Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 12s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Age estimation based on tooth cementum annulations using light and phase contrast microscope

Dr. Apsara[1], Dr. Sushruth Nayak, M.D.S[2]

[1]Post Graduate student, Department of Oral and Maxillofacial Pathology and Oral Microbiology, Maharishi Markandeshwar College of Dental Sciences & Research, Maharishi Markandeshwar (Deemed to be university), Mullana, Ambala, Haryana.

[2]Professor & Head, Department of Oral and Maxillofacial Pathology and Oral Microbiology, Maharishi Markandeshwar College of Dental Sciences & Research, Maharishi Markandeshwar (Deemed to be university), Mullana, Ambala, Haryana.

***Corresponding Author:**

Dr. Sushruth Nayak, M.D.S

Professor & Head, Department of Oral and Maxillofacial Pathology and Oral Microbiology, Maharishi Markandeshwar College of Dental Sciences & Research, Maharishi Markandeshwar (Deemed to be university),Mullana, Ambala, Haryana - 133207, INDIA.

Cite this paper as: Dr. Apsara, Dr. Sushruth Nayak, M.D.S, (2025) Age estimation based on tooth cementum annulations using light and phase contrast microscope. *Journal of Neonatal Surgery*, 14 (12s), 669-676.

ABSTRACT

Background: This study aimed to compare the estimated age with the actual age by analysing cementum incremental lines using light and phase contrast microscopy

Methodology: Conducted in the Department of Oral and Maxillofacial Pathology and Oral Microbiology at Maharishi Markandeshwar (Deemed to be University) Mullana, Ambala, the study utilized 40 extracted teeth (20 males and 20 females), that were stored 10% formalin. Longitudinal ground sections were prepared by using a lathe - cutting machine followed by hand-trimming on the Arkansas stone. These ground sections were mounted with DPX and examined under both light and phase contrast microscopes simultaneously then these slides were transferred to the computer monitor from microscopes under 40x magnification by using NIS Element software. Cementum displayed alternating dark and bright bands, each pair representing a single annulation was counted as one and added at the end with tooth eruption age to estimate the actual age of an individual.

Results: The connection between the actual age and phase-contrast microscopy readings was stronger ($r = 0.938$, $p = 0.0001$) compared to light microscopy ($r = 0.677$, $p = 0.0001$). In male subjects the phase contrast microscopy showed slight underestimation of age (actual: 25.20, estimated: 23.20, $p = 0.001$), whereas light microscopy understated age even more (actual: 25.20, estimated: 20.75, $p = 0.005$). Phase contrast microscopy revealed no variation of age in females (actual: 18.80, estimated: 18.85, $p = 0.942$), but light microscopy revealed understated age (actual: 18.80, estimated: 17.00, $p = 0.181$). These results suggest that while phase contrast microscopy was more accurate in predicting the actual age, its dependability is impacted by gender variations as we can see more disparities in male samples.

Conclusion: The study confirms the stronger association between the cementum annulations and the chronological age, with phase contrast microscopy showing better accuracy as compared to light microscopy.

Keywords: *light microscope, phase contrast microscope, cementum annulations, age estimation*

1. INTRODUCTION

In this contemporary era, when the crimes and other societal developments have become rampant, one of the greatest influential factors in all parts of life is the prediction of age in various fields. Estimation of age or computation using dental traits can considerably improve recognition of a person. It may also be salient in assessing any legal liability for a young adult or an individual of unknown age, facilitating parental procedures and releasing private pensions for the unknown persons, and assisting archaeological evidence with the old-world demography.[1] The dialectic analysis reveals the importance of progression in the growth of the hard tissues. Dentition is better preserved than any other body remnant thus they are often used for determining the age of any deceased person. A mineralised coating that covers the roots of the tooth is called cementum and, due to its appositional growth, provides for easy age calculations.[2] Our dentition can survive every scenario that may arise after death, decay, or any other destructive situation, making the tooth a significant asset for TCA (tooth cementum annulations) techniques. Establishing age by tooth examination depends on events that take place throughout odontogenesis and maturation, or based on a recurrent process that affects the integrity of dental tissues soon after a person's

maturation is concluded.[3]

