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A Comparative Review Analysis of Age Estimation Using Annulations in Cementum and Secondary Dentin

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ABSTRACT:

Forensic age estimation plays one of the key roles in the unknown human remains identification. There are numerous odontological methods to estimate the same. Analysis of incremental lines in cementum and dentin has emerged as a non destructive and reliable approach . This paper discusses the review of studies showing the evolution of studies in dentin and cementum using light microscopy along with the advancement in this regards.

Introduction

Age estimation is a crucial component of forensic science, playing a significant role in the identification of unknown individuals in medico-legal cases. Among various biological tissues, teeth are considered one of the most reliable indicators for age determination due to their resistance to environmental changes, decomposition, and physical damage. Dental tissues such as enamel, dentin and cementum preserve structural and incremental changes throughout life, making them valuable tools in forensic investigations. Among these, dentin and cementum are of particular importance as they exhibit incremental growth lines that can be correlated with chronological age.

Review of Literature

In 1950 Gustafson G. studied age-related changes in teeth and analyzed six factors, including secondary dentin deposition and root transparency, laying the groundwork for forensic odontology, that have been used as age determinants and even dental histological techniques contribute to age determination. The choice to use teeth for age determination is well accepted due to their longevity and ability of being resilient to change. Physiological or biological aging is in many cases not related to chronological aging; therefore, a biological marker independent of any environmental alteration is needed to provide information about the age of an individual. One such tool is secondary dentin, also supported by Gustafson.[1]

Nalbandian J. et al. (1960) study provided insights into the appositional growth of cementum and dentin. By correlating cementum layers with aging, the authors emphasized the utility of light microscopy in forensic age estimation.[2]

Boyde A. (1963) Boyde highlighted the biological basis of incremental lines in enamel and dentin, marking the first step toward quantifying these structures for age estimation. His work demonstrated the regularity of incremental growth patterns and their potential for chronological studies.[3]

Maples WR. Maple & Burns (1978) reported an improved technique of using dental histology for age estimation using multiple regression analysis of Gustafson's parameters.[4]

Stott G. et al. (1982) proposed cementum annulations as a reliable forensic tool for age estimation. Their

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methodology emphasized counting incremental lines using light microscopy, setting a precedent for future quantitative studies, focuses on the use of tooth cementum annulations for age estimation, laying the groundwork for later research. It highlighted the stability of these lines under various environmental conditions, making them a reliable method for age determination in both fresh and historical samples.[5]

Naylor JW, Miller WG, Stokes GN, Stott GG. (1985) conducted a study on teeth obtained from cadavers revealed that Cemental annulations are generally difficult to count, especially when they are numerous and close together. 100-micron thick sections used were strong enough to handle while processing and yet thin enough for light microscopic examination. Sectioning of teeth without decalcification was preferred as decalcification may require days or weeks depending on the solutions used and if not watched may dissolve the specimen. Thus, strict protocol of section taking, cleaning, and staining is essential to make the Cementum annulations appreciable and thus relate to the age of the subject.[6]

Condon K, Charles DK, Cheverud JM, Buikstra JE. (1986) Demineralized longitudinal sections of 7 µm thickness of 80 freshly extracted premolars from the individuals of age ranging from 11 to 70 years were studied. The sections were stained with hematoxylin and observed under light microscope. Cemental annulations were counted and added to the eruption age of the tooth. 78% correlation was found between chronological age and the estimated age with the mean error of 6 years. The study concluded that cementum annulation was the most reliable aging criterion in adults.[7]

Charles D. K. et al. (1986) Charles introduced standardized methods for visualizing and quantifying cementum lines, improving consistency in forensic investigations. The study also addressed potential errors due to environmental and pathological factors.[8]

Solheim T. (1990) focused on dental cementum apposition as a critical factor for age estimation. He developed a technique combining light microscopy and staining methods, improving accuracy in forensic applications.[9]

Solheim T. (1993) studied 1000 teeth by making ground sections & he gave the formula for age estimation using only secondary dentin as the parameter. Maple used a total of 355 teeth obtained from a Florida dental clinic. Longitudinal sections of approximately 250 to 350 rm were cut with a diamond-blade thin-section saw. Root transparency was the best followed by secondary dentin, attrition, paradentosis followed by cementum. Since secondary dentin and transparency are two of the easiest to evaluate of all the other changes, observer error may be lessened[10]

