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Dental Age Estimation of Adults: A Review of Methods and Principals

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Abstract: The aim of the study, is to present the historical development of age estimation in adults from teeth. It should be noted that individuals are separated to those who are below 20 years where tooth development can be used and those above 20 years of age where all teeth are fully formed and regressive age related changes might be used. For each technique developed for the second group since 1950, a basic apprehension of the method is given and its application and reliability is discussed. The techniques are categorized in morphological, radiological and biochemical. In order to reach to reliable estimation different techniques should be used and repeated measurements should be made. Recently, the first software program for age estimation was introduced enabling quicker results.

Key words: Age estimation, dental, adults, methods, techniques

INTRODUCTION

Forensic odontologists are often confronted with the problem of determining the age of unknown bodies, as well as living persons. Age estimation is of great importance for the identification of unknown bodies or skeletal remains of accidents and crimes as well in disaster victims (Willem *et al*., 2002). In the case of living people who have no acceptable identification documents, such as refugees, adopted children of unknown age, verification of chronological age is required in order to be entitled to civil rights and social benefits (Willem *et al*., 2002; Kvaal, 2006; Yang *et al*., 2006; Herchaft *et al*., 2007). In archaeological search, estimation of age at death for skeletal remains serves as an aid in palaeodemography (Kvaal, 2006; Kvaal *et al*., 1995).

A forensic dentist in the cases mentioned before, carries a considerable responsibility since his scientific opinion is frequently asked when all other paths of identification have been exhausted (Willem *et al*., 2002; Bowers and Bell, 1995). There are instances in which teeth are the only preserved human remains and present the only means for age determination (Gustafson *et al*., 1950; Bang and Ramm, 1970; Maples, 1978) in order to narrow down the search within the missing person's file and enable a more efficient approach (Herchaft *et al*., 2007). In these cases final identification may depend on specific odontological matching of pre- and post-mortem dental data, DNA-typing and fingerprinting (Jeffreys, 1993; James and Noraby, 2003; Reppien *et al*., 2006). Teeth have the benefit to be preserved long after other tissues, even bone, have disintegrated and also unlike bones they can be examined directly in living individuals (Kvaal *et al*., 1995; James and Noraby, 2003; Reppien *et al*., 2006). However, one must not forget that the more parameters taken into account the more accurate the determination of age is. For this reason clues from dentition must be correlated with clues found in the bones (Stavrianos and Choudakis, 1983; Stavrianos and Choudakis, 1984; Stavrianos and Metska, 2002; Stavrianos and Vasiliadis, 2002; Stavrianos, 2008; Solheim and Kvaal, 2000).

In our study we chronologically present the various methods published up to our days. The approach, the advantages and disadvantages of each technique are discussed giving an emphasis on the methods applied most. Then the steps that a forensic dentist should follow in order to estimate the age are presented. Finally the latest developments and speculations upon age estimation methods are reported.

HISTORICAL BACKGROUND

The first known attempts which used teeth as an indicator of age originate from England. In the early 19th century, because of economic depression of the industrial revolution, juvenile work and criminality were serious social problems. The social legislation provided that no child under 9 years of age should be employed, while children under 13 should not work more than nine hours the day. The limit for criminal responsibility was seven years of age. However there wasn't a registration of birth, thus making the proof of date of birth difficult. Up to this

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time the determination of age was based mostly on the calculation of height (Stavrianos and Metska, 2002; Stavrianos, 2008; Miles, 1963).

In 1836, A.T. Thomson who was one of the pioneers of medical jurisprudence claims that children, where the first permanent molars had not erupted, it was certain that that they had not reached the age of seven (Kvaal, 2006; Stavrianos, 2008; Miles, 1963). The first scientific study was presented in 1837 by Edwin Saunders, in which he points out that dentition is a more reliable standard than height for determination of age (Kvaal, 2006).

In 1872, Wedl made the first observations of changes with age in the permanent dentition and described fatty degeneration, calcification, colloid deposits, netlike trophy and pigment deposits in the pulp tissue and a notable diminution in the size of the pulp cavity due to continued deposits of new dentine layers (Kvaal, 2006).

METHODS OF AGE ESTIMATION IN ADULTS

Several techniques are described in literature that address age estimation in adults. In general the methods are divided into three categories. The morphological, the radiological and the biochemical methods, which are all based on degenerative processes, observed in the dental structures (Herschaft *et al*., 2007; Valezuela *et al*., 2002; Solheim and Anne, 2006). The methods described below are reproducible and rather accurate methods, some of which are non-destructive for the tooth substance.