Various microscopic techniques, including ground and decalcified tooth sections, have been employed to compute the age by dentition, revealing that light and phase contrast microscopic techniques are more accurate than any other microscopy techniques.[4] Because both males and females have decreasing age estimation accuracy as they age, the cementum annulation approach is recommended for usage with younger age groups because sharpey's fibres and cemental annulations are interrelated. When periodontal health deteriorates by aging, alveolar bone shrinks and sharpey's fibres degenerate. This could prevent the cemental annulus from forming. According to studies, at the age of 60, the cementum content declines by one-third.[5] The precision and reproducibility of the approach are based on the mean, which is obtained by conducting as many counts as feasible, regardless of the variety or position of a tooth. The incremental lines in tooth cementum have the potential to be a useful instrument in medical jurisprudence.[6]

2. METHODOLOGY:

The research was conducted in the Department of Oral and Maxillofacial Pathology and Oral Microbiology, M.M. College of Dental Sciences and Research, Mullana, Ambala. The research consisted of 40 teeth that were removed from healthy individuals (20 males and 20 females) after informed consent was taken. The points included in inclusion criteria were, teeth that were extracted due to orthodontic treatment, teeth that were extracted due to prosthodontic procedures, teeth that were not periodontally affected, tooth that was free of physiological or compositional abnormalities, decay, fractures, anomaly, or degradation, and teeth with intact and undamaged roots. Exclusion criteria for this study included, teeth with gingival recession, teeth with hypercementosis, and teeth with any periapical pathology, caries including root.

A total of 40 teeth were collected for this study 20 from males and 20 from females. The study used the teeth that were extracted for medical procedures. The subjects' age, health records, and previous dental procedures were maintained at the time of surgical removal of the tooth, along with their permission. Each tooth was stored in 10% formalin and given a unique identification tag. Ground sections were prepared in mesiodistal plane longitudinally by using Lathe cutting machine and then hand - trimmed with the Arkansas stone on the rough surface followed by the smoother surface for finer trim. These sections were then washed with xylene thoroughly. And mounted on a glass slide with the DPX mountant. Cementum incremental lines were then observed under light and phase contrast microscope. The main attention was given to the cementum in the center of the root. The images obtained under 40 x magnifications were transferred to the computer monitor by using NIS Element software and evaluated. The number of incremental lines of cementum was manually counted by marking a point next to each visible line. Each recurrent bright and dark band of appositional growth of cementum. were assigned a score of one and then total number of these lines were counted.

The formula used to approximate the subject's age was:

"Total cementum annulations + tooth eruption age = Estimated age"

The estimated number was then compared to the person's exact age.

Results: Data was analysed using the statistical package **SPSS 26.0** (SPSS Inc., Chicago, IL) and level of significance was set at **P<0.05**. **Descriptive statistics** was performed to assess the mean and standard deviation of the respective groups. **Inferential statistics** to find out the difference **Between the group was done by Independent T test. Pearson correlation test** was done for estimation of accuracy.

