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DENTAL AGE ESTIMATION THROUGH TEETH AND CEMENTUM ANNULATIONS: A LITERATURE REVIEW AND UPDATE

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ABSTRACT Teeth can survive for several thousands of years and can reflect age changes from the cradle to the grave. Teeth have been used for age determination in several ways, of which, estimation of cementum annulations is an important technique. Cementum formation is a continuous process that occurs throughout the life of humans and animals. Hence, with age, the cementum increases in width. During this formation, hypermineralized layers of extracellular matrix alternate with less mineralized layers. These changes in cementogenesis result in incremental lines. These incremental lines, or annulations, are similar to the macroscopic visible annual rings that are commonly seen in trees and are useful for estimating the age at death. This article reviews the use of human teeth in general and human tooth cementum annulations for age estimation.

KEYWORDS : age estimation, cementum annulations, dental age, forensic odontology, incremental lines

INTRODUCTION

Forensic odontology is that branch of dentistry which, in the interest of justice, deals with the proper handling and examination of dental evidence, and with the proper evaluation and presentation of the dental findings (Sharma, 2010). The forensic odontologist assists legal authorities by examining dental evidence in different situations (Avon, 2004). In forensics and anthropology, one of the most important issues addressed is the estimation of age at death for the identification of human remains (Gonzalez et al.,2007). In the last couple of decades, the number of unidentified cadavers and human remains as well as the number of cases lacking age documentation and therefore requiring age determination has increased considerably(Gunst et al.,2003). A number of medico-legal reasons exist for determining the age of an individual Leung, 2008). Age estimation of individuals or victims narrows the search for ante-mortem data during the identification process of unknown remains, helps in establishing the difference between juvenile and adult status of an individual in law cases, and aids persons without a birth certificate finding out their presumed age (Thevissen et al.,2009). Teeth offer an attractive source of information with regards to age determination due to their long survivability (Cook et al., 2006).

VARIOUS METHODS OF AGE ESTIMATION FROM TEETH

Teeth can survive for several thousands of years and can reflect age changes from the cradle to the grave (Li and Gi , 1995). Appreciable age changes in teeth include attrition, periodontal disease, deposition of secondary dentin, root translucency, cementum apposition, root resorption, color changes and increase in root roughness(Singh et al., 2004). Various methods have been utilized for age estimation from teeth which include- visual method, radiographic method, histological method, computer assisted method and comparison with ante-mortem data (Shamim et al., 2006). The process of estimating age from teeth is relatively simple in young people and can be made from radiographs or sections through developing teeth (Xiaohu et al., 1992). In adults, dental age prediction is usually done most commonly using-Gustafson's parameters(Whittaker, 1992), dentinal translucency (Babshet et al., 2010) and cementum annulations (Bojarun et al., 2003).

Gustafson G was the first person to bring out a method of age estimation in 1950, based on six characteristics of teeth- degree of attrition, position of epithelial attachment, amount of secondary dentin, thickness of cementum, degree of root resorption and translucency of root dentin(Gustafson 1950). Targeting increased precision, modifications of Gustafson's technique were proposed by Kilian J in 1986 and Kashyap VK & Koteswara Rao NR in 1990 (Vystrcilova and Novotny 2008). In 1992, Lamendin H *et al*. used two of Gustafson's six characteristics, i.e. position of epithelial attachment and translucency of tooth root, in order to estimate age at death (Lamendin et al.,1992). In 2002, Prince DA & Ubelakar DH modified the method of Lamendin H *et al*. and obtained more precise age estimations creating formulas for separate sexes and different ancestries (Prince and Ubelaker, 2002). In 2007, Gonzalez-Colmenares G et al. compared Lamendin's and Prince & Ubelakar's methods in a Spanish Caucasian population and found that the latter method was more accurate (Gonzalez et al., 2007). Metska E *et al*. extensively investigated root dentin translucency for age estimation and concluded that root dentin translucency of single-rooted teeth is the only parameter giving accurate results for age estimation and that there is a lack of uniformity in dentin translucency among the roots of the same molar tooth (Metska et al., 2009).