Morphological methods: In the morphological methods belong all these that use morphological criteria. The samples can be observed sectioned or unsectioned with the eye (Willems *et al*., 2002). At this category belong the methods suggested by Gustafson (1950), Dalitz (1962), Bang and Ramm (1970), Johanson (1971), Maples (1978), Solheim (1993) and Gustafson (1950).

The first technique for age estimation on teeth based on a systematic and statistical approach was published by Gustafson (1950). Once dental development is complete, through assessment of age and function, the dental structures undergo changes. According to Gustafson (1950) and Thoma (1944) described the age changes occurring in the dental tissues and notes attrition of the enamel, sclerosis of the dentin, denticles in the pulp, deposition of cementum, continuous eruption of the teeth and alternations in the periodontal structures (periodontitis).

After observing ground sections of adult human teeth, he designed diagrams of six changes related to age. The age-related changes are the following:

- Attrition of the incisal or occlusar surfaces due to mastication.
- Periodontitis.
- Secondary dentine.
- Cementum apposition.
- Root resorption.
- Transparency of the root.

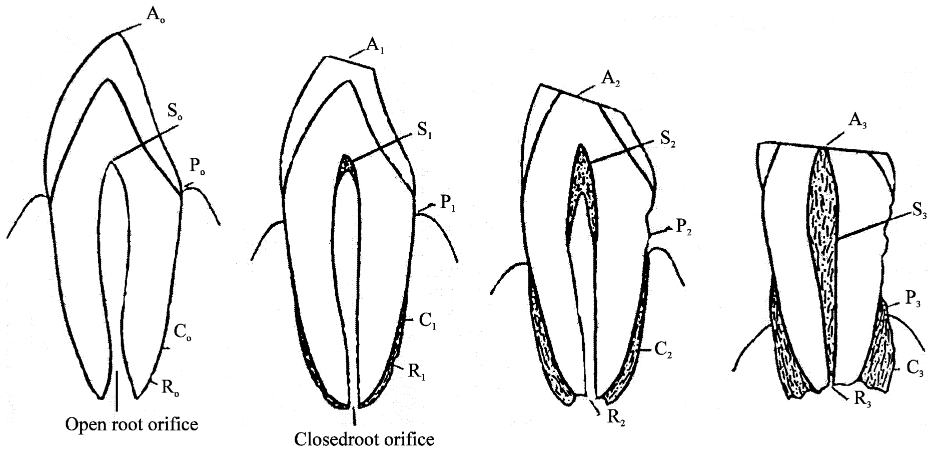
Gustafson suggested the last two changes. In the method proposed, each sign was ranked and allotted 0, 1, 2, 3 points (Fig. 1 and 2); according to degree of development. In forensic dentistry Gustafson's method of age estimation is the best known and most commonly referred to Kvall (2006).

The point values of each age-change are added according to the following formula:

$$A_n + P_n + S_n + C_n + R_n + T_n = \text{points}$$

It was found that an increase in points corresponded to an increase in age and that it was possible to draw a regression line for the correlation between age and points. In order to estimate the age of an individual, the point value is entered in the graph and the corresponding age is found (Fig. 3). The exact equation calculated was: $y = 11.43 + 4.56x$ where, y = age and x = points according to the formula above. The error of estimation as calculated by Gustafson (1950) was ±3.6 years (Gustafson, 1947).

Gustafson's method has for many years been used by forensic odontologists in actual cases. At the same time it has been criticized for a number of reasons. It can not be used in living person, only in dead when extraction of a tooth is allowed (Solheim and Anne, 2006). The assessment of the scores is the result of a subjective evaluation of the changes (Bang and Ramm, 1970). Too many age related changes needed to be considered making the method time-consuming (Maples, 1978). Periodontitis is often impossible to determine due to decomposition of soft tissue (Maples, 1978). One regression line is given for all teeth ignoring eruption time and morphological differences for the various teeth (Maples, 1978; Solheim, 1993). The method assumes that all 6 criteria are age changes of equal importance. Also it ignores any possibility of interrelationship between the criteria themselves ((Bang and Ramm, 1970; Daltiz, 1962; Solheim, 1993). Another problem stated by Solheim (1989) was that the size of the material was small and consisted of only 40 teeth, while many of them originated from the same patient. Since, the variation of age-related changes in teeth from the same individual is evidently less than in



A_0 = no attrition	A_1 = attrition within enamel	A_2 = attrition reaching dentin	A_3 = attrition reaching pulp
S_0 = no secondary dentin	S_1 = secondary dentine has begun to form in upper part of pulp cavity	S_2 = pulp cavity is half filled	S_3 = pulp cavity is nearly or wholly filed with secondary dentin
P_0 = no periodontosis	P_1 = periodontosis just begun	P_2 = periodontosis along first one-third of root	P_3 = periodontosis has passed two-thirds of root
C_0 = normal layer of cementum laid down	C_1 = apposition a little greater than normal	C_2 = great layer of cementum	C_3 = heavy layer of cementum
R_0 = no root resorption visible	R_1 = root resorption only on small isolated spots	R_2 = greater loss of substance	R_3 = great areas of both cementum and dentin affected