Table I and Graph 1 represents the distribution of gender among a sample of 40 individuals, revealing an equal representation of females and males. Specifically, there are 20 females (50.0%) and 20 males (50.0%), resulting in a balanced gender composition. The findings in **Table 2 Graph 2** show a good association with actual age and estimated age with the Pearson Correlation Coefficient of 0.983 measured by phase contrast microscope. The correlation is statistically significant, as indicated by the value of 0.0001, which also suggests the probability of this outcome happening by chance is extremely low. In general, these results provide credence to phase contrast microscopy's accuracy in age estimation **Table 3 and Graph 3** show the relationship among the age determined by a light microscope and real age with a Pearson correlation value of 0.677, these two variables show a moderately to strongly favorable relationship Although not as drastically as with the phase contrast microscope, this implies that the age as predicted by the light microscope tends to be similar with real age of the subject. this correlation's statistical significance is confirmed by the significance level of 0.0001, which also shows that the likelihood that the observed association is the result of chance is extremely low. Overall, these findings suggests that although the light microscope can accurately estimate age, it has considerably lower connection with real age than the phase contrast microscope **Table 4** shows the correlation analysis of real age of subjects with the results from the light microscope and phase contrast microscope it reveals different patterns between genders. For females, the Pearson correlation coefficient between actual age and the light microscope is -0.017, indicating a negligible and non-significant relationship ($p = 0.942$). In contrast, the correlation with the phase contrast microscope is significantly positive at 0.728 ($p = 0.000$), suggesting a strong relationship between actual age and the measurements obtained from this method. **(Graph 4)** For males, the results are notably different. The correlation with the light microscope is significantly positive at 0.780 ($p = 0.000$), indicating a strong association with actual age. The correlation with the phase contrast microscope is even higher at 0.977 ($p = 0.000$), suggesting an almost perfect relationship. **(Graph 5)** Overall, while both genders show significant correlations with the phase contrast microscope, males demonstrate stronger associations with both microscopy methods compared to females. **Table 5**

and Graph 6 Illustrates the comparison of actual age versus age determined by phase contrast and light microscopy revealing significant differences between the two methods for males but not for females. For females, the mean age determined by the phase contrast microscope (18.85) is nearly identical to the actual age (18.80), with a p-value of 0.947 indicating no significant difference. Similarly, the light microscope shows a mean age of 17.00, with a p-value of 0.181, suggesting that this difference is also not statistically significant. In contrast, for males, the phase contrast microscope shows a mean age of 23.20, resulting in a significant p-value of 0.001 when compared to the actual age of 25.20 showing understated age prediction. The light microscope presents an even greater discrepancy, with a mean age of 20.75 and a significant p-value of 0.005. These results highlight that while the microscopy methods provide consistent age estimates for females, they yield notably different results for males as we can observe more discrepancy in males as compared to females which was observed in both the microscopes.

Table 1- Table of gender distribution

		Frequency	Percent
Gender	**F**	**20**	**50.0**
	M	**20**	**50.0**
	Total	**40**	**100.0**

Table 2- Correlation between Actual Age vs Age by Phase contrast microscope

		Age by Phase contrast microscope
Actual Age	**Pearson Correlation**	**.938****
	Sig. (2-tailed)	**.0001***

Table 3- Correlation between Actual Age vs Age by Light microscope

		Age by Light microscope
Actual Age	**Pearson Correlation**	**.677****
	Sig. (2-tailed)	**.0001***

Table 4 – Correlation Analysis -Gender wise

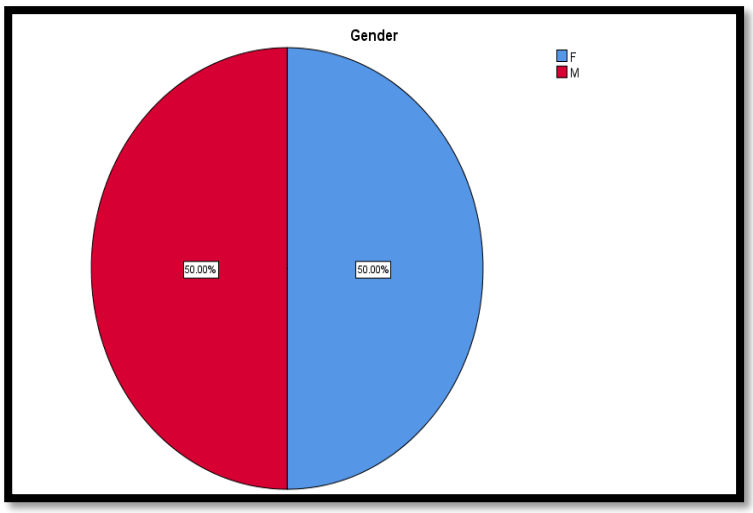
Gender			Light microscope	Phase contrast microscope
F	**Actual Age**	**Pearson Correlation**	**-.017**	**.728****
		Sig. (2-tailed)	**.942**	**.0001***
M	**Actual Age**	**Pearson Correlation**	**.780****	**.977****
		Sig. (2-tailed)	**.0001***	**.0001***

