Age Estimation Using Cemental Lines and Mineral Density Index with Polarized Microscopy

Abstract:

Background: Age estimation from dental tissues plays a crucial role in forensic science, especially in cases involving unidentified individuals or legal disputes. Cementum, a mineralized tissue in teeth, forms incremental lines that serve as biomarkers for age determination.

Aim: The study aims to explore the use of cemental line counts (CLC), mineral density index (MDI), and cementum thickness (CT) for age estimation using polarized light microscopy (PLM).

Methods: A total of 35 pathology-free third molars from individuals aged 16-35 years were analyzed. Teeth were grouped into four age ranges: Group I (16-20 years), Group II (21-25 years), Group III (26-30 years), and Group IV (31-35 years). Cemental line counts, MDI, and CT were measured using PLM. A regression model was applied to estimate age, and correlations between the variables were assessed using Pearson's correlation. ANOVA was conducted to compare MDI across age groups, and paired t-tests were performed to compare estimated and actual ages.

Results: The study found a strong positive correlation between CLC and actual age ($r = 0.82$, $p < 0.01$). The MDI showed a moderate correlation with age ($r = 0.15$, $p = 0.04$), while CT showed a weak correlation ($r = 0.08$). ANOVA revealed significant differences in MDI across age groups ($p = 0.03$), with higher MDI in older age groups. The regression model showed a mean difference of 1.25 years (SD ± 0.72) between estimated and actual ages, with no significant difference ($p = 0.08$) between the two.

Conclusions: The study demonstrates that CLC and MDI, when measured using PLM, are effective in estimating age in individuals aged 16-35 years. Incorporating multiple parameters, including cementum thickness and mineral density, enhances the accuracy and reliability of age estimation. This multifactorial approach provides a promising tool for forensic age estimation, particularly in young adults.

Key-words: Cementum, Cemental Line Count (CLC), Mineral Density Index (MDI), Cementum Thickness (CT), Polarized Light Microscopy (PLM), Age Estimation, Forensic Science, Forensic Odontology, Age Groups, Regression Model

Introduction:

The age estimation has been a part of forensic science that helps in the identification of individuals in various criminal and civil investigations. The estimation of the age of an individual from skeletal or dental remains becomes essential in mass fatalities, missing persons cases, immigration disputes, and criminal cases.[1] Their remarkable hardness, resistance to environmental deterioration, and deep preservation of teeth for a longer time make them some of the most reliable biological tissues designed for age estimation.[2] Hence, in recent years, much focus has been placed on developing the methods to estimate age based on teeth as their sensitivity and suitability must be increased for forensic science application.

Cementum is a calcified tissue that covers the roots of teeth and provides support as well as protection to a tooth.[3] Unlike enamel and dentin, which barely undergoes any post-eruption changes, cementum continually forms throughout life, leading to successive deposition of concentric layers

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[1]JITENDRA SHARMA, [2]KAVITA NITISH GARG, [3]AMIT THAHRIANI, [4]DEEPALI UPADHYAYA, [5]MANOJ MALHOTRA

[1-5]Department of Oral Pathology and Microbiology
Chandra Dental College Safedabad, Barabanki

Address for Correspondence: Dr Jitendra Sharma
Post-Graduate Student
Department of Oral Pathology and Microbiology
Chandra Dental College Safedabad, Barabanki
Email : dr.jitendera@gmail.com

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referred to as cemental incremental lines (CIL)[4] These are commonly compared to tree rings which are annular since they are deposited at nearly an annual rate, making them a significant biomarker for age estimation[4]

Previous studies revealed that simple tabulations of these lines provide adequate chronic age estimation with numerous authors reporting this relationship in differing populations[6]. Traditionally, however, cementum analysis techniques have been accused of being prone to inter-visit variability in line visibility to being observer dependent and environmentally determined in cementum formation activities[7]. Therefore, it is time to combine several additional histological and molecular parameters to make CIL-based age estimation more realistic and reliable.

The cementum composition and structure exhibit extreme variations with age, thus providing potential markers for more accurate age estimation. The main constituents of cementum are collagen type I and hydroxyapatite crystals, and a variety of non-collagenous proteins like BSP, osteopontin, and DMP-1 are required in the process of matrix formation and mineralization[8]. As the cementum ages, cellular activity and matrix turnover decrease, which results in the accumulation of very mature, tightly mineralized cementum[9].

