Original Article

Comparative Study on Pulp to Tooth Volume Ratio, Root Dentin Translucency, and Incremental Lines of Cementum in Age Estimation of Human Premolars

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ABSTRACT

Objective: The present study aimed to compare the validity of different dental markers as pulp to tooth volume ratio, root dentin translucency, and cementum annulation in chronological age determination. The reliability of the results was amended using (multiple linear regression equation) through the measurements obtained from pulp to tooth volume ratio and root dentin translucency.

Materials and Methods: 150 extracted maxillary first premolars were investigated using Cone-beam computed tomography imaging and Mimics software application to obtain pulp to tooth volume ratio, then ground sections were prepared to measure the length of the vertical extension of the root dentin translucent zones using polarized light microscopy. To count incremental lines of cementum, phase contrast microscopy was used. Comparison between the three methods to the chronological age was carried out to obtain the most reliable one.

Results: The linear regression equation using root dentin translucency, and for cementum annulation were both statistically significant ($p<0.001$). The multiple linear regression model showed a significant effect on age prediction ($p<0.001$).

Conclusion: Our comparative study which is age related concluded that cementum annulation using phase contrast microscopy gave the best and most reliable results in correlation to the chronological age (mean value of -0.01) followed by root dentin translucency using polarized light microscopy (mean value -0.018). Pulp to tooth volume ratio using Cone-beam computed tomography imaging and Mimics software revealed the weakest results in relation to the chronological age. Thus, multiple linear regression model in cementum annulation and root dentin translucency can be used to increase the reliability of the obtained results.

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Key Words: Age determination; cementum annulation; cone beam computed tomography; dentin translucency; forensic dentistry.

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INTRODUCTION

Estimating age has a significant function in clinical work, as well as criminal and civil proceedings. Age estimation was generally utilized in determination of clinical treatment plans, judgement of criminal responsibility, and identity verification of human remains[1]. The cornerstone of forensic science is the recognition of either the living or the deceased individuals through using the distinctive qualities and features of the teeth and jaws[2].

Non-invasive radiographic approaches were developed for the measurement of secondary dentin deposition, thus causing reduction of the dental pulp cavity with the advancement of age[3]. Cone beam computed tomography (CBCT) has shown to be a reliable and practical approach for measuring the reduction of the pulp volume of the teeth[4]. On the other hand, dentin translucency that occurs in the root portion is one of the changes that could be traced with increasing age[5]. Dentin translucency is a term that describes the dentin sclerosis where the dentinal tubules have become occluded with minerals deposition[6]. Different approaches were previously used to observe and quantify root dentin translucency using the vernier caliper, different types of microscopes, and computer-based customized software[7]. On the flip side, cementum annulation refers to alternation of light and dark bands that results from variations in the relative orientation of collagen fibers and/or their mineralization[7]. In compliance with the literature, cemental annulations have shown positive correlation with age estimation in human studies[8].

"Multiple linear regression model" is a statistical equation which allows for the inclusion of different parameters (independent variables) to predict an outcome (dependent variable) and evaluate the combined effect of the parameters on the result[9].

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$$Y = \beta 0 + \beta 1\ X1 + \beta 2\ X2$$

Where, (Y) is the dependent variable, (β0) is the intercept, (β1) is the slope for (X1), and (X1) is the independent variable.

To foster the previous trials, the present study aimed to compare the accuracy of the mostly used dental markers (pulp to tooth volume ratio, root dentin translucency, and cementum annulation) to determine the chronological age. The reliability of the results was amended using (multiple linear regression equation) through using the measurements of root dentin translucency and pulp to tooth volume ratio.

MATERIALS AND METHODS

The present study was applied on a sample of 150 freshly extracted, sound maxillary first premolars with fully developed roots with age range 20-39 years old. The study was encompassing a heterogenous samples of both females and males. obtained from the department of Oral & Maxillofacial surgery (Faculty of dentistry-Ain Shams University), and Giza Orthodontic Foundation. The study was proceeded after receiving an ethical clearance from the Research Ethics Committee of Faculty of Dentistry-Ain Shams University (FDASU-RecEM012157).

Teeth grouping

The teeth samples were allocated into two groups based on the age; group (I) included age group 20 to 29 years old (45 females and 30 males), while group (II) included age group 30 to 39 years old (45 females and 30 males). Inclusion criteria were teeth extracted for orthodontic reasons, with intact root surface.