Table 5- Comparison of Actual Age vs Age by Phase contrast/Light Microscope-Gender

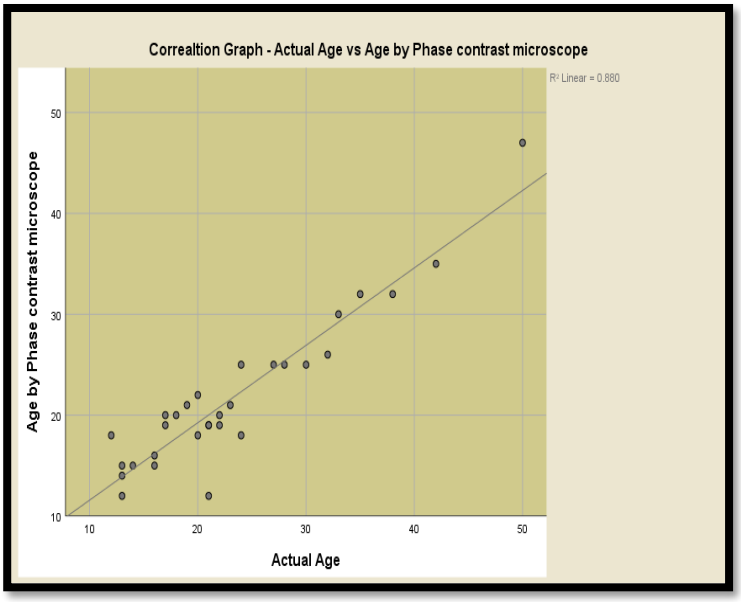
		Mean	Std. Deviation	Std. Error Mean	Mean Difference	T Value	P Value	95% CI	
								Lower limit	**Upper limit**
Female	**Actual Age**	**18.80**	**4.830**	**1.080**	**-.050**	**-.067**	**.947**	**-1.602**	**1.502**
	Phase contrast microscope	**18.85**	**3.731**	**.834**					
	Actual Age	**18.80**	**4.830**	**1.080**	**1.800**	**1.388**	**.181**	**-.915**	**4.515**

	Light microscope	**17.00**	**3.129**	**.700**					
Male	**Actual Age**	**25.20**	**9.796**	**2.190**	**2.000**	**3.752**	**.001***	**.884**	**3.116**
	Phase contrast microscope	**23.20**	**8.389**	**1.876**					
	Actual Age	**25.20**	**9.796**	**2.190**	**4.450**	**3.178**	**.005***	**1.519**	**7.381**
	Light microscope	**20.75**	**6.373**	**1.425**					