Stein TJ, Corcoran JF. (1994) Similar study conducted on longitudinal sections of thickness 500µm of 52 non- restorable teeth including lower bicuspids & central incisors extracted from 42 individuals of age ranging from 27 to 84 years with a mean of 61 years, indicated that quantitation of cementum annulation was a moderately reliable means of age estimation in humans. Age prediction showed greater divergence from actual age in person older than 55 years .[11]

Jagannathan N, Neelakantan P et al. (1995) The apposition of secondary dentin leads to a gradual reduction in size of the pulp chamber and can affect the obliteration of the root canal. No influence of periodontal recession on secondary dentin formation was found. Hence, its amount can be used to estimate the age of an individual. Various methods have been developed to study the size of the pulp chamber, including tooth cross sections and radiographs. Many studies have shown that with advancing age, the size of the dental pulp cavity is reduced because of secondary dentin deposition. The assessment of pulp/tooth area ratio and pulp/tooth volume ratio are methods to indirectly quantify secondary dentine deposition.[12]

Miller CS, Dove SB, Cottone JA. (1997) The reliability of cemental annulations count in teeth was examined to determine its value as an age indicator for humans in 100 extracted teeth using ground sections. The age of the specimen was determined by adding the number of annulations counted by the observers & the eruption age of the specific tooth that was used. The data analyzed by simple regression indicated that determining chronologic age in humans from cemental annulations was possible.[13]

Singhal A et al. (2001) After attaining maturity, teeth undergo changes making age estimation possible

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among adults mainly by visual examination, radiographic methods, and structural changes in teeth and by means of chemical methods. Accurate estimated age helps the search and enables a more efficient and time saving approach to individual identification.[14]

Kagerer P, Grupe G. (2001) Amongst the four types of cementum, the acellular extrinsic fiber cementum is located around the cervical and middle third of teeth and serves to anchor the teeth in the alveolar socket. As long as it is surrounded and nutritionally supported by an intact periodontium, AEFC undergoes appositional growth resulting in even layers of alternating dark and light bands depicted in histological sections. Depending on individual age, normal AEFC varies in thickness between 20 to 250 microns. The alteration of dark and light layers is due to an underlying seasonal rhythm which is probably triggered by several factors like ultraviolet radiation dose, climatic parameters, differential food quality and hormonal status. One pair of dark and light bands each constitute one incremental line, the number of which added to the year of eruption of the respective tooth, results in the histological age of the individual under study.[15]

Singh A et al. (2004) This study describes the age estimation methods from various physiological changes of teeth. Age estimation of adults using secondary dentin and cemental annulations are described below**.**[16]

Guy W. (2005) Various studies have been carried out to find the accuracy of different dental age estimation methods. Changes that were appreciable in dentition with increasing age were attrition, periodontal disease, secondary dentin deposition, root secondary dentin, cementum apposition, root resorption and alterations in the color. Amongst the hard tissues of the tooth, unlike enamel, dentin and cementum are continuously synthesized and maintained throughout all stages of a person's adult life and can provide valuable information for the estimation of age.[17]

Kinney, J. H., Nalla, R. K., Pople, J. A., Breunig, T. M., Ritchie, R. O. (2005). Provided insights into how dentin properties change with aging.[18]

Backofen UW, Gampe J, Vaupel JW. **(2005)** A similar study conducted by Backofen, Gampe with a sample of 363 teeth concluded that difference in gender, intra individual correlations & periodontal disease did not have any quantitative effect on the number of annulations. The study concluded that cementum annulation technique was reliable method for estimating a subject's age with 95% accuracy and mean error of <2.5 years.29 Contradictory to the above finding, Dias PEM and coauthors studied 31 ground cross sections of approximately 30µm thickness from 25 teeth obtained from individuals of age ranging from 17 to 77 years with the mean of 44.2 years. The accuracy of the technique decreased with increasing age of the individuals analyzed. The technique was reliable for periodontally sound teeth with the mean error of 1.6 years, but not for periodontally diseased teeth which showed a mean error of 22.6 years.[19]