CBCT Imaging & Mimics Software Application

Teeth Coding and preparation

Each tooth sample was numbered using a permanent marker in order to be identified and to specify it to its original data (age and gender). Then the samples were arranged and mounted on modeling wax (to overcome the radiation hazards of handling each sample by the researcher) to be scanned using CBCT as represented in (Figure 1).

Fig. 1: A Photograph showing teeth samples arranged and mounted on modeling wax to be scanned using CBCT

CBCT Images data Processing and importing

CBCT images were acquired using Rayscan alpha plus (Ray S Korea), at RAY SCAN Center, Cairo, Egypt. Through applying the following settings; the tube current: 85 kVP, exposure time: 14 s, the field of view: 5 X 10 cm, voxel size 100 µm. Before acquisition, a scout view was obtained, and corrections were done to guarantee that every tooth was accurately positioned in the scanner in accordance with the light beam adjustment. Images exporting was done in digital imaging and communications in medicine images format (DICOM). Further steps of segmentation and calculation of pulp to tooth volume ratio (PV/TV) as was previously described in the literature[10].

Histological Analysis

Ground sections preparation

Once the CBCT images were obtained, the teeth were prepared for histologic analysis. Teeth were first cleaned using running tap water for 20 minutes, sectioned manually using diamond discs (BLYOJGSSP (C00/190-Sliver)), diameter: 19mm, thickness: 0.25mm under continuous sufficient irrigation by saline in a bucco-lingual plane to 100µm through measurements obtained using vernier caliper , in a close proximity to the central axis of the pulp[8]. The sections were placed on a glass slide after being cleaned with xylene using Dibutylphthalate Polystyrene Xylene mountant (DPX) and a cover slip, then slides were dried, examined using both polarized light, and phase contrast microscopes. An individual identification number was used to uniquely identify each image.

Measuring The Length of the Vertical Extension of the Root Translucent Zones Using Polarized Light Microscopy:

Each prepared ground section was examined using the light microscope (Olympus BX60) in the mode of Polarized light field for observation of the translucent root dentin under magnification 4X, in the Precision Measurement Unit, Oral Pathology Department, Faculty of Dentistry, Ain Shams University. A digital camera was used to take photographs of the root translucent zones (EOS 650D, Canon Japan) mounted on the light microscope. Image J 1.53e (java_version 1.8.0_172) a program for image analysis, was used to measure the captured images. On both sides of the midline, the length of the vertical extension of the translucent zones was measured independently, and the average of both sides was calculated. Under a constant source of light, the root total vertical length of each section was measured using a digital plastic vernier caliper (INGCO, HDCP16150). The length of the vertical extension of the root dentin translucent zone to the total root length (TL/RL) ratio was subsequently calculated. (TL/RL) was used in a statistical regression analysis to obtain an age estimation formula[11,12].

Counting the Incremental Lines of Cementum Using Phase Contrast Microscopy:

To facilitate the incremental lines of cementum counting, we selected the middle third of the root cementum[13,14]. An area where the lines appeared to be relatively parallel[14–16], and acellular cementum was present[15–17]. Apical third of the root cementum was ruled out due to increased cellularity and thickness of cementum in that area[18]. The prepared sections were examined and studied using microscope Leica Microsystems CMS GmbH (Version 4.12.0) in phase contrast mode with (×200 magnification)[19]. Micrographs for each section were taken with a 200× objective with the help of DMC4500 digital camera mounted on the microscope. Photomicrographs were visualized using Image J 1.53e (java_version1.8.0_172) image analysis software. Cementum thickness was measured in three different regions where the lines are approximately parallel, and calculations were made using the average of the three measurements as "X" i.e., the total width of cementum (from the cemento-dentinal junction (CDJ) to the outermost layer of cementum). The alternating light and dark bands were observed in photomicrographs, then, the width occupied by two successive bands (i.e., one dark and one light) was measured and designated as"Y". The following formula was used to determine the total number of incremental lines in the total thickness of cementum: (n) = X/Y.

The individual`s estimated age was determined by the summation of the incremental lines of cementum and the average age of the eruption in years (t)[20] as follow:

The estimated age (E) = Incremental lines of cementum total number (n) + average age of eruption (t)[17,19].