Recent studies have demonstrated that with progressive ages, the expression of major cementum-specific proteins like BSP and DMP-1 is downregulated to govern the mineralization process[10]. Moreover, some other cytokines like IL-1 and TNF-α have been reported to intervene in cementogenesis that further contributes to changes in the transmission, thickness, and cemental line density during pathology as well as non-pathology conditions[11]. These molecular changes can also be used in the protocol of forensic science to maximize the validity of the present methods of age estimation being used.

Recent advances in molecular biology also opened the avenue for using DNA methylation patterns in cementum for age estimation. DNA methylation is an epigenetic marker whose alterations are predictable with age and thus can complement traditional CIL counting as a dual histological and molecular framework for forensic application[12]. This is still in its infancy and needs further investigation before it can be spread out in forensic practice.

Use of conventional light microscopy has led to poor contrast for cemental lines, particularly when aging persons or teeth which have exposed their structures to the environmental deterioration. Polarised light microscopy (PLM) provides increased contrast, improving the visual identification of cemental layers with significantly fewer biases in observations.[13] Based on the fact that the birefringence of mineralized tissues has been exploited by PLM, observer bias is diminished and the countability of lines is much improved to the extent of increasing reliance, especially on forensic issues where accuracy would be emphasized more.PLM has shown great superiorities over traditional microscopy in analyses of dense or mineral-rich tissues; its use in cementum-based age estimation is receiving more recognition. Research into the technique indicates that it tends to minimize the margin for error in age estimation by almost 15% as provided by the conventional methods.[14] This increased visibility enhances the reliability of CIL counts but also helps to assess other ancillary parameters like cemental thickness and density, further increasing the precision of estimations.

Forensic odontology has a specific requirement for precise age estimation methodologies, especially in populations bearing hefty medico-legal case loads.

In the Indian context, ageing issues occur more frequently and significantly in the legal sectors of juvenile justice, the marital laws, and criminal capacity.[15] Demographics-based statistics show that a large amount of forensic age estimations include subjects who are approximately between 16 and 35 years old, and the field becomes very important regarding research aimed at enhancing accuracy in dental age estimation.[16] The procedure of orthodontic extraction of teeth along with third molar excision under conditions of being free from infection by pathology creates an opportunity for the examination of cemental changes within a well-controlled cohort. This specific demographic and dental classification are thus considered in this study. A consistent, replicable method for age estimation in such a subject would, no doubt be rigorously scrutinized by the forensic and legal professions.

Even while being considered a core component in age estimation based on cementum, the counts associated with cementum can be used, besides novel variables like thickness, line density, and even optical properties, to add enormous validity to the method of estimating ages. Cemental thickness was demonstrated to increase regularly according to age, and determination with PLM can allow measurement and add another quantitative measurement as supplementary support for age.[17] Line density-number of lines per unit area also proves an insight into the pace of cementogenesis and association of chronological age.[18]

Further, studying the refractive index and birefringence patterns of cementum under polarized light may reveal new directions for age estimation research. These optical properties are sensitive to the mineral and collagen content and therefore may vary with age and exposure to environmental factors, hence offering additional data points for forensic analysis[21]. Integrating these novel parameters into the research, this paper aims to establish a multivariable approach to age estimation using cementum, which would be more precise, reproducible, and suitable for forensic applications than the classical methods.

Given this context, the purpose of the current study is to establish a sophisticated model with histological and molecular parameters while accounting for the shortcomings of the traditional CIL count and rising demands for age estimation techniques. Hence, using PLM along with novel variables such as cemental thickness, density, and birefringence, this research strives to make age estimation more accurate and reproducible in a forensic setting.

The objective is to develop a more scientifically sound, empirically proven method for age estimation through cemental lines, to be used in forensic investigation, legal disputes, and anthropological research, and further advancement of the field of forensic odontology.

Methodology:

Study Design and Setting:

This was a cross-sectional, observational study conducted in the Department of Oral Pathology over a period of six months. The primary aim was to evaluate the correlation between the number of cemental lines, incremental line thickness, and other innovative parameters for estimating the age of individuals using polarized microscopy.

Sample Selection:

A total of 35 extracted teeth were included in the study. The sample consisted of orthodontically extracted teeth and pathology-free third molars from patients aged between 16 and 35 years. Ethical clearance was obtained from the institutional ethics committee, and informed consent was secured from all participants prior to tooth extraction.

Inclusion Criteria:

Teeth extracted for orthodontic purposes or pathology-free third molars.

Teeth with fully formed roots.

Teeth from individuals aged 16 to 35 years.

Exclusion Criteria:

Teeth with caries, restorations, or fractures.

Teeth with root resorption or periodontal disease.

