.18 + (-413.41 \times PTV)$ PTV= pulp/tooth volume ratio	Mean absolute error (MAE)= ±8.54 years
Drusini (Drusini 2008)	1991 Italy	n= 846 intact teeth from 433 Caucasian individuals OPGs were used to measure the tooth-coronal index (TCI).	Only mandibular posterior teeth where the pulp chamber is normally fully visible were considered excluding third molars.	$TCI = (CPCH \times 100)/CL$ CPCH= coronal pulp cavity height or length, CL= coronal length	Standard error varies from ±5 to ±20 years.

[a] Note that the method of error estimate in all the studies is not uniform

in different populations, and it led to different regression formulae which were specific to the study population. This led to a difference in the error estimate. However, even when the same linear regression formula was applied to different populations, it resulted in a greater error estimate (Marroquin et al. 2017).

Mental foramen and mandibular canal

Bhardwaj et al. in 2014 used digital panoramic radiographs for assessing gonial angle, antegonial angle, mental foramen, mandibular canal, and mandibular foramen for age estimation and found mandibular canal and mandibular foramen as highly significant parameters. But they considered the age range from only 25 to 54 years and excluded the extremes of age. Many researchers have also tried the same using CBCT, but the results have not been quite significant (Bhardwaj et al. 2014).

Recently, technological advancements have made the application of artificial intelligence (AI) possible in the forensic world. One such study by Kim et al. on data set of 1586 individuals considered only first molars from OPGs. They obtained an accuracy of 89.05 to 90.27% indicating excellent outcomes. The AI utilized a convolutional neural network (CNN) that focused on tooth pulp, alveolar bone level, and interdental space depending on age and location of tooth (Kim et al. 2021).

To summarize, the radiographical methods are highly accepted due to their ease, reproducibility, reliability, and usefulness in living individuals. It is nowadays used with one of the biochemical methods, and the combined method is said to give the best results.

Biochemical methods

The biochemical methods are mainly based on the racemization of amino acids and uptake of radioactive carbon by enamel. Both these methods depend on the uptake and deposition of respective chemicals in vivo as a result of aging.

Aspartic acid racemization

The racemization of amino acids is relatively rapid in living tissues whose metabolism is slow. It involves the conversion of L-isomer to D-isomer toward the equilibrium and can be related to the chronological age. Aspartic acid has the highest rate of racemization and is stored during aging. The levels of D-aspartic acid increase in human enamel, dentin, and cementum with age (Senn and Stimson 2010).

Helfman and Bada in 1976 were the first to show that age estimation can be done in human enamel and dentin by quantifying the relative amounts of D- and L-isomers. They concluded that the estimated age of an individual falls around ±10 years the chronological age. Further, the age of death of an individual can be estimated using the extent of aspartic acid in tooth enamel from the skeletons (Helfman and Bada 1976). Mornstad et al. in 1991 demonstrated the use of high-performance liquid chromatography (HPLC) instead of amino acid analyzers. The advantage was the method was quick and less expensive (Mornstad et al. 1995).

Carolan et al. concluded that the reliability of racemization was similar to that of other methods. However, Waite et al. showed that high accuracy with an error of ±3 years can be obtained with proper sampling and analysis (Senn and Stimson 2010). Ohtani and Yamamoto in 1995 conducted a study where racemization of aspartic acid in cementum, and they summarized 5 cases as follows: (a) age of a female skeleton estimated to be 21±3 years (actual age=18 years); (b) age of an adipocere female body estimated to be 39±3 years (actual age=41 years); (c) age of an adult female skeleton estimated to be 19±3 years (actual age=21 years); (d) age of a female body estimated to be 57±3 years (actual age=55 years); and (e) age of a decomposed female body estimated to be 67±3 years (actual age=66 years) (Ohtani et al. 1995).

Furthermore, Ritz et al. in 1995 applied aspartic acid racemization on dentinal biopsy from living individuals and standard error estimate obtained as ±2.9 years with a correlation of $r=0.99$. The good outcome of the study made this method a hallmark as it gave the least error range and most accurate result while being used in living individuals (Ritz et al. 1995).