Statistics

Categorical data was presented in the form of percentage and frequency values. Numerical data was highlighted as mean with 95% confidence intervals, minimum, maximum values, and standard deviation. The data explored for normality by checking the data distribution using Shapiro-Wilk test. All numerical data were distributed normally except for age difference. Age prediction was done utilizing linear regression models. Difference between methods for parametric data was analyzed using paired t-test while for the age difference it was analyzed using Freidman's test. Reliability analysis was done using Intra-class correlation coefficient. Statistical analysis was performed with R statistical analysis software version 4.1.3 for Windows. The significance level was set at $p \leq 0.05$ within all tests[21].

RESULTS

CBCT Results

Age Estimation Equation

The variables for the regression model predicting the chronological age presented in (Table 1). The model was statistically nonsignificant (p=0.709) for the estimate age compared with the actual ages, indicating nonsignificant prediction of (PV/TV) in estimating age for both age groups (I) and (II).

The regression model equation was:

$$\textbf{Age}=41.11(PV/TV)+25.64$$

The equation is obtained from the output data of the R statistical analysis software, where, the estimated age is obtained from the summation of the intercept (β0) with value (25.64) and the slope coefficient regression (X1) with value (41.11) multiplied by the independent variable (β1) which is the value of the (PV/TV) ratio.

Descriptive statistics

Descriptive statistics for (PV/TV) measurements presented in (Table 2).

Histological Analysis Results

Polarized Light Microscopy Results

Examination of the ground sections of the extracted teeth at their root segments from the apex toward the crown using the light microscopy in the mode of polarized light field revealed the normal structure of pulp, dentin and cementum in different colors due to difference in refractive indices of different tissues. Translucent zones were seen as clear, whitish zones devoid of any apparent dentinal tubules extending from the root apices toward the crown at different regions on both sides of the midline of the roots. Photomicrographs were demonstrated using Image J 1.53e image analysis software to apply tools to measure the vertical length of the translucent root dentin extending in different regions on both sides of the midline of each sample as presented in (Figure 2).

Age Estimation Equation

Parameters for the regression model predicting age from the ratio of the length of the root dentin translucent zones to the total root length (TL/RL) were presented in (Table 3). The model showed that (TL/RL) significantly predicted age ($p<0.001$) for both age groups (I) and (II).

The model equation was:

$$\textbf{Age}=12.01(TL/RL)+248.47$$

The equation is obtained from the output data of R statistical analysis software, where the estimated age is obtained from the summation of the intercept (β0) with value (248.47) and the slope coefficient regression (X1) with value (12.01) multiplied by the independent variable (β1) which is the value of the (TL/RL) ratio.

Descriptive statistics

The descriptive statistics for (TL/RL) measurements presented in (Table 4).

Phase Contrast Microscopy Result

The examination by microscope Leica Microsystems in phase-contrast mode of the incremental lines of cementum in the middle third region (acellular cementum) of the prepared sections of this group specimens' roots revealed the normal structure of cementum, showing the incremental lines of Salter parallel to each other and in a parallel direction to the cemento-dentinal junction which appear well defined and perpendicular to the dentin structure containing well defined dentinal tubules and showing Tome`s granular layer adjacent to the cemento-dentinal junction. Photomicrographs were visualized using Image J 1.53e image analysis software to apply a ruler to measure the total width of cementum (from cemento-dentinal junction (CDJ) to the outermost layer of cementum). Another ruler was applied to calculate the width that two adjacent incremental lines occupied (i.e., one dark and one light) which were most easily recognizable as represented in (Figure 3).

Age Estimation Equation

The equation used for age estimation based on cementum annulation was:

Age=Total number of cementum annulations+Average age of eruption of that tooth[17,19]

Descriptive statistics

The descriptive statistics for cementum annulation measurements were presented in (Table 5).

Difference of results of all methods from the chronological age.

Results according to T-test showed that for (PV/TV) the mean difference of estimated age was lower than that of the actual age. For (TL/RL) and cementum annulation, the estimated and the actual ages both had close mean difference values. Reliability analysis showed a strong statistical significance agreement between the estimated and the actual age for (TL/RL) and cementum annulation (ICC>0.9, $p<0.001$), while for (PV/TV) the statistical agreement was nonsignificant ($p=0.443$). The difference in reliability of age estimation results in the error bars shows that (PV/TV) ratio estimated age in relation to actual indicate gross difference, while that of (TL/RL) ratio and cementum annulation shows nearly low difference presented in (Table 6, Figure 4).