Stages of tooth development and eruption, structural changes on teeth and the changes in the chemical composition of teeth can help determine dental age (Nambiar, 1995). In children and adolescents, age can be estimated by means of development and eruption of deciduous and permanent teeth (Ajmal, 2001). Because of the presence of a multitude of developing teeth, the accuracy of dental-age estimation in children is much greater compared to adults (Willems et al., 2002). In 1998, Whittaker DK *et al*. orthodontically reconstructed the illustration of a victim of murder in which the victim's age was determined to be between 15 years and 17 years based on the fact that the skull radiographs revealed the presence of partially developed and unerupted third molars and slightly open apices on the lower second molars (Whittaker et al., 1998). Tooth color can also be used as an age indicator. Ten Cate AR *et al*. reported that using root dentin color as an indicator, most teeth can be estimated within ±10 years of chronological age (TenCate et al., 1977). According to de las Heras SM *et al*., determination of dentin color by spectroradiometry is a potentially useful objective method to estimate age in forensic studies in combination with other methods (Heras et al., 2003).

Dental radiographs can be used for age estimation in certain cases. The method of Demirjian *et al*., which is most commonly used in estimating the chronological age of children, is based on orthopantomograms (Demirjian et al., 1973). Kvaal & Solheim presented a method which combined radiological and morphological measurements. These measurements involved extraction of teeth (Kvaal and Solheim et al, 1994). Kvaal *et al*. later reported a method based on radiological measurements that did not require tooth extractions (Kvaal et al., 1995). Bosmans N *et al*. reported that comparable results were obtained by applying Kvaal's technique on orthopantomograms instead of the typical periapical radiographs (Bosmans et al., 2005). Bosmans Olze A *et al*. used variations in radiographically determined DMF index to estimate the age of unaccompanied minors. However, this method was deemed unsuitable (Olze et al., 2006). Cameriere R *et al*. estimated age from radiographs using pulp/tooth area ratio in canines (Cameriere et al., 2009). The correlation between the reduction of coronal pulp cavity and chronological age was also shown as a potential method of age estimation by Drusini AG (Drusini, 2008). The chronological age of individuals was estimated based on the dental developmental stages of

third molars evaluated on orthopantomograms by Mesotten K (Mesotten, 2002) and Thevissen PW (Thevissen *et al*., 2010). Vandevoort FM *et al*. calculated volume ratio of pulp versus tooth volume, in order to estimate dental age, using X-ray microfocus computed tomography (Vandevoort et al., 2004). Yang F *et al*. used a custom-made voxel counting software and successfully estimated dental age by calculating the ratio between pulp canal versus tooth volume based on cone-beam CT tooth images (Yang et al., 2006).

Racemization of aspartic acid from human dentin has been used in the estimation of chronological age (Ohtani and Yamamoto, 1992) (Ohtani and Yamamoto, 1991). Yekkela R *et al*. demonstrated the efficiency of high performance liquid chromatography (HPLC) coupled with fluorescence detection for dentin-aspartic acid racemization (Yekkela et al., 2006). Ohtani S studied age estimation using racemization of aspartic acid in cementum and concluded that accurate estimation of age is practically possible using the amino acid racemization method for cementum (Ohtani, 1995). de las Heras SM *et al*. introduced the determination of deoxypyridinoline in human dentin extract as a reliable tool to estimate human dental age (Heras et al., 1999). The determination of Gelatinase A in human dentin is also a useful marker for age estimation (Heras et al., 2000). Rai B *et al*. (Rai et al., 2006) and Bocutoglu O & Yakan B(Bocutoglu and Yakan, 1997) suggested that the coronal displacement of cementum, which occurs with age, can be used to estimate an individual's age. Solheim T indicated that, for some types of teeth, the cementum thickness gives a significant contribution to statistical methods of age assessment (Solheim, 1990).