Aggarwal et al. (2008) - This research confirmed the reliability of counting incremental lines for age estimation, even in forensic cases. It emphasized the method's independence from periodontal disease and supported its use across various tooth types.[20]

Meinl et al. (2008) – A significant study compared incremental lines with alternative age estimation methods and found tooth cementum annulations (TCA) to be more predictable when used with standardized procedures.[21]

Aggarwal P et al. (2008) Age can be estimated in children and in adolescents by means of development and eruption of deciduous and permanent teeth up to 14 years. For most age estimation methods, the developing teeth are assessed on radiographs. After the age of 14 years, the third molar is the only remaining tooth that is still developing, and consequently dental age estimation methods have to rely on the development of this tooth until the age of 20.[22]

Acharya AB et al. (2009) Age estimation to establish the identity of a person for ethical, humanitarian, and official records, particularly in legal and criminal investigations in the field of forensic science, is of paramount importance. Various modalities are available in the assessment of age of a person such as skeletal and dental changes. Age estimation using dentition is an important subspecialty of forensic. Dental age is one of the few measures of physiological development that are uniformly applicable from

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infancy to late adolescence.[23]

Pundir S, Saxena S, Aggarwal P. (2009) The biological explanation for the alternating layers was given by Lieberman and Schroeder who suggested that the dark lines are the stop phases of mineralization during the continuing growth of fibroblasts, leading to change in mineral crystal orientation which is visible under microscope as a series of alternating light and dark lines or bands, which are known as incremental lines of cementum.[24]

Vikas Bhakar, Vandana shah, Nivedita Pachore. (2010) Age estimation using secondary dentin: Gottlieb was the first person to correlate the secondary dentin changes with an age. Among the traditional methods for estimating age in adults, the morpho-histological parameters suggested by Gustafson continue to find widespread use. Of the six variables that Gustafson suggested, secondary dentin is perhaps the easiest to assess and also relatively accurate in age prediction. In fact, later Johanson found that secondary dentin was best correlated to age when used alone.[25]

Dias PEM, Beaini TL, Melani RFH. (2010) conducted on 25 teeth from patients of known age & devoid of any pathology such as attrition & hypercementosis to determine the relation between incremental lines of cementum and age of the individual. Half of the selected teeth were sectioned longitudinally & the other half, were cross sectioned. The mid root region was selected for counting the annulations. Age was determined by adding the eruption age of the tooth to the annulations. Estimated age varied by about 2-3 years from the actual age of the patient. Reliability of the method was found to be 94.73%, with good inter observer agreement in counting annulations.[26]

Schmitt A, Saliba B, Tremblay M, Martrille L. (2010) Cementum is a bone-like connective tissue that grows in increments around the roots of teeth. This incremental deposition manifests as incremental lines with alternating phases of matrix production and mineralization.[27]

Kasetty S, Rammanohar M, Ragavendra TR. (2010) A study was conducted on 200 extracted teeth from individuals of age ranging from 20 to 60 years. Two longitudinal sections of 100µm thick were prepared from each tooth using a hard tissue microtome. One section of each tooth was stained with Alizarin red stain and the other was left unstained. The stained sections were examined under stereomicroscope and cementum thickness was measured at four areas of the tooth and the average was taken. The unstained sections were observed under polarized microscope and age estimation was done using TCA method. Statistically significant positive correlation was observed between estimated age and known age.[28]

A study by **Vikas bhaskhar (2013)** included 200 extracted teeth, 100 were single rooted teeth and 100 were multirooted teeth. The comparison of the mean actual age and mean calculated age, using the formula for Secondary dentin deposition was found that mean calculated age (40.79 ± 9.87) was slightly higher as compared to the mean actual age (40.30 ± 12.04) but statistically this difference was non-significant. He found that secondary dentin can give comparable results for age estimation.[29]