"Multiple linear regression equation" using pulp to tooth volume ratio and root dentin translucency

Parameters for the regression equation predicting age from both ratios (PV/TV) and (TL/RL) presented in (Table 7). The model showed that the estimated age of (TL/RL) is significant in correlation to the actual age ($p<0.001$), yet the effect of estimated age of (PV/TV) in relation to the actual age was statistically nonsignificant ($p=0.297$). As a result, the (TL/RL) ratio was only used in the multiple linear regression equation to amend the reliability of the results.

The model equation was:

Age=12.01(TL/RL)+248.47

The equation is obtained from the output data of R statistical analysis software, where, the estimated age is obtained from the summation of the intercept (β0) with value (248.47) and the slope coefficient regression (X1) with value (12.01) multiplied by the independent variable (β1) which is the value of the (TL/RL) ratio.

Comparison of reliability of the different methods

There was not a significant difference between the chronological age and the measurements of the used methods (root dentin translucency and cementum annulation) ($p<0.001$) as the obtained results of the estimated age for both methods show close relation to the actual age, while for pulp to tooth volume ratio the results show nonsignificant prediction of estimated age in relation to actual age ($p = 0.443$).

Comparison of the reliability of the different methods presented in (Table 8, Figure 5).

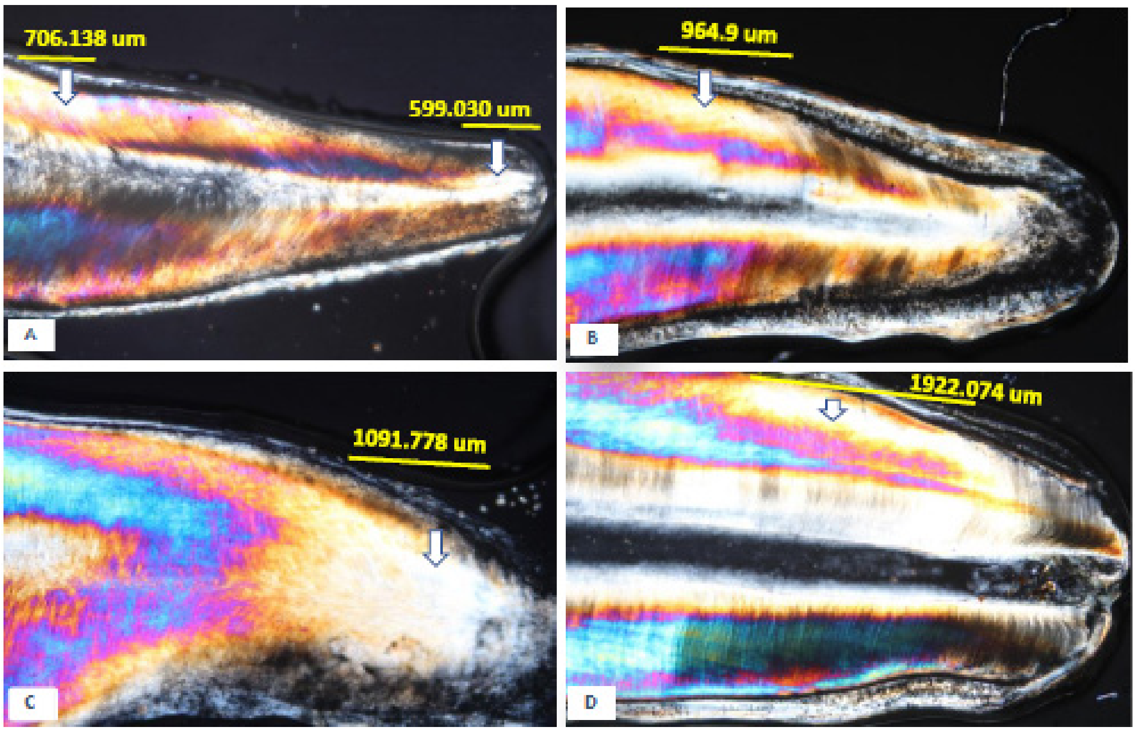

Fig. 2: Photomicrographs showing translucent dentin under polarized light microscopy. Ground sections of study sample roots group I (A (female), B (female)); short vertical extension of translucency, group II (C (male), D (male)); long vertical extension of dentin translucency. Apico-coronal vertical extension of translucency at different zones (white arrow) which is whitish areas devoid of dentinal tubules. The ruler measures the length of the vertical root dentin translucent zones extensions (yellow line), (the original magnification 4X).

