

ORIGINAL ARTICLE

Age Estimation with Cemental Incremental Lines- A Phase Contrast and Stereomicroscopic Study.

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Abstract

Background : Age estimation is an important factor in forensic science for human identification. Teeth resist decomposition at death unlike other tissues. The gradual structural changes that take place throughout the life have made teeth useful indicators for age estimation. Cemental annulation is a microscopic method for the determination of age based on the analysis of incremental lines of cementum. Light microscopy as well as specialized microscopic techniques have been employed to enhance the assessment of the cemental annulations.

Aim : To estimate age with cemental incremental lines in human teeth using phase contrast and stereomicroscope.

Materials and methods: A total of 51 normal teeth were included in the study (sound teeth without any associated pathologies). Longitudinal ground sections were observed under phase contrast microscope and stereomicroscope. The number of incremental lines in the total cementum width was calculated with the following formula:

Number of incremental lines (n) = X/Y

where X is the total width of cementum (from DCJ to cementum surface) and Y is the width of cementum between the two incremental lines.

Estimated age (E) = number of incremental lines (n) + eruption age of tooth (t)

Results : The measurements obtained were subjected to statistical analysis for age estimation. Statistically significant results were obtained with phase contrast microscope as compared to stereo microscope.

Conclusion : Age estimation by cemental annulation using phase contrast microscope is a reliable method and can be easily applied in forensic odontology.

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INTRODUCTION

Human identification is the most significant aspect of civilization. It has been of utmost importance to the society in many medicolegal cases.[1] Forensic Odontology is an evolving branch of dentistry and plays an important role in medicolegal matters. The term forensic is derived from a Latin word “forensis” which literally means “before the forum”. Federation Dentaire International (FDI) defines Forensic odontology as “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 dental findings.” [2] It mainly deals with examination, evaluation, and documentation of injuries to teeth, jawbones, oral and paraoral soft tissues, thereby facilitating identification of individuals in investigations related to crime analysis, mass disasters, age estimation. [4]

Age estimation warrants an important role in identification of deceased as well as living individual and can be done depending on body’s biological maturity. Teeth are considered to be most resistant to decomposition as they undergo least amount of degenerative changes on exposure to environmental factors.[6] The first ever known evidence of teeth being used as an age indicator originated in England in 1837 by Edwin Saunders who after examining 1049 children stated that teeth were better indicator of age than height.[5] Other systems used for age estimation are bone development, secondary sexual characteristics, and stature and weight.[7]

Estimation of age using dentition is classified into three stages:

- Estimation of age in prenatal, neonatal and early postnatal period
- Estimation of age in children and adolescents
- Age estimation in adults

Age estimation in humans can be done using following methods:

1) Morpho histological methods, (2) radiological methods, and (3) biochemical method

Under morphological method certain characteristics are observed in sectioned or unsectioned teeth like - Given by Gosta Gustafson in 1947 and 1950 which include: A] Attrition of enamel; B] Secondary dentin deposition; B] Recession of PDL ; C]Cemental annulations ; D] Root resorption ; E] Dentin Translucency. [5]

Cementum is deposited throughout the individual’s life even after completion of growth spurt. This takes place annually in regular rhythmic pattern appearing as alternate light and dark bands. Single pair of this light and dark bands equals to one cemental annulation. They are counted using the tooth cemental annulation method (TCA) and has been frequently employed for age estimation.[6]

Microscopic examination of cemental annulations is done using thin sections of tooth. These thin sections can be either ground sections or decalcified and stained sections subjected to histological examination. Light microscopy as well as specialized microscopy such as stereo and phase contrast have been successfully employed to enhance the appearance of cemental annulations. Results also have shown the significant positive correlation between cemental annulations and age. The present study has been done to determine age by analysing cemental annulations using ground sections of teeth.

MATERIALS AND METHOD

The study was carried out over a period of 2 months and sample size was selected based on the available resources. 51 human extracted teeth that were free from caries, periodontally sound were obtained and kept for fixation in 10% neutral buffered formalin. They were thoroughly washed under tap water. Thin longitudinal sections of 2 mm were cut with diamond disc. Ground sections of 100 um were prepared after

grinding on Arkansas stone. Following mounting using DPX, ground sections were observed under phase contrast and stereomicroscope. The middle 3rd of the root surface was considered for analysing cemental annulations. They were then subjected to image analysing software (TC capture).

Cemental annulations were counted for each tooth using the following formula-

$$\text{Number of incremental lines (n)} = X/Y$$

where X- Total width of cementum

Y -Width of the cementum between two incremental lines.