Radioactive carbon-14 uptake (carbon dating)

Carbon dating which is conventionally used by archaeologists to measure the age of geological matter found its application in dental age estimation also. Spalding et al. in 2005 showed a path-breaking alternative to determine age from the enamel of teeth. The levels of radioactive C^{14} isotope levels and radioactive CO_2 which is formed by the reaction of C^{14} with oxygen have drastically increased after the nuclear tests. This radioactive CO_2 which has a radioactive signature is up-taken by plants. It is deposited in human dental enamel while consuming these plants. By calculating these levels of deposited C^{14} in enamel, the date of birth can be calculated. Initial reports claim the accuracy of estimated age by the mean error of ±1.5 years (Spalding et al. 2005).

Alkass et al. in 2009 proposed a combined method where both aspartic acid racemizaton and radioactive carbon uptake could be used. The age at death determined by racemization and the date of birth from C^{14} levels provide the closest estimation of age with a difference of 1–1.5 years (Alkass et al. 2010).

Miscellaneous biochemical methods

Dentin showed a higher capacity for fluoride uptake as compared to enamel. The concentration of fluoride mainly occurs in dentin, close to the pulp, and it depends on the fluoride administration. Steinecke et al. in 1995 evaluated the age-dependent fluoride content in enamel and dentin and found a correlation of $r=0.31$ for enamel and $r=0.8$ for dentin (Rer. nat 2007). Schramm in 2002 compared both dentin transparency and fluoride concentration in dentin to check their validity for dental age estimation. 69% of the cases gave close estimation with a deviation of ± 5 years using the fluoride method whereas dentin transparency resulted in 61% of cases to give close prediction with ± 5 years deviation. However, increased age ≥ 75 years was not well estimated. The biggest limitation is variations in the concentration of fluoride in drinking water, and the use of fluoride supplements and dietary habits alters the results making it less reliable (Rer.nat 2007).

De-las Heras et al. in 1999 quantified the crosslinking of deoxypyridinoline (DPD) in the collagen matrix of dentin. Further, they correlated it with the age of an individual and obtained an error estimate of ± 14.9 years (Martin-de et al. 1999).

Maillard reaction is a type of non-enzymatic reaction of carbonyl compounds, mainly reducing sugars with compounds possessing a free amino group, such as amino acids, amines, and proteins resulting in browning. It is chemically termed as glycation, due to which, advanced glycation endproducts (AGEs) are produced and they get accumulated with age. They have been correlated with complications derived from aging and associated diseases by Ager et al. in 2006. But they failed to give an accurate result with the error of ± 13.7 years (Ager et al. 2006).

Lead is one of the most significant pollutants in the environment. The levels of lead in blood indicate instant exposure while the concentration of lead in teeth encompasses earlier exposure also. Dentin is the main site for lead deposition. Measurement of accumulation of lead in dentin has been considered for age estimation. Al-Qattan et al. in 2010 gave a linear regression formula calculated for dentin lead concentration and age in the Kuwaiti population as follows:

$$y = 1.2x + 17.6 \; (y = \text{subject's age and } x = \text{dentine lead concentration})$$

The standard error obtained was 5.95 years. However, more research is required to confirm the utility of this method in determining age (Al-Qattan and Elfawal 2010).

Genetic and epigenetic methods

The advanced technological developments gave chance to adopt newer methods and improve the accuracy and reliability of age estimation.

Telomeres at the end of the chromosomes shorten with each cell division, limiting the proliferation of human cells and inducing senescence, differentiation, or cell death. Telomere shortening may be an effective method to estimate age. Takasaki et al. in 2003 determined the terminal restriction fragment (TRF) length as telomere length in dental pulp DNA and determined the age. However, the mean error of this technique was found to be about 10 years, which was greater than previous methods (Takasaki et al. 2003).