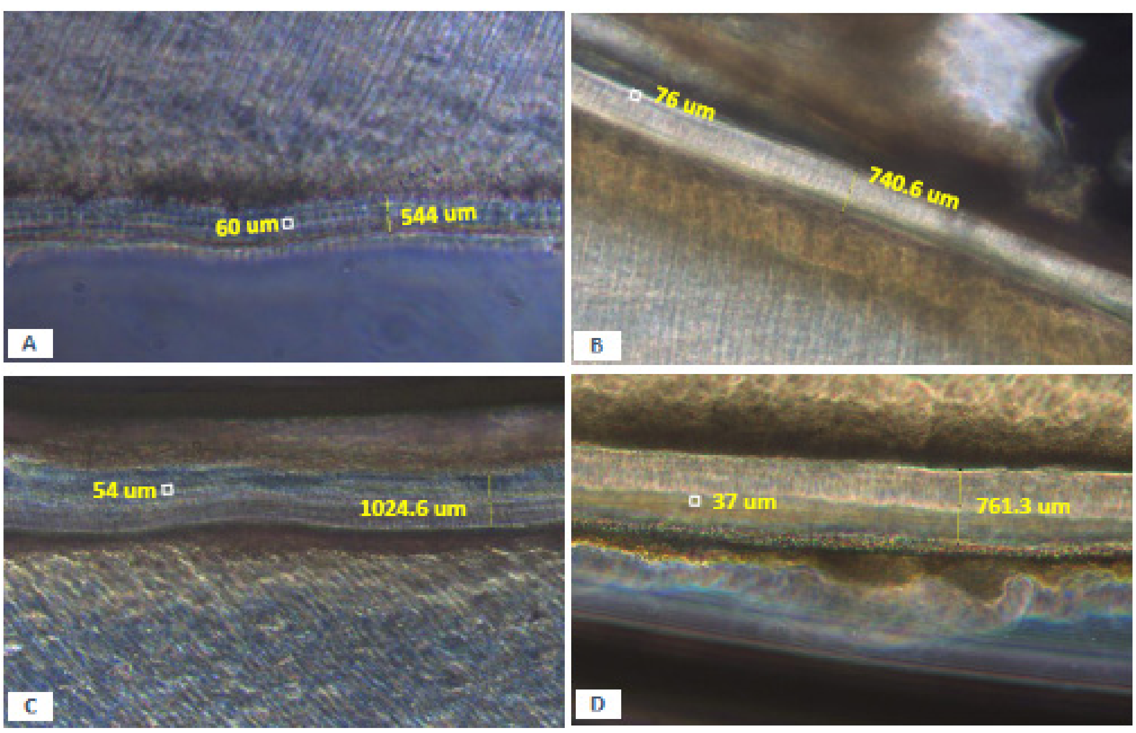

Fig. 3: Photomicrographs showing incremental lines of cementum under phase contrast microscopy. Ground sections of root cementum of study samples root in group I (A (female), B (female)); Narrow total thickness of cementum and less count of incremental lines and, group II (C (male), D (female)); Increased total thickness of cementum with higher count of incremental lines, showing the ruler measuring the total width of cementum (yellow line), the width occupied by two successive bands (i.e., one dark and one light) (white square), (the original magnification 200X).

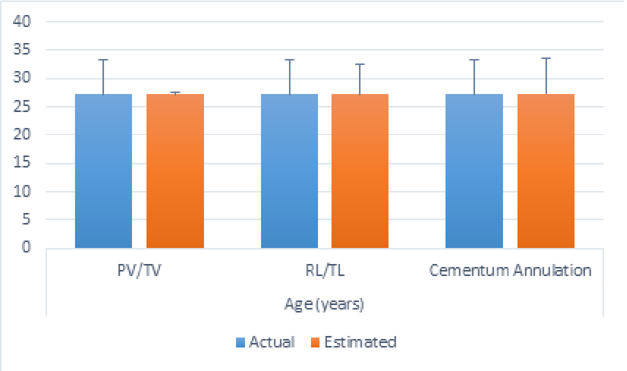

Fig. 4: Bar chart showing mean and standard deviation values (error bars) for actual and estimated ages (years).