Fig. 1: Gustafson's method (1950)-point values

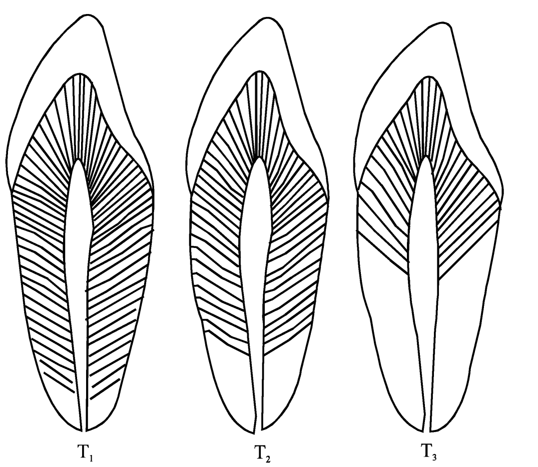


Fig. 2: Gustafson's method (1950). T1 = noticeable root transparency, T2 = root transparency extends over the apical third of the root, T2 = root transparency extends over the apical two-thirds of the root

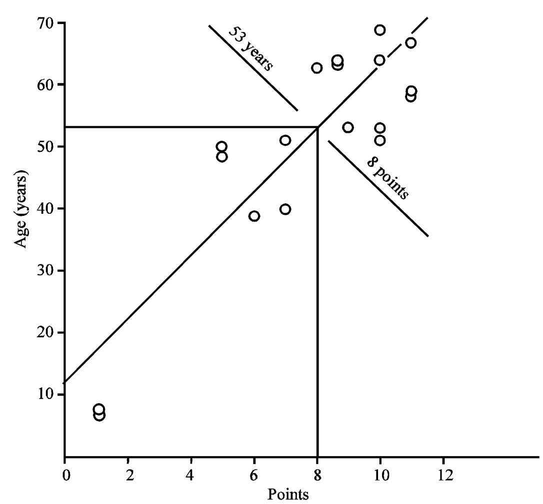


Fig. 3: Relation between point value and age of 19 teeth. Gustafson (1950)

teeth from different individuals, this affects the statistical analysis and contributed to an even more favorable deviation. Also, the regression formula was incorrectly calculated. Maples and Rice (1978) Dalitz (1962), found the new formula:

$$y = 13.45 + 4.26 x$$

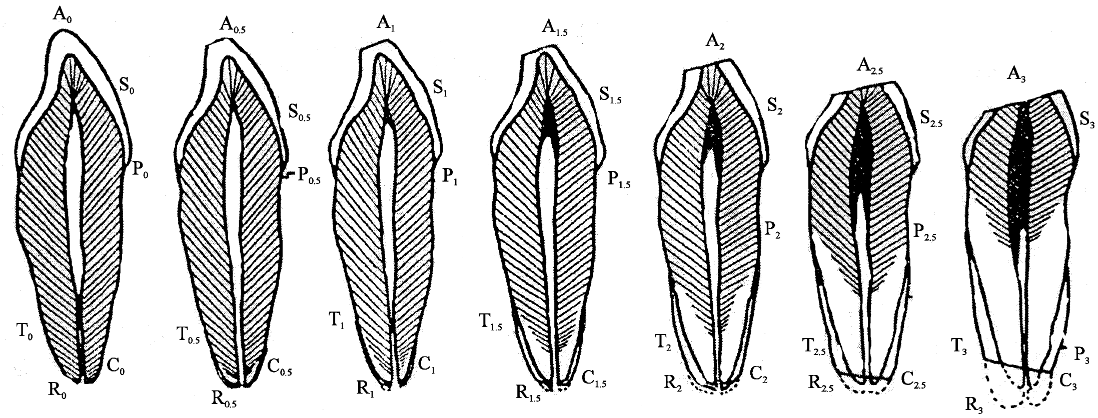


Fig. 4: Johanson's method (1971)

Dalitz in 1962 re-examined Gustafson's method and suggested a 5 point system from 0-4, instead of the 4 point system that was previous used. This change was proposed in order to give a slightly greater accuracy.