A variety of T cell receptor (TCR) molecules is generated when each immature T lymphocyte undergoes somatic rearrangement. This process leads to the elimination of DNA sequences of TCR and circulation of TCR into "Signal joint TCR excision circles (sjTRECs)". Zubakov et al. in 2010 conducted a study on the Dutch population where they quantified sjTRECs level and found a standard error estimate of ± 8.9 years (Zubakov et al. 2010).

According to the mitochondrial theory of aging, mitochondrial DNA (mtDNA), near the inner membrane of the mitochondria, is more susceptible to damage generated by the free radicals released by the electron transport chain. This produces mtDNA mutations. Zapico et al. in 2016 quantified the mutations in mtDNA from pulp and demonstrated a strong correlation with increasing age (Zapico and Ubelaker 2015).

Genetic methods are recent, expensive, and technique-sensitive—yield variable results which are not reliable. Hence, further research is needed to determine standardization and improve accuracy.

Recently, following genetics, even epigenetic modifications have been tried out such as DNA methylation. Bekaert et al. in 2015 carried out DNA methylation on both blood and teeth DNA. The associated error for blood samples was of ± 3.75 years, and for dentin, it was ± 4.86 error (Bekaert et al. 2015). Guillani et al. in 2016 studied DNA methylation in DNA from three tissues- dentin, pulp, and cementum. They demonstrated a median absolute difference of 1.20 years. This technique is highly technique-sensitive and requires sophisticated and expensive equipment, so is not quite common in routine setup (Guiliani et al. 2016).

Conclusions

Age estimation has from its inception been one of the most central subfields in forensic science. Age estimation by teeth has become the most accepted means

mainly due to the high accuracy most methods offer. Adult dental age estimation is extensively varied due to the application of numerous technologies to study regressive or aging changes.

It is also observed that not all the studies have homogeneity in the statistical calculation of error estimation which may employ either standard error, mean error, average error, or mean absolute error. Further, not all studies have a similar sample size. The study population depends on the country where the study is conducted, and hence, the variations in the results are difficult to generalize as it gets population-specific. Regardless of the method used, a population-specific formula would always give better accuracy than a non-population-specific formula. Hence, more standardization is required in order to improve reliability and reduce the range of error estimates.

Despite these many methods, there has always been a question regarding the most appropriate methods for various scenarios. According to the present review, in case of living individuals—the most appropriate is the radiological methods. However, the use of biochemical methods in living can be explored based on dentinal biopsies in selected cases. The combination of both of them has been suggested as the best possibility, whenever possible. For the deceased or in mass disasters, the histological methods remain the first priority due to its high accuracy and reliability. Biochemical methods can also be used as an adjunct to improve accuracy.

In summary, how the adult dental age estimation methods have evolved over the years is quite fascinating. Appreciating the evolution of these methods helps us increase our understanding and thereby improve the efficiency of these methods in the forensic application. Although the newer methods lack the standardization and ease of application, more studies with a significantly large sample size and appropriate statistical error estimates may help improve the accuracy and reliability.

Abbreviations
SEM: Scanning electron microscope; CBCT: Cone beam computed tomography; IOPA: Intraoral periapical; OPG: Orthopantogram; TCI: Tooth-coronal index or tooth coronal pulp cavity index; CH: Crown height; CPCH: Coronal pulp cavity height or length; CL: Coronal length; μCT: X-ray microfocus scanning computed tomography unit; AI: Artificial intelligence; CNN: Convolutional neural network; HPLC: High-performance liquid chromatography; DPD: Deoxypyridinoline; AGE: Advanced glycation endproducts; TRF: Terminal restriction fragment; TCR: T cell receptor; sjTRECs: Signal joint TCR excision circles; mtDNA: Mitochondrial DNA.

Authors' contributions
RP: Conceptualization, Visualization, Project administration, Supervision, Writing— reviewing and editing. ED: Data curation, Resources, Investigations, Writing—original draft. The authors have read and approved the final manuscript.

Declarations

Ethics approval and consent to participate
Not applicable

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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