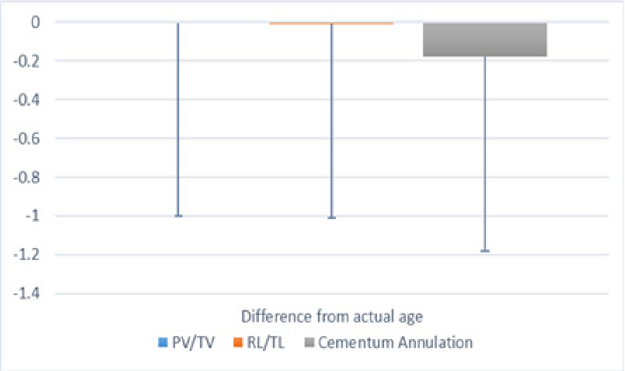

Fig. 5: Bar chart showing mean and standard deviation values (error bars) for the difference in each method from actual age.

Table 1: Regression model predicting age from pulp to tooth volume ratio (PV/TV)

Parameter	Regression coefficient	95% CI		SE	Statistic	*p-value*
		Lower	Upper			
Intercept	25.64	17.03	34.26	4.21	6.1	<0.001*
PV/TV	41.11	-182.54	264.76	109.18	0.38	0.709ns

*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

Table 2: Descriptive statistic for (PV/TV).

Parameter	Mean	95% CI		SD	Minimum	Maximum
		Lower	Upper			
Pulp volume (mm^3)	21.51	19.96	23.07	4.35	13.49	27.82
Tooth volume (mm^3)	598.00	573.44	622.55	68.62	424.00	734.50
PV/TV	0.04	0.03	0.04	0.01	0.02	0.06
Estimated age	27.16	27.00	27.32	0.44	26.40	28.14

Table 3: Regression model predicting age from (TL/RL)

Parameter	Regression coefficient	95% CI		SE	Statistic	*p-value*
		Lower	Upper			
Intercept	12.01	8.4	15.61	1.76	6.83	<0.001*
TL/RL	248.47	192.79	304.15	27.18	9.14	<0.001*

*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

Table 4: Descriptive statistic for (TL/RL) measurements.

Parameter	Mean	95% CI		SD	Minimum	Maximum
		Lower	Upper			
Dentin translucency average length (mm)	0.82	0.72	0.91	0.27	0.36	1.30
Total root length (mm)	13.60	13.08	14.12	1.45	12.00	16.70
TL/RL	0.06	0.05	0.07	0.02	0.02	0.09
Estimated age	27.17	25.24	29.11	5.41	17.90	34.52

Table 5: Descriptive statistic for cementum annulation measurements.

Parameter	Mean	95% CI		SD	Minimum	Maximum
		Lower	Upper			
Total thickness of cementum in middle part of root (µm)	807.65	675.46	939.83	369.39	329.00	2232.00
Distance between two alternating incremental lines of cementum (µm)	52.57	43.12	62.02	26.40	25.00	118.00
Ratio	16.57	14.34	18.80	6.23	9.00	25.00
Estimated age	27.35	25.11	29.59	6.27	19.55	35.94

Table 6: Difference in reliability of age estimation.

Parameter	Mean±SD		Mean difference (95%CI)	ICC (95% CI)	*p-value*
	Actual age	Estimated age			
PV/TV	27.17±6.25	27.16±0.44	0.00 (-2.32:2.33)	0.053 (-0.989:0.549)	0.443[ns]
RL/TL	27.17±6.25	27.17±5.41	0.01 (-1.16:1.17)	0.925 (0.844:0.964)	<0.001*
Cementum Annulation	27.17±6.25	27.35±6.27	0.18 (0.00:0.37)	0.998 (0.996:0.999)	<0.001*

*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

Table 7: Regression model predicting age from both ratios

Parameter	Regression coefficient	95% CI		SE	Statistic	*p-value*
		Lower	Upper			
Intercept	9.79	4.2	15.38	2.72	3.6	0.001*
PV/TV	58.22	-54.1	170.53	54.74	1.06	0.297[ns]
TL/RL	249.45	193.78	305.12	27.13	9.19	<0.001*

*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

Table 8: Comparison of the reliability of the different methods

Difference from actual age (Mean±SD)			*p-value*
PV/TV	RL/TL	Cementum Annulation	
0.00±6.23	-0.01±3.13	-0.18±0.50	0.648[ns]

*; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$)

DISCUSSION

Teeth are known to be the most effective human body part for forensic age determination. since they are usually not significantly altered by environmental factors or the process of decomposition[22]. The reduction of the size of the pulpal cavity is one of the remarkable dental indicators that can be used to estimate age. caused by the deposition of secondary dentin[23,24]. Several earlier reports support that with age, the dentin translucency and cemental apposition readily and gradually increase which prove that dentin and cementum are the ideal tissues to investigate the aging process because of the gradual and ongoing changes that occur through them[15].