Age estimation was done using following formula –

$$\text{Estimated age (E)} = \text{Number of incremental lines (n)} + \text{Eruption age of tooth (t)}$$

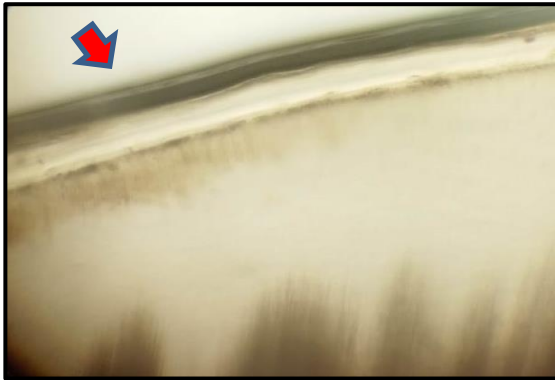


Fig 1. Cemental annulations in Phase contrast microscope

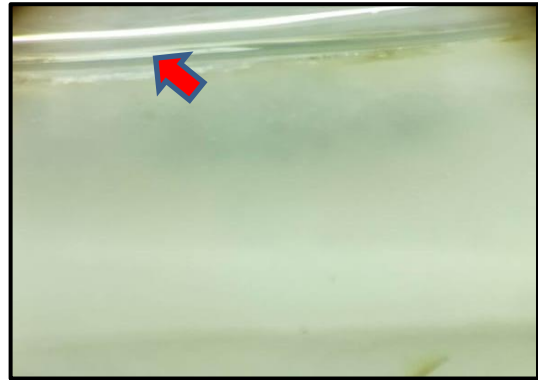


Fig 2. Cemental annulations in stereomicroscope

RESULT

Total 51 ground sections of healthy human teeth were examined under stereo and phase contrast microscope to evaluate cemental annulation for age estimation. The data obtained was subjected to statistical analysis using SPSS v 26.0, IBM software and efficacy of phase contrast and stereomicroscope was determined. A Pearson product-moment correlation was run to determine the relationship between one variable (x: Total width of cementum) and the second variable (y: Width of the cementum between two incremental lines). Number of incremental lines(X/Y) was observed to have significant positive correlation with age ($r = 0.959$) while width between two incremental lines(Y) showed high negative correlation with age ($r = - 0.715$) as shown in table 1. Based on the regression analysis the following regression equations were derived to enable age estimation of teeth by TCA method-

$$\text{Age} = 11.359 + (-.052 \times \text{Total width of the cementum in um(X)}) + .247 \times \text{Width of cementum between two incremental lines(Y)} + 1.024 \times \text{No. of incremental lines (X/Y)}$$

When comparing phase contrast with stereomicroscope, a significantly higher correlation was observed using phase contrast microscope regarding age estimation from cemental annulation(Table 4).

It was observed that phase contrast microscope allowed better visualization of cemental annulations as compared to stereo microscope. Thus age estimation could be done with greater accuracy with the help of phase contrast microscopy that showed 100% and 90.2 % accuracy rate within 10 years and 5 years of actual age respectively.

Table 1: A Pearson product-moment correlation to determine the relationship between one variable (x) and the second variable (y).

| Variables | | Actual age |
|---|-----------------------------|---------------|
| Total width of the cementum in um(X) | Pearson Correlation r value | 0.096 |
| | P value | 0.503 |
| Width of cementum between two incremental lines (Y) | Pearson Correlation r value | -0.715 |
| | P value | 0.000 |
| No.of incremental lines (X/Y) | Pearson Correlation r value | 0.959 |
| | P value | 0.000 |

Table 2: Accuracy of estimated age within a cut off of 10 years

| | Phase contrast | | Stereo microscope | |
|-----|----------------|---------|-------------------|---------|
| | Frequency | Percent | Frequency | Percent |
| Yes | 51 | 100.0 | 35 | 68.62 |
| No | -- | --- | 16 | 31.37 |

Table 3: Accuracy of estimated age within a cut off of 5 years

| | Phase contrast | | Stereo | |
|-------|----------------|---------|-----------|---------|
| | Frequency | Percent | Frequency | Percent |
| No | 5 | 9.8 | 16 | 31.37 |
| Yes | 46 | 90.2 | 35 | 68.62 |
| Total | 51 | 100.0 | 51 | 100.0 |

Table 4: Difference in age for Stereomicroscope as compared to Phase contrast microscope

| | Mean | Std. Deviation | T value | p value |
|--------------------------------|--------|----------------|---------|---------|
| Diff in age for Phase contrast | 2.4204 | 1.5692 | -6.444 | 0.000** |
| Diff in age for Stereo | 5.134 | 2.5664 | | |