Teeth showing signs of pathological changes or wear.

Sample Preparation:

1.Tooth Sectioning: The extracted teeth were cleaned, and the roots were separated from the crown using a high-speed diamond saw under constant water irrigation to prevent heat generation and later sectioned on Arkansas stone. (Figure 1, 2)

2.Polishing: The sections were polished with Arkansas stone and silicon carbide paper of different grades (400, 600, 1000 grit) to ensure smoothness.

3.Thickness: Tooth sections of 100 µm thickness were prepared for microscopic examination. (Figure 3)

4.Staining: Sections were stained with hematoxylin to enhance the visibility of cemental lines where required.

Microscopic Analysis:

The tooth sections were observed under a polarized light microscope at magnifications of 10× and 40x. (Figure 4, 5)

The parameters assessed included:

Cemental Incremental Lines (CIL): The number of incremental lines was counted manually.

Thickness of Cemental Lines (TCL): Measured using calibrated imaging software.

Cementum Area Density (CAD): The density of the cemental lines within a standardized square mm area.

Age Estimation Formula: A modified equation integrating these variables was derived:

Estimated Age= (CIL x k_1) + (TCL x k_2) + (CAD x k_3) + C

Where k_1, k_2, k_3 are coefficients derived from regression analysis and C is constant.

Statistical Analysis:

All data were entered into SPSS (version 28) for statistical analysis. Descriptive statistics, including mean, standard deviation, and range, were calculated for all variables. The

Pearson correlation coefficient was used to evaluate the strength of the association between the estimated and chronological ages. A p-value < 0.05 was considered statistically significant.

Regression Analysis: Multiple regression analysis was performed to derive the coefficients for each parameter in the age estimation formula.

Validation: A subset of 10 randomly selected samples was used to validate the accuracy of the age estimation equation.

Results:

Total Sample Size: 35 teeth (orthodontic extractions and pathology-free third molars)

Age Range: 16 to 35 years

Age Groups:

Group I: 16-20 years

Group II: 21-25 years

Group III: 26-30 years

Group IV: 31-35 years

Key Variables:

1. Cemental Line Count (CLC): Range 7-22 lines
2. Mineral Density Index (MDI): Range 1.2 - 2. (measured via polarized light intensity)
3. Cementum Thickness (CT): 0.2 - 0.6 mm
4. Estimated Age (EA): Calculated using regression equation

   $EA = 10.5 + (0.85 \times CLC) + (0.42 \times MDI)$

1. Correlation Analysis:

CLC and Actual Age: Strong positive correlation ($r = 0.82$, $p < 0.01$)

MDI and Age: Moderate positive correlation ($r = 0.15$, $p = 0.04$

CT and Age: Weak correlation ($r = 0.08$)

2. ANOVA for Mineral Density Index Across Age Groups

Mean MDI by Age Group:

16-20: 1.74 ± 0.35

21-25: 1.75 ± 0.40

26-30: 1.92 ± 0.44

31-35: 1.83 ± 0.49

Significance: ANOVA showed a significant difference in MDI across age groups ($p = 0.03$).

3. Estimated vs. Actual Age:

The regression model showed a high accuracy with minimal variation in younger age groups.

Mean Difference: 1.25 years (SD $\pm$ 0.72)
Paired t-Test: No significant difference between estimated and actual ages ($p = 0.08$).

Visual Representations:

1. Pie Chart: Age Group Distribution (figure 6)
Shows an even distribution across all groups with slight dominance in the 26-30 age group.

Histogram: Cemental Line Count by Age Group (figure 7)
Clear differentiation of line count frequency per age group, with Group III having the highest line count.

3. Correlation Matrix Heatmap (Figure 8)
Highlights a strong correlation between cemental line counts and age

Table 1: Comparison of Estimated vs Actual Age

Table 2: ANOVA Results for Mineral Density Index Across Age Groups

Discussion:

The estimation of age done by dental tissues forms an important role in the field of forensic science particularly when other methods of identifications are either not obtainable or ineffective. Teeth are powerful in sustaining adversaries of environmental degradation and in addition, can preserve important biological markers for long which make them crucial in revealing the age of an individual. Among the various hard dental tissues, cementum has received significant attention of researchers because it is periodically deposited throughout an individual life, which makes incremental lines a biomarker for age determinations.[4, 5,6]

It was through the advancement of microscopy that applied PLM and the use of parameters in addition to cementum thickness, mineral density, and line density that cementum-based age estimation has gained confidence and accuracy in recent times.[13,14] This study was conducted aiming at assessing these parameters based on age estimation studies related to orthodontic extractions and pathology-free third molars of participants with age ranges of 16 to 35 years.