His results showed that root resorption and secondary cementum formation could be disregarded. The other criteria, attrition, periodontitis, secondary dentine deposition and transparency of the root of the 12 anterior teeth, are related appreciably to age and to a similar degree. Dalitz suggested that it is preferable to use up to 4 of the 12 anterior teeth from the one individual for age estimation, but if using a greater number of teeth does not necessarily increase the accuracy of the determination. This overruled Gustafson who considered that his error in estimation would decrease when more teeth examined. Dalitz's method results in a standard deviation in age determination of ±6 years.

The regression equation suggested by Dalitz (1962) was:

$$E' = 8.691 + 5.146A' + 5.338P' + 1.866S' + 8.411T'$$

The improvement is that Dalitz used weights for each factor.

One of the problems is that it does not take into account bicuspids and molar teeth. This is critical for the application of the method since in many cases the only teeth left are molars and bicuspids as a result of severe external force Johanson (1971).

Gustafson's technique had been improved first by Dalitz in 1962 and finally by Johanson in 1971. The improvements implemented by Johanson are actually the most appreciated among forensic odontologists. He differentiated for seven different stages (Fig. 4); instead of four originally and evaluated for the same six criteria, mentioned earlier, attrition (A), secondary dentine formation (S), periodontal attachment loss (P), cement apposition (C), root resorption (R) and apical translucency (T). Johanson made a more detailed study of the root transparency and stated that it is more clear when the thickness of the ground section of the tooth was 0.25 mm. The following formula was recommended:

$$Age=11.02+(5.14*A)+(2.3*S)+(4.14*P) + (3.71*C)+(5.57*R)+(8.98*T)$$ (Johanson, 1971)

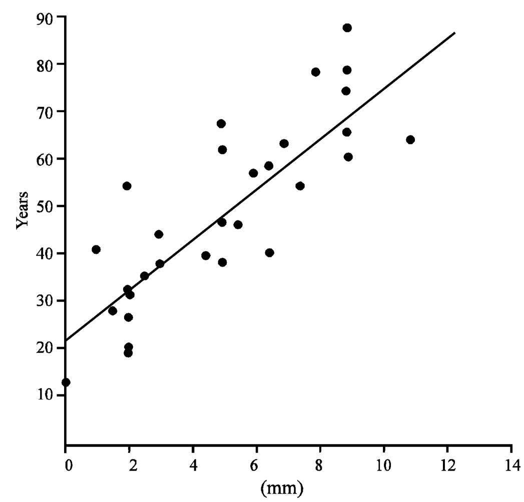


Fig. 5: The extent of root transparency at various ages in intact roots of 31 upper, right lateral incisors. Bang and Ramm (1970)

Bang and Ramm (1970) suggested a totally new approach in age estimation. They found that the root dentine appears to become transparent during the third decade starting at the tip of the root and advancing coronally with age (Fig. 5). This alteration is believed to be caused by a reduction of the diameter of the dentinal tubules caused by increasing intratubular calcification (Vasiliadis, 1981).

The examination of the teeth is done in two ways. First the teeth were examined unhurt and then, 400 μ thick labiolingual sections were cut. The total length of the root was measured bucally in the midline from the cemento-enamel junction to the apex. The transparent root dentin was measured from the apex of the root in coronal direction to the borderline between transparent and opaque dentin (Bang and Ramm, 1970).

From the material collected, they found that it was difficult to make accurate measurements in molars and bicuspids and thus the survey included only incisors and cuspids (Bang and Ramm, 1970).

In spite the difficulties a small number of molars and bicuspids were examined. The mesial and distal roots showed good correlation in the degree of root transparency. However, there was difference between mesial, distal and palatial roots. For practical reasons Bang and Ramm (1970) recommend the exclusion of upper first premolars and all the molars in order to arrive at the best estimation. They also suggest 2 different equations, one when the transparent length is less than or equal to 9 mm and a second when it exceeds 9 mm.

A great advantage of the method is that good results are obtained by measuring intact roots only. The measuring of one factor-degree of root transparency-makes the method simple and rather fast compared to previous methods. It is an objective method because it is not based on a point system but on measurements. It can be applied without previous extensive training or expensive equipment. Also no differences were found between living and dead persons in the degree of root transparency and storage of specimens in 10% neutral formaldehyde caused no significant changes (Bang and Ramm, 1970).

Various studies have been made about the influence of different conditions upon the degree of root transparency. Gustafson (1947) and Nalbandian *et al.* (1960), pointed out that root transparency is less influenced by pathological processes, while Bang and Ramm (2006) found the opposite. Kvaal (2005) states that studies in archaeology show root transparency to be reduced in the presence of metal. Also according to Reppien (2006) root transparency increases in diabetes and drug addicts Maples (1978).

Maples (1978) suggested the use of only 2 criteria of the total 6 Gustafson recommended-secondary dentine formation and root transparency, in order to make the method more simple and accurate.