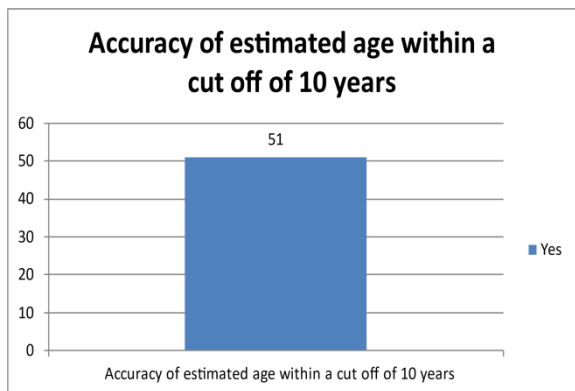


Figure 2: Bar graph showing accuracy of estimated age within cutoff of 10 years

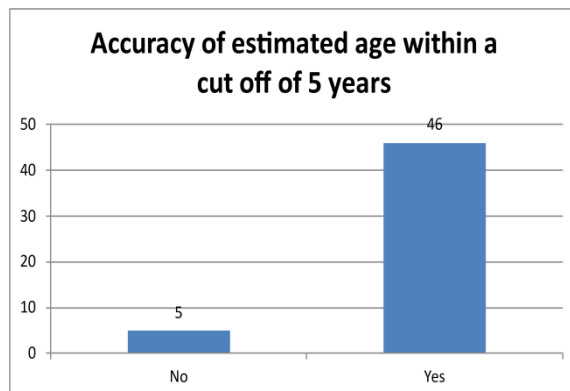


Figure 1: Bar graph showing accuracy of the estimated age within cut off of 5 years

DISCUSSION

Forensic odontology is the emerging branch of forensic medicine. Crime rate has been continuously increasing in our country which corresponds to the evolution in the field of forensic odontology. The significance of dental identification has been increasing in recent years.[4]. Thus teeth become the most important evidence of forensic investigation. Forensic dentistry is the most important tool in estimating age, gender determination, dental DNA fingerprinting, tooth prints, bite mark analysis and in determining blood group from dental pulp. [8] It has widely helped in recognition of bodies, age of victim and /or suspect in criminal cases. [4,9]

Age estimation has been popularly carried out using distinct tooth features as suggested by Gustafson like attrition (A), secondary dentin deposition (S), apical migration of periodontal attachment (P), cementum apposition at the root apex (C), root resorption at the apex (R) and root dentin translucency (T). Studies have reported cemental annulations to be more reliable in estimating age as compared to other skeletal and morphological characteristics. Avadhani et al. (2009) suggested that cemental annulation are better indicator of age.

Zander and Hurzeler reported that cementum is potentially a better tissue for age estimation. Cementum is a calcified tissue that's covers the root portion of the tooth. Its thickness is highest at the apex and least in the cervical region. Early cementogenesis marks the deposition of thin acellular cementum before tooth eruption. This follows subsequent addition of layers of cementum during and after tooth eruption appearing as incremental lines. Its presence shows that cementum is an anisotropic material responsible for birefringence which can be effectively observed using various specialized microscopy techniques. Orientation of these lines produces an optical effect of alternate light and dark bands. The first biological explanation for these alternating bands was given by Lieberman and Schroder. They concluded that dark lines were produced due to the termination of the mineralization during the continual growth of the fibroblasts changing the mineral crystal orientation. [12, 14]

Studies carried out by Pundir et al. and Kaur et al stated that variation in modality can enhance visualization of cemental annulation. Wilson et al (2016) stated that using TCA method with high quality microscopes and digital image analysis lead to accuracy within 4 years of true age for 80.9%.[13] Light microscopy and specialized microscopy like stereo, phase contrast and polarized microscopy have been employed with either

decalcified sections or ground sections recently. Agrawal et al (2008) reported polarizing microscope to be more reliable in visualizing cemental annulations as compared to light microscope. [14] Sowmya et al (2010) correlated cemental annulations and age using polarized and stereomicroscope in 200 teeth found a positive correlation between two studied variables. The predicted age range was observed to be ± 12 years with only 1.5% of cases showing exact correlation between known age and predicted age especially for polarizing microscope.[7] Recently Pradeep et al (2021), Patil A. et al, Vibhute N. et al (2022) stated that phase contrast microscopy along with ground sections of teeth are reported to be reliable and highly significant regarding age estimation. Also Thomas S et al (2020) reported accuracy within 4 years of actual age 70% of times while analyzing cemental annulations using phase contrast microscopy. [10,11,12] These nearly corresponds to the present study which also shows accuracy rate of 100 % within cut off of 10 years while accuracy is observed to be 90.2% within cut off of 5 years. This interprets that the estimated age was ± 5 years of actual age with 90.2% accuracy using phase contrast microscope which is statistically significant when compared with stereomicroscope showing accuracy of 68.62%.