CEMENTUM AND CEMENTUM ANNULATIONS

Cementum is the calcified tissue of continuous apposition that surrounds the root portion of dentin and forms the attachment site for the periodontal fibers that links the tooth to the alveolar bone (Aggarwal et al., 2008) (Dias et al., 2010). Formation of cementum is a continuous process that occurs throughout the life of humans and animals. Hence, with age, the cementum increases in width (Huttner et al., 2009). It has been shown to triple in thickness between the ages of 20 and 60 years. The maximum thickness is seen at the apex and the minimum near the cementoenamel junction (Pundir et al., 2009). During this process of formation, hypermineralized layers of extracellular matrix alternate with less mineralized layers (Wittwer et al., 2004). The changes in cementogenesis result in incremental bands that are correlated with seasonal growth in many species (Lieberman, 1994). These incremental bands, or tooth cementum annulations (TCA), are similar to the macroscopic visible annual rings that are commonly seen in trees (Wedel et al, 2007). Usually, cellular cementum increments are useful for estimating the age at death and the acellular bands are useful to estimate the season of death (Lieberman, 1994) (Wedel et al, 2007).

METHOD OF COUNTING TCA

Incremental lines in cementum can usually be observed in decalcified or undecalcified sections by using toluidine blue (Pundir et al., 2009), cresyl violet (Naylor et al., 1985), hematoxylin (Condon et al., 1986), alizarin red (Charles et al., 1986), or periodic acid-Schiff (Pundir et al., 2009) stains under conventional light microscopy. Obertová Z & Francken M used unstained sections for TCA analysis (Obertová and Francken, 2002). Sousa EM *et al*. compared age estimates from cementum annulations in three groups- unstained, stained with Villanueva's blood stain, and stained with acridine orange. Results obtained from unstained sections provided the most countable lines (Sousa et al., 1999).

The method commonly followed for counting TCA is the one specified by Stott GG *et al*. in 1982 (Stott et al., 1982). A low-speed saw with a precision diamond wafering blade was used to make cross-sections of tooth. Sections were undecalcified. After histological preparation, these sections were photographed and the TCA were counted either manually or through a 10x magnifier. Aggarwal P *et al*. studied longitudinal ground sections of teeth for counting the TCA instead of cross-sections. They indicated that polarizing microscopy is superior to light microscopy in visualizing TCA (Aggarwal et al., 2008). Pundir S *et al*. also used longitudinal ground sections to study TCA and demonstrated that the incremental lines are best viewed through phase-contrast microscopy compared to polarizing microscopy and light microscopy (Pundir et al., 2009). Avadhani A *et al*. compared transverse and longitudinal sections for counting TCA and found that transverse sections are better than longitudinal sections (Avadhani et al., 2009). Kaur I *et al*. recommended that incremental lines in human dental cementum are best viewed under polarizing microscope and the visibility can be further enhanced by adding different imbibing media like quinoline (Kaur et al., 2011). Rao NG & Rao NN counted cemental annulations by light microscope using CCTV Screen, at the junction of cervical with middle third of root. They determined age by adding the eruption age in years of tooth in study to the annulations counted. This was found to be matching with actual age almost to an accuracy of ±1-2 years (Rao and Rao, 1998). Lipsinic FE *et al*. stated that there is a correlation between the number of incremental lines in cementum and age, and with a large enough specimen size, a computer-generated formula for age prediction may be possible (Lipsinic et al., 1986). Czermak A *et al*., in 2006, developed software for the automated evaluation of TCA images in order to substitute manual counting and save time. They also felt that this software could reduce human error and supply consistent and reproducible results (Czermak et al., 2006). The advancement of digital photography today has made easy the process of acquiring images of the incremental lines and further enhancing them, thus increasing image quality and precision.