As a result of relative deficiency regarding current research comparing the reliability of pulp-tooth volume ratio (PV/TV), root dentin translucency extension, and cementum annulation methods in the estimation of age of adults, the present study aimed to compare which method gives a greater degree of reliability for forensic investigations. Pulp to tooth volume ratio and root dentin translucency methods collectively were used to obtain more reliable data for age determination.

In the current study, we selected premolars as the study sample as they are reliable age indicators[25].

The reduction in the size of the pulp cavity was correlated with the chronological age, through which regression equations to estimate age were developed[3]. We used Cone Beam Computed Tomography (CBCT) to calculate (PV/TV) as CBCT is known to be reliable for studying the anatomy of the root canal system providing a three-dimensional volumetric analysis through using a single scan of a living person's teeth and can be operated with non-destructively[10]. Mimics software was used in this study instead of other softwares as most studies stated that it requires about 2-3 hours per tooth to complete the process of importing the DICOM information into the software, thresholding, and 3D reconstruction[26–29], but through using mimics software less than 15 minutes were needed to complete each tooth's dental volume measurements, and we could complete the process of 3D reconstruction in almost 20 minutes[29]. The dimensional accuracy of the craniofacial measurements obtained through the application of Mimics and InVesalius softwares showed nonsignificant difference (P>0.05) in the accuracy of both softwares[30].

Dentin translucency occur when numerous tubules in a single position are affected, the dentin takes on a glassy appearance and become translucent. It is demonstrated that sclerotic dentin is a physiological response and the occlusion of the dentinal tubules is enhanced by continuous deposition of peritubular dentin in the root dentin without any evident external influences[6]. We used root dentin translucency as a histological method to estimate age as it serves as a reliable technique to help determine both the living individuals and the deceased's age[30]. As age advances, the translucency gradually spread in the direction of the crown, eventually has an impact on the entire root[11].

Each prepared ground section was examined using the light microscope in the mode of Polarized light field for observation of the translucent zone of the root dentin in accordance to a study performed by Mohan *et al*., in 2020[31] which concluded that polarized light microscope complements the information available from the light microscope about the dentinal changes in teeth with attrition and normal teeth. We obtained the translucency data by measuring vertical length of the root dentin translucency because if the translucent zone vertical length is compared to translucent zone area, the vertical length shows more reliability to estimate age when compared with that of translucent zone area[12].

We used cementum annulation for age estimation because the cementum of teeth can be used for determining human chronologic age[32]. Summing up the incremental lines of cementum and the average age of tooth eruption can possibly provide a reliable technique to estimate the chronological age[7].

Phase contrast microscopy was used to assess the cemental annulations in agreement with the study conducted by Kaur *et al*., in 2015[16] who explained that the annulations may be seen more clearly using a phase contrast microscope than light or polarizing microscope. Incremental lines of cementum in the middle third region of the prepared sections of the roots were chosen for counting the annulations, since the cementum in the middle region of the root is typically acellular which is contradicted to the apical region, less compressed than the cementum near the cementoenamel junction (CEJ), and the annulations are easier to resolve under the microscope[8]. Our study followed the "mathematical approach" used in earlier studies[32] using the average eruption age for the maxillary first premolar in years according to the Egyptian population as presented by Elkhatib *et al*., in 2021[20].

Considering the obtained results of the present study, the (PV/TV) ratio linear regression equation model results of estimated age in correlation to chronological age was not statistically significant (p=0.709) as p value resulted from the regression equation is greater than the significance level indicating insufficient evidence in my study sample to be able to conclude that a correlation exists for (PV/TV) ratio with age estimation. According to the study findings, the (PV/TV) of maxillary first premolars in a heterogenous sample of males and females cannot be used to estimate age. Reliability regression analysis showed that there was no probability for pulp–tooth volume ratio to have a relation to the actual age. Age estimation was not accurate for the whole study sample may be due to using a single type of teeth (maxillary first premolar) to predict age rather than an average of different types of teeth, which is in disagreement with Previous research that demonstrated a significant inversely proportional relationship between age and (PV/TV) in all the teeth assessed and significant correlations in various types of teeth demonstrate that pulp volume decreases with aging[29,33].