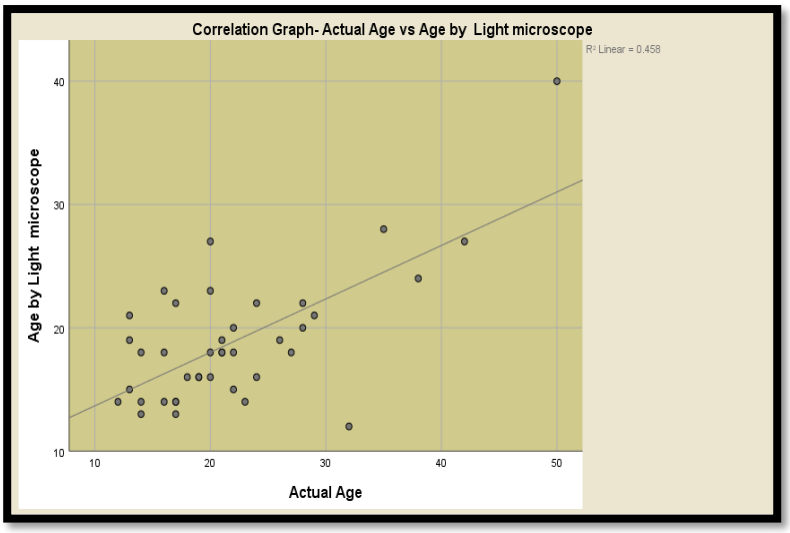
Graph 1 – Graph of gender distribution



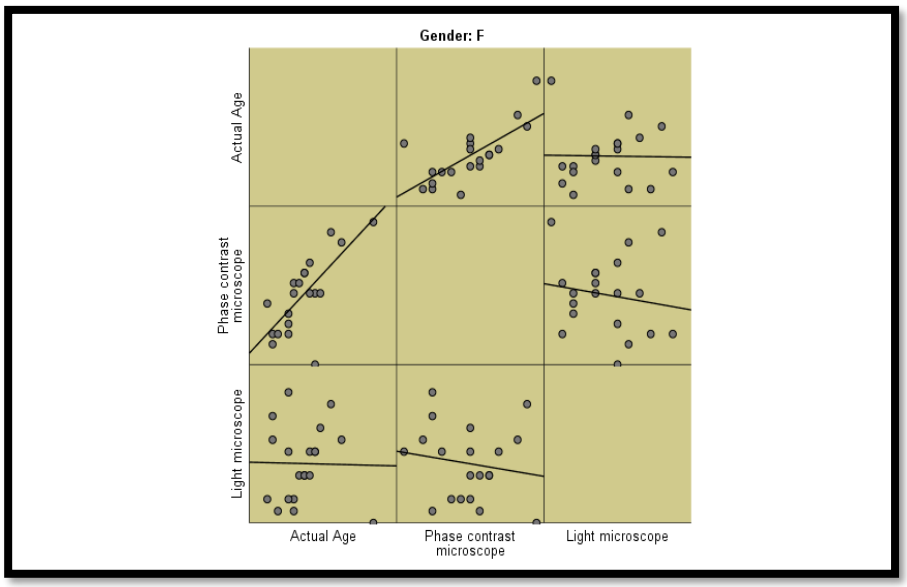
Graph 2 – Correlation between Actual age vs Age by Phase contrast microscope



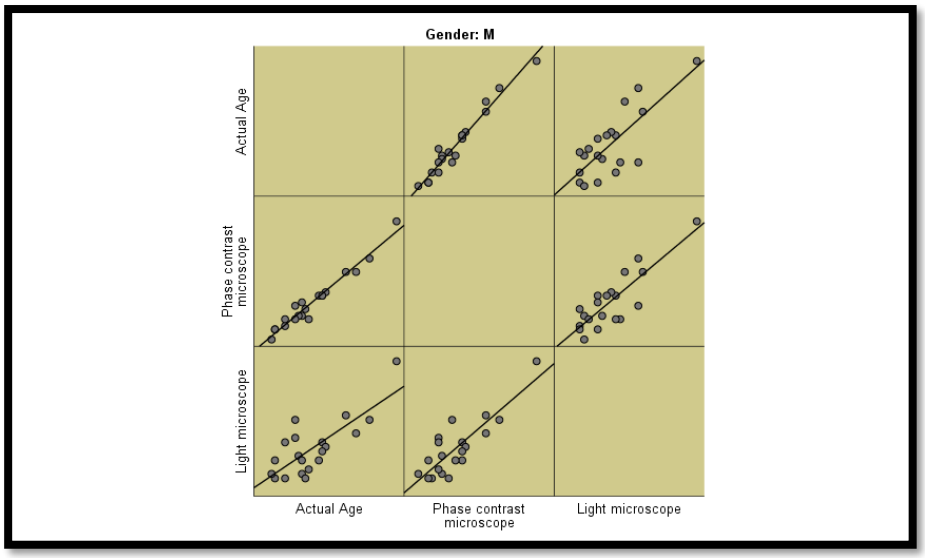
Graph 3- Correlation between Actual Age vs Age by Light microscope