The results of his research showed that root resorption was negative correlated to age. Elimination of root resorption improved the results and the error of the estimate was reduced 20-30%. Periodontitis was not used because it was difficult to determine it long after soft tissue decomposition. In the same way attrition was excluded because differences among populations were found as a result of diet habits or abnormal occlusion (Johanson, 1971).

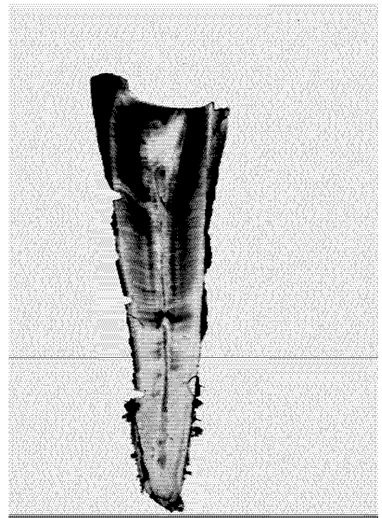


Fig. 6: The extent of root transparency in a 77 year old person (Stavrianos, 2008)

As said before the method requires the scoring of secondary dentin and root transparency. This means that teeth with broken crowns, no evidence of periodontal attachment and lost cementum may still give accurate age estimates. Also the technique can be used on other populations, contemporary and prehistoric, with less fear that dietary differences will decrease the estimation. Since, secondary dentin and root transparency (Fig. 6); are easy to evaluate, observer error may be lessened (Maples, 1978).

For age estimation Solheim (1993) used five of the changes that Gustafson recommended (attrition, secondary dentin, periodontitis, cementum apposition and root transparency) and added another three new changes which showed significant correlation in different types of teeth. The three new age-related changes were surface roughness, color and sex (Solheim, 1993).

Solheim after examining a collection of 1000 teeth, excluding molars came down to good correlation between age and the whole number of changes. He found that mandibular canines and second premolars had the weakest relationship between the parameters and age, so when possible it was recommended to avoid the use of these teeth. Two sets of formulas were presented, one including sex and color and the other without them, because these factors were not always determinable in deceased individuals (Solheim, 1993).

In Solheim's (1993) study there was a variety of origin of teeth (cadavers, forensic cases, living) which might better reflect the biological variation. Compared with teeth from living individuals, teeth removed from deceased bodies were darker, possibly owing to the changes or reactions to the environment after death. The finding indicates the need for cautiousness in using color as a factor in estimating the age of a corpse and this depends upon the condition of the teeth. In order to estimate color a shade guide is needed. Regarding sex as factor depends upon the condition of skeletal remains. The reported formulae are recommended for deceased bodies for identification purposes (unsectioned teeth) and in archaeology (Solheim, 1993).

Radiological methods: Dental radiographs have been used quite recently in dental age estimation methods for adults. Kvaal and Solheim (1994) were the pioneers on this subject. They estimated the age of an adult from measurements of the size of the pulp on full mouth dental radiographs, without tooth extraction and destruction. The size of the dental pulp cavity is reduced as a result of secondary dentine deposit, so the measurements of this reduction can be used as an indicator of age Kvaal and Solheim (1994).

Kvaal and Solheim (1994) presented a method where radiological and morphological measurements are combined in order to estimate the age of an individual (Willems, 2001). Using the radiographs they measured pulp length and width as well as root length and width. Then different ratios between the root and pulp were measured. These ratios were found to be significant correlated with age. They also found inclusion of the length of the apical translucency and periodontical retraction in some types of teeth. The results showed the strongest correlation with age to be in the ratio between the width of the pulp and the root. This may indicate that the rate of deposition of dentine on the mesial and distal walls is more closely related to age than that on the roof of the pulp cavity. However, the correlation between age and the ratios between pulp and the root length was significant for only maxillary cuspids and premolars (Kvaal and Solheim, 1994).

The method is non-destructive (ASFO) and can be applied in living people or dry skeletal material, where single-rooted teeth are often loose in the jaw or can be removed easily (Herschaft *et al*., 2007). It can be employed when the preservation of the material is requested, as in archaeological studies and in forensic investigations. Formulae for premolars showed a stronger correlation with age and this may be an advantage, because these teeth are less prone to damage by trauma or fire and also more often retained in skeletal material. Also the measurements require a fairly short time compared to methods where the teeth have to be sectioned Kvaal (1995).

Kvaal (1995) however proposed a method based totally on radiographic measurements (periapical radiographs) and didn't depend on other factors such as root transparency and periodontical retraction, thus not requiring extraction of teeth. Measurements on dental radiographs may be a non-invasive technique for estimating the age of adults, both living and dead in forensic work and in archaeological studies (Kvaal and Solheim, 1994).