EVIDENCE FOR AGE ESTIMATION FROM TCA

Stott GG *et al*. stated that TCA can provide a close estimate of the actual age of individuals from which the teeth are acquired (Stott et al., 1982). Naylor JW *et al*. recommended the enhancement of TCA as a method of age determination in man (Naylor et al., 1985). The age thus determined in an Indian population by Avadhani A *et al*. in 2009 varied about 2-3 years from the actual age of the patient and the reliability of the method was 94.73% (Avadhani et al., 2009). According to Condon K *et al*., inaccuracy of the TCA technique for age estimation is 6 years, with a bias of 0.26 years. Also, the technique provides significantly better estimates for females than for males (Condon et al., 1986). Jankauskas R *et al*. compared age estimation by incremental lines of dental cementum with other methods such as endocranial suture ossification, pubic symphysis morphology and the "combined" method of Nemeskéri in a European population. All four methods yielded a similar correlation and it was concluded that the incremental lines have a similar use as other common methods (Jankauskas et al., 2010). Stewart REA *et al*. compared counts of growth layer groups of dentin and cementum in ringed seals (Phoca hispida) and found that the growth layer groups in cementum provided higher counts and were more reliable than those in the dentin (Stewart et al., 1996). This finding may also be extended to the TCA in humans.

Wittwer-Backofen U *et al*. studied a German population and stated that the TCA technique is a reliable method for estimating a subject's age. Error bounds for age estimates did not exceed 2.5 years. Sex differences, intra-individual correlations, and the effects of periodontal disease had no quantitative effect on the number of TCA bands (Wittwer et al., 2004). Kagerer P & Grupe G, also in a German population, found that the annual production of incremental lines was not influenced by carious lesions, functional disorders, occlusal disturbances, prosthetic pre-treatments, hypercementosis, general disease or even previous pregnancies (Kagerer and Grupe, 2001).

Obertová Z & Francken M proposed that TCA counts should be used for age estimation only in association with macroscopic examination as a considerable underestimation of age occurs in German individuals older than 40 years (Obertova and Francken, 2009). Meinl A *et al*. compare the accuracy, precision, and bias of three age-at-death estimation methods- Lamendin *et al*. (LAM) method, Bang and Ramm (BR) method, and TCA method in a Central European population. TCA method was found to be most accurate in all age groups. LAM method displayed the highest precision in the young and the old age group whereas TCA method was more precise in the middle age group (Meinl et al., 2008).

EVIDENCE AGAINST AGE ESTIMATION FROM TCA

Negative results have also been reported in relation to the use of TCA for age estimation. In 1982, Phillips CJ *et al*. found that incremental lines in dentin and cementum were highly variable and did not correspond to age. However, this study was conducted in bats and was not a human study (Phillips et al., 1982). Miller CS *et al*. in 1988 studied an American population and stated that determining chronologic age in humans from cemental annulations is not possible (Miller et al., 1988). In 1994, Stein TJ and Corcoran JF estimated pararadicular cementum deposition as a criterion for determination of age in an American population and indicated that the quantization of TCA is a only a moderately reliable means for age estimation (Stein and Corcoran, 1994). These negative reports caused due to the

inconsistency in counting TCA may be attributed to the lack of advanced histological techniques during that period. However, in 2006, Renz H & Radlanski RJ studied a European population and reported that a tooth differs markedly in the number of TCA counted in different sections as well as in different regions of the same sections and there is immense difficulty in counting reproducible line numbers in the same cementum area at repeated counts. Hence, they do not recommend counting annulations in root cementum as a method for determining the age of human teeth (Renz and Radlanski, 2006). In 2010, after studying an Indian population, Kasetty S *et al*. reported that estimation of cementum thickness and cementum annulations is not sufficiently accurate for the estimation of age. They listed the problems encountered during the counting of cementum annulations- (1) Incremental lines are not always seen as distinct lines as there is incomplete separation of lines, (2) There is variation in thickness of lines, (3) Same lines in deeper planes may get projected as another line, (4) Resorption of cemental surface reduces thickness of cementum and (5) Cemento-dentinal junction is not always distinct and so the lines here are not distinct (Kasetty et al., 2010). It was also revealed in a Brazilian study by Dias PEM *et al*. that age estimation by counting TCA is reliable only in periodontally sound teeth and cannot be applied to periodontally diseased teeth (Dias et al., 2010).