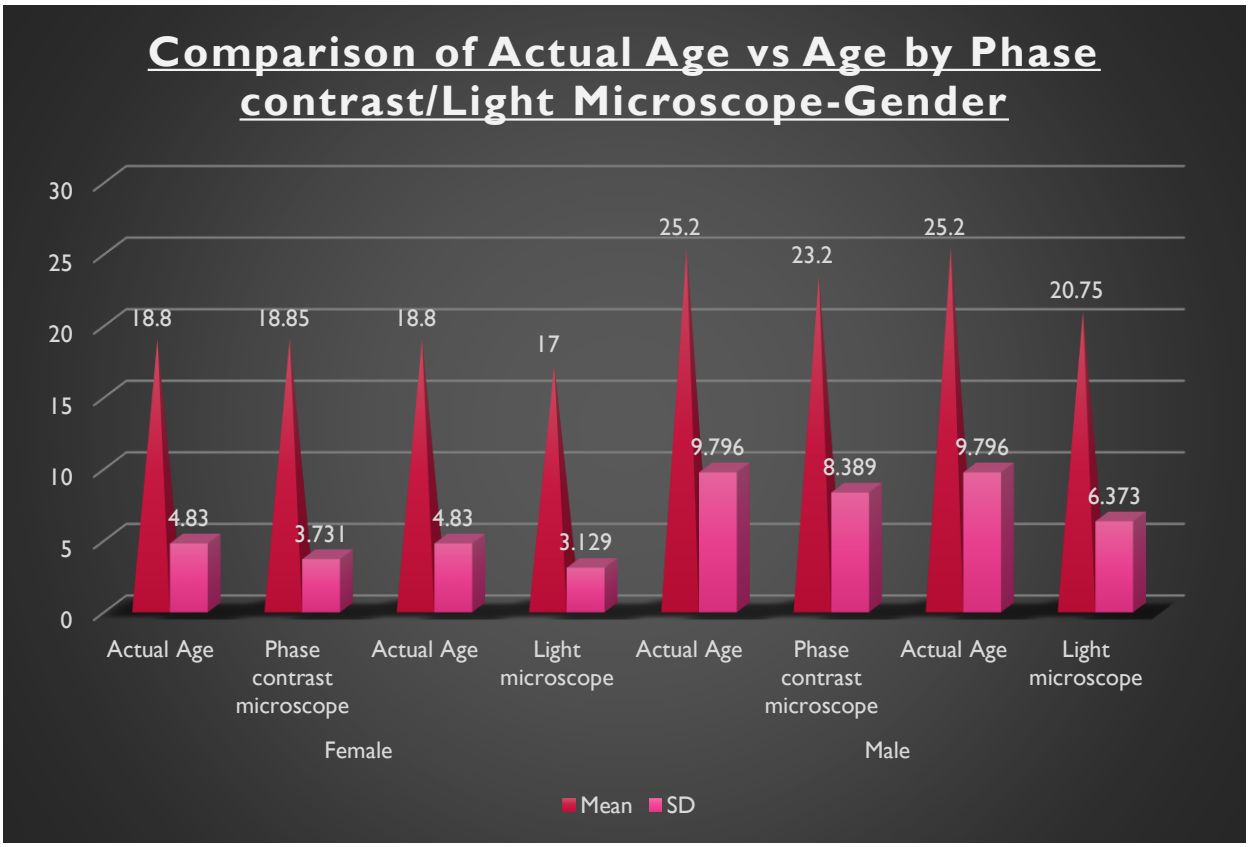
Graph 4 – Correlation Analysis – Female



Graph 5 – Correlation Analysis – Males



Graph-6 - Comparison of Actual age vs Age by Phase contrast/Light Microscope gender analysis



3. DISCUSSION:

Our teeth are the most invaluable tool for the forensic and anthropological studies as our dentition can withstand every extreme environmental condition even after all the tissues are decomposed teeth remain indestructible their durability makes them the best tool for the age estimation as the progressive changes in the tooth structure throughout life provides the significant amount of information regarding a person's growth.[3]