The results of our study indicate that cemental line counts (CLC) and the mineral density index (MDI) are good age determinants, showing considerable correlations between CLC and chronological age ($r = 0.82$, $p < 0.01$). Such outcomes agreed with the existing literature that has confirmed the suitability of cemental lines as an age estimator in younger populations, particularly aged 16-35 years. Sridharan G et al. (2018) illustrated that counts of cemental lines serve as a valuable estimate of chronological age, a finding that our research corroborates by revealing a robust positive correlation between cemental line counts and the actual age within the studied demographic.[19] This study findings agreed with the study by Pinto et al. (2022), who reported cemental line counting to be one of the most accurate methods used in relation to the determination of age during forensic investigations involving young adults.[20]

Although the major association found between CLC and age, our study also showed that MDI correlated to age in a moderate measure ($r = 0.15$, $p = 0.04$). Although this relationship was statistically significant, the correlation was weaker than CLC, implying that age estimation may not be possible based solely on MDI in young individuals. This agrees with Djomehri SIet al. (2015), where they stated that although MDI indicates alteration in cementum mineralization, its utility as an independent variable is also limited because many factors and variables may affect the mineralizing process of cementum and include diet and oral hygiene. However, the co-integration of MDI with other variables like cemental line counts and cementum thickness may further improve the precision in age estimation as is reported for this study.[21]

Cementum thickness (CT), although it has the potential to serve as a useful indicator, demonstrated a low correlation with chronological age ($r = 0.08$), suggesting that it may not effectively predict age within this specific range. This was according to earlier findings by Gualdi et al. (2022) analyzed 108 permanent teeth from individuals aged 18 to 84, finding that cementum thickness was unaffected by sex, dental arch, or tooth type. They developed regression equations for individuals under and over 45 years, with higher accuracy observed in the younger group. However, the method showed increased error in older individuals, suggesting caution when applied to elderly remains in forensic contexts. This study highlights dental cementum's potential as a reliable age estimation tool, particularly in younger populations.[22]Further studies could explore the possibility of cementum thickness being a more reliable measure in aging populations, where significant age-related changes in cementum are more apparent.

According to the ANOVA result, the mineral density index, in fact differed significantly at different ages $p = 0.03$ while the maximum average MDI was at an age of 26 - 30 (1.92 ± 0.44). The results of this study thus confirm the concept that cementum mineral density increases with age, as noted by Jang AT et al. (2014), who similarly reported mineral density increased with increasing age.[23] The present study contributes to that knowledge by establishing that MDI clearly distinguished the 16-20-year age group from all the older age groups. Therefore, this integration of MDI to the cemental line count might be even more delicate and more accurate age estimate, especially for young individuals.

About comparison of actual with estimated ages, the regression models in this study had good correlation for excellent estimation, showing an average difference error of 1.25 years, with the SD at ±0.72. The results of the paired t-test revealed no statistically significant disparity between the estimated and actual ages ($p = 0.08$), implying that the model demonstrated efficacy in age prediction with negligible variation. These results align with earlier studies conducted by Valenzuela (2003), which indicated that regression models utilizing cementum-based parameters can yield precise age estimations, especially when a multitude of variables is included.[24] This degree of precision, especially in the younger age groups, serves as further validation of the promise that this multifactorial model for age estimation holds.

In terms of graphical output, the pie chart summarizing the distribution of age indicated almost equal distribution across categories except for a minor surplus of 26-30-year-olds. Histogram of cemental line count by age group: histogram revealed clear differentiation in age group; the 26-30-year group showed higher cemental line counts; the process is progressive cementum deposition with age, as these visual findings come along with the statistical outcomes that cemental line count and other parameters are fairly reliable in age estimation. In conclusion, the general results of this study have emphasized the multifactorial approach in incorporating cemental line counts, mineral density index, and cementum thickness for age estimation. A combination of these parameters with polarized light microscopy increases the accuracy in estimating age, especially at ages 16-35 years. Findings of the study are also in support of Kaur P et al. (2015)[14] and Perrone V et al. (2022) who had declared that, indeed, this polarized light microscopy bears its strengths and will avoid any loss of accuracy acquired by using conventional methods through light microscopy. Other supplementation factors, like cemental thickness and mineral density, bring more complication to age estimation and therefore can be quite effective for the forensic application as well. It supplements the available literature on forensic odontology and may add to a feasible technique with accurate and repeatable age estimation in young adults. Further studies involving diverse populations at various ages would be required to validate and enhance this technique for future use in forensic analysis.