Another important area where estimation of TCA may have a major role to play is the estimation of age in archaeological populations. In 2009, Roksandic M *et al*. studied TCA in adults from the Iron Gates Gorge Mesolithic/Neolithic series. The results pointed to several errors and it was suggested that the effect of taphonomy on dental histological structures needs to be researched before applying TCA for age estimation in archaeological populations (Roksandic et al., 2009). Not many studies have been conducted in this field and a lot of research is needed before a high degree of accuracy can be attained.

CONCLUSION

The forensic dentist today has a major role to play in the identification of unidentified individuals who cannot be identified by other means. Age estimation is an integral part of this identification. Of the several methods of age estimation from teeth, counting the number of TCA is a frequently used method. In spite of the few negative reports published, majority of the studies which have been done till date support this technique. In the present day, advanced equipment and techniques have been developed which make the estimation of TCA easy. Precision, applicability and reproducibility are the center of focus and continuous research is on in order to make this process more easy and cost-effective. The future might see "TCA estimation" as the most commonly and routinely used technique for age estimation in unidentified dead individuals.

Studies conducted on TCA analysis as a tool for age estimation in humans

Year	*Author(s)*	*Purpose of Study*	*Population*	*Result*
1982	Stott GG *et al*.[58]	Cemental annulation as an age criterion in forensic dentistry	American	Positive
1985	Naylor JW *et al*.[53]	Cemental annulation enhancement technique for age determination in man	–	Positive
1986	Condon K et al.[54]	Estimates and accuracy of cementum annulation for age determination in Homo sapiens	American	Positive
1986	Lipsinic FE *et al*.[62]	Correlation of age and incremental lines in the cementum of human teeth	–	Positive
1988	Miller CS *et al*.[69]	Failure of use of cemental annulations in teeth to determine the age of humans	American	Negative
1994	Stein TJ & Corcoran JF[70]	Pararadicular cementum deposition as a criterion for age estimation in human beings	American	Negative
1998	Rao NG & Rao NN[61]	CCTV analysis of cemental annulations in determining age	Indian	Positive
1999	Souse EM *et al*.[57]	Determination of age from cemental incremental lines for forensic dentistry	–	Positive
2001	Jankauskas R et al.[64]	Incremental lines of dental root cementum for biological age estimation	European	Positive
2001	Kagerer O & Grupe G[66]	Age at death diagnosis and determination of life history parameters by incremental lines in human dental cementum	German	Positive
2004	Wittwer-Backofen U et al.[50]	Tooth cementum annulation for age estimation	German	Positive
2006	Czermak A *et al*.[63]	New method for automated age-at-death evaluation by TCA	German	Positive
2006	Renz H & Radlanski RJ[71]	Incremental lines in root cementum of human teeth as a reliable age marker	European	Negative
2008	Aggarwal P *et al*.[46]	Role of incremental lines of root cementum of human teeth in age estimation using polarizing microscopy	Indian	Positive
2008	Meinl A et al.[67]	Comparison of the validity of three dental methods for the estimation of age at death	European	Positive
2009	Avadhani A et al.[59]	Reliability of TCA for age estimation	Indian	Positive
2009	Obertová Z & Francken M[56]	Accuracy and applicability of tooth cementum annulation method	German	Positive
2009	Pundir S *et al*.[49]	Estimation of age based on tooth cementum annulations using three different microscopic methods	Indian	Positive
2010	PEM *et al*.[47]	Age estimation from dental cementum incremental lines and periodontal disease	Brazilian	Negative
2010	Kasetty S *et al*.[72]	A polarized light and stereomicroscopic study of dental cementum in age estimation	Indian	Negative
2011	Kaur I *et al*.[60]	Efficacy of age estimation in forensic dentistry using cemental annulations as a criteria	Indian	Positive

Positive – In favor of using TCA analysis for age estimation
Negative – Not in favor of using TCA analysis for age estimation

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