The determination of age is among the most focused aspects of forensic studies it is crucial for cases involving unidentified human remains, situations such as mass disasters, certain criminal investigations, facilitating parental procedures, it can also play an important role in living individuals by verifying eligibility for pensions and inheritance claims after the death of a

family member. With advancing technology, age estimation from a single tooth will be a ground-breaking milestone in the forensic and legal fields, providing a reliable and easy method for identification of an individual.[1]

The fundamental basis for estimating age by teeth is the appositional changes that are encountered over the course of a lifetime. In our study roots of the teeth are the focal point.[4] The whole surface of the root is covered in cementum, also known as root cementum, it is a mineralized tissue. According to **Denton**, cementum was originally shown under a microscope by **Retzius (1836)** and **Fraenkel** and **Raschkow (1835),** and since then it has become an integral part of the odontology.[7]

Throughout life, appositional growth of cementum persists, especially while the physiological stress response. For instance, among humans, fresh cementum is being produced upon the roots, when the crown of the tooth deteriorates, enabling the tooth to progressively elongate in the socket while preserving proper occlusion. Tooth cemental annulations were successfully applied as a technique for predicting the real age of both terrestrial and marine animals in numerous investigations. Cemental annulations have been employed by researchers as a trustworthy technique of growth determination over the past 30 years. Because of its special place in the alveolar process, **Zander and Hurzeler** believe that the cementum that covers the root is a superior structure for measuring age.[4]

Periodic incremental growth of cementum is formed by Parathyroid hormone which, in conjunction with Vitamin D, controls the absorption of calcium.[8] Different dental cementum types may be identified; they serve distinct purposes and are found in various topographical regions of the tooth root as determined by **Renz and Radlanski,** in **2006**. The three primary forms are cellular mixed stratified cementum in the transition area (CMSC), cellular intrinsic fibers cementum (CIFC) accumulated in the vicinity of the apex and furcation, and acellular extrinsic fibers cementum (AEFC), which is primarily located in cervical and mid thirds of the root. A combination of cellular and acellular cementum makes up the latter. The dental cementum begins to form as the tooth root develops and keeps building up in layers. (**Bosshardt and Selvig, 1997, Lieberman, 1994, Renz and Radlanski, 2006)** [7]

Various techniques have been used to demonstrate these appositional growths of cementum in order to determine real age of an individual. A variety of microscopic methods, including transmitted light, polarized light, or phase contrast (**Bosshardt, 2005, Bosshardt and Selvig, 1997, Renz and Radlanski, 2006)** has been used for this purpose throughout these years, it has been demonstrated that the regular formation of acellular extrinsic fibers can be seen by cementum layers also known as incremental lines that run parallel to the root surface. Although the cellular cementum likewise exhibits these lines, the uneven development causes the layers to differ in breadth and distribution determined by **Bosshardt and Schroeder** in **1996.**[7]

Under the microscope these annulations of cementum appear as alternating bright and dark lines these optical formations are caused due to collagen fibrils that are uncalcified, these bundles get mineralized by crystals of hydroxyapatite, the organization of these fibrils changes time to time causing hyper mineralised layer and a hypo mineralised layer of cementum thus forming bright and dull bands making it an easier approach towards determining age and seasonal changes of an individual.[6] Also, certain factors can influence the appositional growth of cementum such as stress levels of a person, environmental factors, low nutrition, amount of UV radiation perceived by a person, and injury to the tooth root while in developmental stages of life, pregnancy , menopause also researchers found that Human dental cementum's production rate is found to be affected by both degenerative processes and mechanical stimuli, such as increased masticatory forces and bruxism concluded by **Lieberman, 1994, Schroeder, 1992**.[9] Still TCA is renowned to be a reliable, non-invasive and cost-effective method because teeth are present in abundance at any archaeological sites, teeth can also be found in severely mutilated bodies and it is easier to assess the dentition as our teeth are better preserved and we have 32 of them.