Regression formulae for all six teeth together was proposed and also for each one of the 6 different teeth (11/21, 12/22, 15/25, 32/42, 33/43, 34/44). According to the survey there was a better age estimation when several teeth were included. Maxillary first premolars and molars were excluded because accurate measurements were difficult to perform. In order to compensate for differences because of magnification and angulation on the radiographs, ratios of measurements were used. Also correlation between age and the ratio of tooth to root length was insignificant to all types of teeth, indicating that attrition on the occlusal surface was so weak that it could not be related to age. As in the previous survey the width ratio was found to have a stronger correlation than the length ratio (Kvaal and Solheim 1994).

Bosmans *et al.* (2005) applied the original formulas of Kvaal's technique (1995) using measurements made on panoramic radiographs instead from the typical periapical radiographs as originally described. The age estimations were comparable to those based on the original technique (Kvaal and Solheim, 1994; Bosmans *et al*., 2005).

Kvaal states that when dealing with radiographs several complicating factors are encountered since the curved arch of the jaws is projected on to a flat film thus giving a certain amount of distortion. When periapical radiographs must be taken, the parallel technique should be used, because if the film is at an angle to the root the ratio will be influenced resulting in wrong estimation (Kvaal and Solheim, 1994). For this reason when dealing with orthopantomographs the patient should be correctly positioned to the x-ray machine (Bosmans *et al*., 2005). In many cases the bone overshadowed the apical third of the tooth, so that the width from this area of the tooth could not be measured with sufficient accuracy. This necessitates the use of a stereomicroscope (Willems, 2002). Rotated teeth, teeth with enamel overlap, teeth with restorations, cavities, attrition and periapical pathological processes cannot be used. With all the restrictions mentioned above in the older age groups it was difficult to find patients who retained all the six teeth that were measured in their study (Willems, 2002; Kvaal and Solheim 1994; Bosmans *et al*., 2005).

Vandevoort *et al*. (2004), reported a morphometric method pilot study using microfocused computer tomography (CT) on extracted teeth to compare pulp-tooth ratios in the determination of age. Yang *et al*. (2006) using cone-beam CT scanning acquired the 3D images of teeth in living individuals. Using the 3D images the ratio of pulp/tooth volume can be calculated. Promising results for age estimation based on the pulp/ tooth volume ratio were obtained.

Biochemical methods

Racemization of aspartic acid in human teeth: The biochemical methods are based upon the racemization of amino acids. The racemization of amino acids is a reversible first-order reaction and is relatively rapid in living tissues in which metabolism is slow. Aspartic acid has been reported to have the highest racemization rate of all amino acids and to be stored during aging. In particular, L-aspartic acids are conversed to D-aspartic acids and thus the levels of D-aspartic acid in human enamel, dentine and cementum increase with age. The D/L ratio has been shown to be highly correlated with age (Ohtani *et al*., 1997).

Helfman and Bada (1975, 1976) were the first that reported studies that focused on the racemization of amino acids and obtained a significant correlation between age and ratio of D-/l-enantiomers in aspartic acid in enamel and coronal dentine (Helfman and Bada, 1975, 1976). Ohtani and Yamamoto (1987) showed that even higher correlations were obtainable using longitudinal sections of whole dentine. Ohtani *et al*. (1995) applied the racemization method to cementum, which has higher water content (Brudevold, 1962; Yamamura, 1981).

The racemization rates of the cementum, enamel and dentine samples showed that cementum had the fastest reaction, followed by dentine and then enamel. This may be because cementum is surrounded by periodontal tissue and dentine and possibly has a highly environmental temperature, which would speed up the rate of reaction, as well as a known higher water content. Also the cementum was found to have a relative irregularity of the increase in the D/L ratio compared with that of dentine. As dentine is covered with cementum, enamel and periodontal tissue, it is presumably exposed to a relatively constant environment. In addition, the D/L ratio in dentine is assumed to increase linearly with aging (Ohtani *et al*., 1995). Although, cementum showed the fastest reaction, dentine showed the highest correlation with actual age. The results show that in cementum the racemization reaction proceeds in a relatively constant manner and that cementum is, like dentine, a tissue that has low metabolism. As a result the racemization method with cementum is, as with dentine, sufficiently useful for precise age estimation (Ohtani *et al*., 1995).

Ritz *et al*. (1995) used the racemization method in dentinal biopsy specimens in order to estimate the age of living individuals. This method emerged from the need to identify the age of living individuals without extracting teeth. In Germany for example extraction of a tooth exclusively for age estimation when it is not medically indicated is regarded as ethically and legally problematic. The results were hopeful and showed a close relationship between the extend of aspartic acid racemization in dentinal biopsy specimens and age, thus facilitating age estimation. However, for accurate results the performance of biopsies must occur under strictly standardized conditions (Ritz *et al*., 1995).