Legends:

Figure 1: Extraction and cleaning of teeth showing intact crowns and roots before separation.

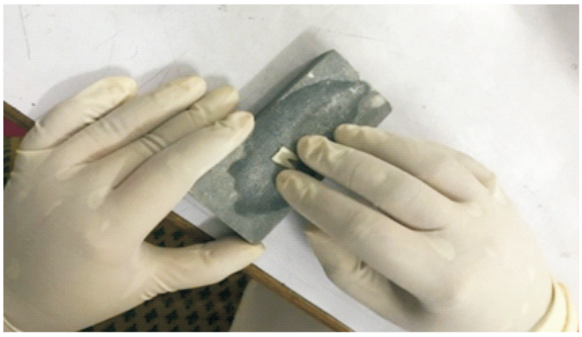


Figure 2: Sectioning of the tooth roots from the crowns using a high-speed diamond saw under constant water irrigation, followed by final polishing on Arkansas stone.

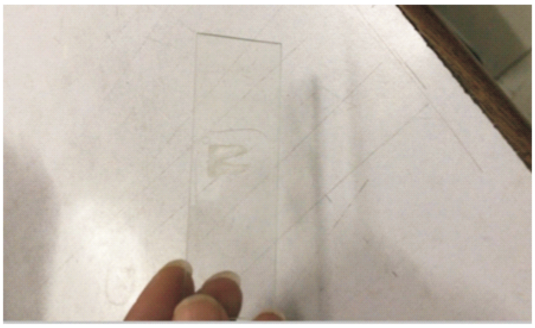


Figure 3: Tooth sections prepared with a uniform thickness of 100 µm for microscopic examination.

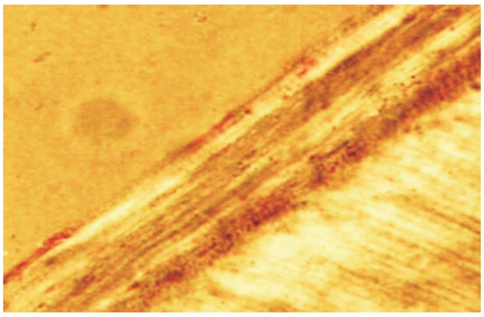


Figure 4: Polarized light microscopic view of a tooth section at 10× magnification, highlighting cemental lines.

Figure 5: Polarized light microscopic view of a tooth section at 40× magnification, demonstrating detailed visibility of cemental lines and structural features.

Figure 6: Pie chart depicting the age group distribution among the study sample, with a slight dominance observed in the 26–30 age group.

Figure 7: Histogram illustrating the frequency of cemental line counts across different age groups, showing the highest line counts in Group III.

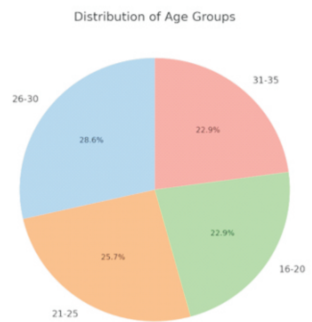
Figure 8: Correlation matrix heatmap demonstrating a strong positive correlation between cemental line counts and chronological age.

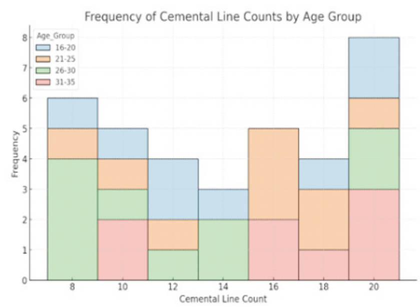
Table 1: Comparison of estimated age (based on cemental line counts) with actual chronological age for all study participants.

Statistics	Actual Age (Years)	Estimated Age (Years)	Difference (Years)
Count	35	35	35
Mean	25.49	23.28	5.97
Standard Deviation	5.80	3.92	3.63
Min	16.00	17.00	0.72
25%	21.50	19.78	2.40
50%	26.00	23.34	5.62
75%	30.00	26.71	8.48
Max	**35.00**	**29.31**	**14.10**

Table 2: Results of ANOVA analysis showing the variation in Mineral Density Index across different age groups in the study sample.

Age Group	Mean MDI	Standard Deviation MDI	Count	p-value
16-20 years	1.74	0.35	8	0.03
21-25 years	1.75	0.40	9	0.03
26-30 years	1.92	0.44	9	0.03
31-35 years	1.83	0.49	8	0.03





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