Firstly, cementum width was taken into account for measurements in TCA method by **Gustafson** in **1950** and was first applied to extracted teeth that were freshly obtained. **Grobkopf 1989 /1990** applied this method to archaeological remains and other forensic cases, cremated bodies supporting the authenticity and reliability of cementum as an age estimating factor.[7] Keeping all this in mind we compared the actual age of an individual with the estimated age under light and phase contrast microscope by using ground sections of 40 teeth sample that were equally divided by gender (20 males, 20 females) and statistically phase contrast microscope was most accurate for counting cementum annulations.

Prospective research carried out by **Pradeep et al., 2021** included ground section of 50 teeth that were studied for the incremental growth of cementum under phase contrast, light and polarised microscope. When the mean as well as the standard deviation (SD) values of estimated and actual age were compared using the Z-test, it was found that there was no statistically significant variance between the two in ground sections. Their study also proposed that phase contrast microscopy came out to be more accurate among other microscopic technique showing the age difference of only 1 year between the actual and the estimated age also Phase-contrast microscopy had the greatest Karl Pearson's correlation coefficient (0.98).[2] Our observations also corroborated with their study as we observed a Karl Pearson correlation coefficient 0.938 for phase contrast microscope and it was lower in light microscopy with the Pearson Correlation coefficient 0.667. In another investigation led by **Kaur et al., 2015**[3] they used 60 extracted human teeth to assess the efficiency of age prediction through TCA using different microscopy techniques. The study employed phase contrast microscope, light microscope, and polarised microscopy to estimate the real age of subjects. The results showed that phase contrast microscopy provided the highest correlation with actual age ($r = 0.989$, $P > 0.05$), followed by polarized microscopy ($r = 0.557$, $P > 0.05$), while light microscopy showed a lower correlation ($r = 0.347$, $P < 0.001$). Regression analysis further confirmed that phase contrast microscopy yielded the most accurate age estimation, with a regression coefficient of 0.989, followed by polarized

microscopy (0.542) and light microscopy (0.3337). These findings align with our study, as we also observed phase contrast microscopy outperforming light microscopy.

4. CONCLUSION:

The study confirms the stronger association between the cementum annulations and the chronological age, with phase contrast microscopy showing better accuracy as compared to light microscopy. The study focused on how the incremental growth of cementum can play a significant role in predictions of actual ages. Light microscopy revealed considerably greater disparities, especially in males which imply that gender affects the efficacy of various microscopic techniques by playing a varying role in age determination. Therefore, to confirm these findings and enhance the accuracy of age estimate methods in forensic and anthropological applications, additional research with bigger and more varied samples is required.

REFERENCES

[1] Geetha HL, Baghisath VP, Vinay HB, Sudheer B, Kumar VJ, Gayathri CH. Age estimation using tooth cementum annulations method by different types of microscopes: A comparative study. Int J Oral Health Sci 2018; 8:73-80.

[2] 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.

[3] Kaur P, Astekar M, Singh J, Arora KS, Bhalla G. Estimation of age based on tooth cementum annulations: A comparative study using light, polarized, and phase contrast microscopy. J Forensic Dent Sci. 2015;7(3):215-21.

[4] Patil A, Satish B N, 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; 26:192-98.

[5] Kulkarni N, Mahesh S, Sridharan G. Dental age estimation using incremental lines of cementum. International Journal of Oral Health Dentistry. 2018;4(3):202-04.

[6] Gupta P, Kaur H, Shankari GSM, Jawanda MK, Sahi N. Human age estimation from tooth cementum and dentin. J Clin Diagn Res. 2014;8(4):ZC07-10.

[7] Hinrichs, C., Nicklisch, N., Mardare, C. C., Orechovski, B., Hassel, A. W., Kleber, C., & Alt, K. W. (2022). Incremental lines in human acellular tooth cementum – New insights by SEM analysis. Anatomischer Anzeiger [Annals of Anatomy], 243(151933), 151933.

[8] Mohan N, Gokulraj S, Thomas M. Age estimation by cemental annulation rings. J Forensic Dent Sci. 2018;10:79-83.

[9] Kagerer P, Grupe G. Age-at-death diagnosis and determination of life-history parameters by incremental lines in human dental cementum as an identification aid. Forensic science international. 2001 Apr 15;118(1):75-82.