The specimens taken by the biopsy technique were approximately 1mm in diameter and 1 mm long. Specimens were taken from posterior teeth (molars). These amounts of dentine proved sufficient for the determination of the extend of aspartic acid racemization in all cases. The cavities were then treated with conventional filling materials. The only cases in which the technique was not applicable were in teeth with extensively destroyed crowns. In these cases, the biopsies had to be taken in deeper dentine layers, often close to filling materials or carious lesions (Ritz *et al*., 1995).

As said before, in order to use the formulas proposed by Ritz *et al*. (1995), the biopsies must be taken from a specific region of the tooth. The study showed that the biopsy layer had a significant influence on the results, since the extension of aspartic acid racemization is higher in deep layers (Ritz *et al*., 1995).

The biopsy technique is a low-risk procedure that causes only minor discomfort to the affected person (Ritz *et al*., 1995).

STEPS OF AGE ESTIMATION

Kvaal (2006) describes the approach using different methods in order to reach to relatively accurate estimation.

Visual assessment: Initially a gross "clinical" examination ought to be performed which include the condition of the soft tissues as well as the dentition. Dental attrition, tooth colour and stains, periodontal status as well as quantity and quality of dental restoration may be employed (Willems *et al*., 2002; Kvaal, 2006; Reppien *et al*., 2006;

Solheim and Anne, 2006). From this visual impression an experienced dentist may give a good estimate of chronological age.

Radiographs: Periapical radiographs or orthopantomographs (OPG) will give additional information in the size of the pulp. In cases of fragile tissue e.g. burnt bodies or skeletal remains from archaeological excavations the radiographs ought to be made with minimal handling of the remains to avoid further destructions of tissue (Kvaal, 2006).

Extraction and preparation of single teeth: Age estimation methods that cause irreversible destructions of tissues are used last. Different methods require single intact teeth, half sectioned teeth, or ground sections (Kvaal, 2006).

It is recommended that age is calculated using preferable two independent scientific methods (Willems, 2006; Kvaal, 2006; Solheim and Anne, 2006). This may either be one method using the whole dentition, selected teeth from the dentition or the same method applied to two or more teeth from the same dentition (Kvaal, 2006). In all cases repetitive measurements should be made in order to verify the reproducibility of the calculations performed (Willems, 2006). The final age estimate ought to be based on the results of the methods and the initial visual age assessment (Kvaal, 2006; Solheim and Anne, 2006). To this direction the following software program was developed.

Dental age calculating software (Willems, 2000). developed a software program in order to automate dental age calculations. The program is named “Dental Age Estimation”. It includes the most accurate and often referenced morphological and radiological techniques that are reported in literature and which demand extensive calculations, Bang and Ramm’s (1970), Johanson’s (1971), Solheim’s (1993), Kvaal and Solheim’s (1994) and Kvaal’s (1995).

The great advantage of the program is the immediate dental age estimation results and the avoidance of calculating errors. All that is needed is to measure the required parameters and enter their values into the program. Also it enables the forensic odontologist to apply different techniques and not to stick to one age estimation technique thus providing a more reliable result.

DISCUSSION

The estimation of chronological age in living human beings and dead persons has been performed by forensic dentists for almost more than 50 years. During the last years, numbers of papers were published analyzing the various methods applied and comparing the accuracy and reproducibility of each one of them.

An optimum method for age determination in living individuals should fulfill the following conditions: age determination in all age groups and the procedure must not impair the health of the affected person (Ritz *et al*., 1995). Tooth development and the sequence of eruption have been used extensively as a method of age estimation in children and adolescents (Herschaft *et al*., 2007).

In adults, following completion of the growth period, age estimation by morphological methods becomes difficult -and particularly when nondestructive methods are used- it is not sufficiently accurate (Willems, 2006; Solheim and Anne, 2006; Ritz *et al*., 1995; Hongwei, 1989; Kambe *et al*., 1991; Drusini, 1993; Mincer *et al*., 1993). Previously the methods of choice in adults were, theoretically, the method according to Gustafson (1950) and its modifications. These techniques are destructive, need extracted teeth and can be used only in dead (Solheim and Anne, 2006). Gustafson’s optimistic standard deviation of ±3.4 years has never been confirmed (Gustafson, 1970; Solheim and Anne, 2006). Also Johanson’s standard deviation of ±5.6 years seems to be too optimistic (Solheim and Anne, 2006; Johanson, 1971). Investigation has shown that a standard deviation of around ±10 years is normal for most methods (Solheim and Anne, 2006; Solheim and Sundnes, 1980), while others report that these methods have 95% confidence intervals of approximately ±12 years at best (Daltiz, 1962; Endris, 1979). The formulae are generally most accurate around 40-50 years and with increasing inaccuracy in younger and especially in older age groups. Also another difficulty is that there is a pronounced tendency for overestimation of younger persons and underestimation of older persons (Solheim and Anne, 2006).