Variation in the age estimation accuracy as seen in various other studies as well as the present study within the range of ± 5 to ± 10 years can be attributed to the variation in cementogenesis. Several factors like environmental (temperature, biomechanical forces, nutrition), physiological (hormonal changes), eruption age of teeth, periodontal disease can induce changes in the appearance of cemental lines. [4,13,14]

Present study evaluates the efficacy of stereomicroscope as compared to phase contrast microscope. Thus assessment of cemental annulation by phase contrast microscope has shown better correlation with age than stereomicroscope. Our study also showed that number of incremental lines increase with age and width of cementum between two incremental lines decreases with increase in age which are in accordance with the literature review.

CONCLUSION

Teeth display a large number of observable age-related variables. Cemental annulations proves to be an important parameter in this regard. Study of cemental annulations of teeth using phase contrast microscope increase accuracy of age estimation than stereomicroscope. This can be effectively used for forensic purposes with great accuracy.

Conflict of Interest:

NIL

Financial Disclosures:

NIL

REFERENCES

1. Shamim T. Forensic odontology. J Coll Physicians Surg Pak. 2012 Apr 1;22(4):240-5.
2. Vyas T. Forensic Odontology: An Overview. International Journal of Drug Research And Dental Science. 2020 Apr 20;2(2):1-2.
3. Jeddy N, Ravi S, Radhika T. Current trends in forensic odontology. J Forensic Dent Sci 2017;9:115-9.
4. : Pradeep L, Kokila G, Gopinathan PA, Guruswamy S, Nazir SH, Chatterjee A. Age estimation with cemental annulation using light, phase contrast and polarized microscopy. J Microsc Ultrastruct 2021;9:55-60.

5. Verma M, Verma N, Sharma R, Sharma A. Dental age estimation methods in adult dentitions: An overview. *J Forensic Dent Sci* 2019;11:57-63.
6. Sultana A, Zainab H, Jahagirdar P, Hugar D. Age Estimation with Cemental Incremental Lines in Normal And Periodontally Diseased Teeth Using Phase Contrast Microscope. *Indian Journal of Forensic Medicine & Toxicology*. 2022;16(1):213-23.
7. Kasetty S, Rammanohar M, Raju Ragavendra T. Dental cementum in age estimation: a polarized light and stereomicroscopic study. *Journal of forensic sciences*. 2010 May;55(3):779-83.
8. Shah P, Velani PR, Lakade L, Dukle S. Teeth in forensics: A review. *Indian J Dent Res [serial online]* 2019 [cited 2023 Mar 17];30:291-299.
9. Dutta S. Awareness of Forensic Dentistry among Dental Practitioners. *Indian Journal of Forensic Medicine & Toxicology*. 2020 Oct 29;14(4):545-50.
10. Patil A, Satish BN, Kondody RT, Wali V. Cemental annulation: An approach to age estimation in transverse and longitudinal sections using three different microscopes. *J Oral Maxillofac Pathol* 2022;26:192-8.
11. Vibhute N, Kamate W, Sankpal D, Mulla HM. EVALUATING AGE USING PULP TO TOOTH AREA RATIO USING RADIOGRAPHY AND TOOTH CEMENTAL ANNULATIONS USING THROUGH PHASE CONTRAST MICROSCOPY. *NeuroQuantology*. 2022;20(16):3114.
12. Thomas SC, Corby JH, UNIFORMED SERVICES UNIV OF THE HEALTH SCIENCES BETHESDA MD. The Histological Analysis of Tooth Cementum Annulations to Determine Age and Season of Death of Active Duty Military Personnel: Comparative Study Using Brightfield, Polarizing and Phase Contrast Microscopy (Doctoral dissertation, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814).
13. Wilson, N., K. KoelAbt, and K. Schmidt, The Analysis of Tooth Cementum for Histological Determination of Age and Season at Death of Active Duty Military Members, in AEGD2 Residency. 2016, Uniformed Services University.
14. Aggarwal P, Saxena S, Bansal P. Incremental lines in root cementum of human teeth: An approach to their role in age estimation using polarizing microscopy. *Indian J Dent Res* 2008;19:326-30.



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