Obertova Z, Francken M. (2013) Similar results were obtained in a study conducted on undecalcified, 60–80µm thick cross-sections from the middle third of the root of 116 single-rooted teeth from 65 individuals of age ranging 20 to 75 years with a mean of 42.2 years. Study stated that reasonably accurate age estimates were obtained in young adults using tooth cemental annulations (TCA) method compared to older age group. A considerable underestimation of age was noticed in individuals older than 40 years.28 Incremental lines of acellular cementum of 80 freshly extracted teeth were investigated for verification of the suitability of pathological teeth for a valid age at death diagnosis. For all patients, a detailed query concerning a variety of life history parameters was established. Digital images of each section were taken and cemental annulations later added to the eruption age of the respective tooth. The histological age thus obtained was then compared to the actual known calendar age of the patient. Study concluded that previous pregnancies, skeletal trauma & renal diseases had influence on calcium metabolism and resulted in hypomineralized incremental lines.[30]

Bhondey et al. (2015) - This study focused on the accuracy of using phase contrast microscopy for

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counting incremental lines in the middle third of the root. It demonstrated a significant correlation between estimated and chronological ages, improving reliability.[31]

The comparative study using cemental annulations and secondary dentin was done by **Priya Gupta (2016)** to examine the correlation between age and the number of incremental lines in human cementum and to correlate age with thickness of secondary dentin. The study sample consisted of 100 teeth extracted because of periodontal disease and orthodontic and prosthetic reasons were used in the study. The age of the individuals at the time of tooth extraction ranged from 25-60 years. Longitudinal ground sections of each tooth were prepared and examined. A strong positive correlation was found between the estimated age, which was calculated by using cemental lines and thickness of secondary dentin and actual age. The author concluded quantification of cementum annuli is a moderately reliable means which is used for age estimation in humans. As the age advances, the thickness of the secondary dentin also increases; hence, the amount of secondary dentin can also be an indicator of age of an individual.[32]

Naji S. et al. (2016) This work compared various staining techniques to enhance the visibility of cementum annulations. The authors concluded that optimized staining significantly improves line clarity, aiding forensic accuracy.[33]

Berkovitz BKB, Holland GR, Moxham BJ. (2017) Cementum contains 65% inorganic component i.e., hydroxyapatite crystals, 23% organic component i.e., collagen and non- collagenous matrix proteins and 12% water by weight and 45% inorganic, 33% of organic and 22% water by volume. There are four types of cementum based on the presence of cells and type of fibers. They are Acellular extrinsic fiber cementum (AEFC), Cellular, Mixed and Acellular afibrillar cementum.[34]

Kulkarni et al. (2018) - This research evaluated age estimation using cementum lines in 10 extracted teeth. It highlighted challenges in older age groups but showed a strong correlation between estimated and actual ages in younger specimens.[35]

Wager J. M. et al. (2018) Wager introduced image processing methods for counting root cementum incremental lines, bridging traditional microscopy with modern computational tools. This study marked a transition toward automation in forensic odontology.[36]

Kamal M. (2020) explored computational techniques, integrating artificial intelligence to quantify cementum lines. The study demonstrated enhanced precision, especially in forensic cases with degraded samples.[37]

Menon, P. Aishwarya, Kumar, N. Anoop (2021) establishes the basic understanding of forensic odontology and focuses on recent advances in forensic odontology, especially in investigating and interpreting dental evidence.[38]

Gupta N., et al. (2022) This research highlighted the role of machine learning in analyzing dentin incremental lines. The study emphasized the synergistic potential of AI and microscopy, particularly for large datasets.[39]

Gupta et al. (2023) reviewed advancements in cementum-based microscopy, emphasizing its forensic and medical applications.[40]

Koehler JJ, Mnookin JL, Saks MJ. (2023) discussed the ways to move towards a more empirically grounded scientific culture for the forensic sciences impacts testing, error rate analyses, procedural safeguards, and the reporting of forensic results. [41]

Li Z. et al. (2023) Li advanced multimodal approaches combining microscopy with spectroscopy. This innovative study showcased the potential of cross-disciplinary techniques to refine dental age estimation.[42]

N Upadhyaya et al(2025) explored the use of light microscopy in north Indian population for age estimation with incremental lines from root cementum reinforcing the scientific rigor of cementum- based age estimation as a reliable adjunct in forensic odontology.[43]

CONCLUSION

Incremental line analysis in root cementum and dentin is a reliable, cost-effective, and reproducible method for age estimation. Cementum annulations showed a higher accuracy compared to dentin. Further standardization and advanced imaging techniques can

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enhance its applicability in forensic practice.

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