For most methods of age calculation one regression formula for all types of teeth is given. This is inaccurate because teeth emerge at different times. Also the contribution of each tooth in chewing varies and thus the extended changes. So, according to Solheim (2006) the best method should have one formula for each type of tooth (Solheim and Anne, 2006). Some methods based their results from teeth collected from the same individual. Since, the variation of age related changes is less than in teeth from different individuals, this may have contributed to a more favorable deviation (Solheim and Anne, 2006; Solheim, 1993).

In living individuals ethical and legal regulations must be considered, so it is not possible to extract teeth unless a valid medical reason for removing a tooth exists

Table 1: Recommended dental age estimation procedures in adults (ASFO, 2007)

Status	Examination type	Specific techniques or methods
Living	Radiographs/Morphological	Kvaal and Solheim (dental radiographs)
	Extracted Tooth-Biochemical	Aspartic Acid Racemization
	Post-Formation Changes	Johanson Sectioning
	Post-Formation Changes	Lamendin *et al.* (1992)
	Post-Formation Changes	Bang and Ramm
Deceased	Biochemical	Aspartic Acid Racemization
Anthropological/Historical Collections		
Skeletal	Non-Destructive	Kvaal/Solheim Intact Methods

(Willems *et al.*, 2002; Herschaft *et al.*, 2007; Kvaal *et al.*, 1995; Solheim and Anne, 2006; Ritz *et al.*, 1995; Yekkala *et al.*, 2006). Thus the techniques are limited to radiological complemented by visual examination (Willems *et al.*, 2002; Herschaft *et al.*, 2007). For deceased persons where no permission was granted for tooth sectioning , both radiological techniques mentioned and an estimation based on the length of the translucent zone of the root apex (Bang and Ramm, 1970) are techniques that can be used (Table 1); in addition to visual age estimation (Willems *et al.*, 2002).

On the other hand biochemical age determination procedures based on aspartic acid racemization in dentin have been demonstrated to be highly reproducible and accurate: 95% confidence intervals of approximately ±3-8 years are described (Herschaft *et al.*, 2007; Ohtani *et al.*, 1995; Ritz *et al.*, 1995; Ogino *et al.*, 1985; Ohtani and Yamaoto, 1987, 1991, 1992; Ritz *et al.*, 1990). Ohtani *et al.* (1997) investigated the influence to racemization rate in dentine from teeth stored in fixatives and found that age was almost identical determined like at the time of extraction. An influence of iatrogenic and carious lesions on the composition of the surrounding dentin and thus on aspartic acid racemization cannot be excluded (Ohtani *et al.*, 1997). Hence, teeth with extensive crown destruction are not suitable for age determination based on the extent of aspartic acid racemization in biopsy specimens (Ritz *et al.*, 1995). In deceased individuals, investigators must consider the post mortem environment from which the body was recovered since the D/L conversion rate may be influenced by elevated temperature (Herschaft *et al.*, 2007). However, studies have shown that there was no difference in accuracy regarding post-mortem circumstances in w hich bodies where found (Reppien *et al.*, 2006). The technique requires relatively complex chromatography capability. Gas chromatography (GC) has been most often used (Herschaft *et al.*, 2007).

During the last decade, aspartic acid racemization rate is used even more in chronological age determination and seems to be the method of choice for the next years (Yekkala *et al.*, 2006).

Even though the morphological and radiological techniques have bigger confidence intervals than biochemical techniques, recent studies showed that they are still applicable. Given the fact that Gustafson/ Johanson is a method from 1950 (reviewed in 1971) it only underlines that teeth are consistent in their morphology (Reppien *et al.*, 2006).

CONCLUSION

Age estimation from human teeth is well established. Different techniques and numerous studies have been published for age estimation, each one demonstrating various accuracy, precision and reliability. In all cases reproducible and reliable estimation results are possible when the appropriate methods for each case are properly applied and used. Error is present in every approach. For this reason when investigating a case the forensic odontologist should apply different techniques available and perform repetitive measurements and calculations in order to reach to reliable conclusion. Research into age estimation is ongoing. Forensic odontologists should continually watch the scientific presentations and journals that report new developments and validate or challenge existing techniques.

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