Considering the results of the present study, the linear regression model revealed that root dentin translucency has a strong significance on age prediction (p<0.001). For dentin translucency vertical length to the total root length ratio, the actual and estimated ages both had nearly the same mean values. The outcomes of the current study are in agreement with Loganathan *et al*., in 2019[12] who concluded that root dentin translucencies and aging have a significant direct relationship. The ratio of the root dentin translucency (vertical length as a criterion) to age increases gradually and obviously and this pattern was especially noticeable in different decades of age. An individual's age can be determined by the amount of translucency observed in the root dentin region, and in agreement with Parra *et al*. in 2020[34] who found that one of the degenerative indicators that has demonstrated relevance and value in adults is dentin translucency, but that the combination of additional markers has strengthened the research. In contrast, this study results are in disagreement with Acharya *et al*. in 2011[35] who concluded that root dentin translucency didn`t show any correlation to the actual age, which revealed the lowest results among all markers, possibly caused by periodontal pathology and being applied on un sectioned teeth.

Our study model showed that the estimated age had a high mean value for cementum annulation in correlation to the actual age. Reliability analysis showed a strong statistical significance between the estimated and the chronological age (ICC>0.9, p<0.001), which is in agreement with Natesan *et al*. in 2017[36], Le Cabec *et al*. in 2019[37] who both concluded that cementum annulation showed a positive correlation to the actual age of the individual, and Swetha *et al*. in 2018[18] who concluded that the cementum annulation method is less time-consuming, an easy to perform, economically feasible, and not a technique sensitive method. In contrast, our study results are in disagreement with Colard *et al*. in 2018[38] and Padavala *et al*. in 2015[39] who concluded that cemental annulations cannot be used as a reliable age criteria as there is no significant correlation between the ages among all samples.

Reliability analysis showed that a strong statistical significance agreement between estimated and chronological age for root dentin translucency ratio and cementum annulation (ICC>0.9, *p*<0.001). Our study results are in agreement with Shruti *et al*. in 2015[15] who concluded that dentin translucency and cementum annulation are reliable in the middle age groups as that used in this study, and in agreement with Suciyanie *et al*. in 2022[7] who concluded that, the dentin translucency method is preferably used for age estimation, and subsequently the cementum annulation method. Since both methods reliably predict the actual age, utilizing just one dental marker to estimate age is likely to be promising.

The lack of sound teeth fulfilling the necessary inclusion and exclusion criteria, hindered us from collecting a greater number of extracted teeth. Upon using Mimics software, the need for some manual segmentation processes to fine-tuning the mask of the tooth and reduce any mask noise may be one of the factors that affect the reliability of the obtained data (pulp volume and tooth volume) which may have affected the final outcome. Although conventional tooth sectioning with microscopic observation was the gold standard, it is more time-consuming, destructive, and frequently necessary for postmortem cases where the utilization of numerous teeth is possible but not practical for living individuals where teeth must be extracted in order to use this technique of identification in the cases of medical-legal disputes. Our literature search (Pubmed, Google search: keywords (root dentin translucency using polarized light microscopy)) did not find any study for observation and measuring of the translucent zones of the root dentin which elongated the search and the study process of the dentin translucency.

CONCLUSION

Our comparative study which is age related concluded that cementum annulation by phase contrast microscopy gave the best and most reliable results in correlation to the chronological age with (mean value of -0.01). The middle third of the root was appropriate for counting the incremental lines of cementum and can be used as a reliable tool for age estimation in forensic dentistry. The multiple linear regression model in cementum annulation and root dentin translucency can be used to increase the reliability of the obtained results.

RECOMMENDATIONS

Future recommendation for saving the patients data and the establishment of a national data base for easier and more accurate dental identification using the antemortem records. When the antemortem dental records become accessible or can be collected rapidly, subsequently, the role of the forensic odontologist will be less challenging. Future studies on the Egyptian population are required to verify the precision of the digital approach technique without sectioning the tooth or compromising its integrity. in order to estimate age. Future analysis may require larger sample sizes to lower the estimation's standard errors.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة مقارنة عن نسبة حجم اللب إلى حجم السن، شفافية عاج الجذر، وخطوط تطبق الملاط في تقدير العمر للضواحك البشرية

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الهدف: تهدف هذه الدراسة إلى مقارنة دقة الدلالات المختلفة مثل نسبة حجم اللب إلى حجم الأسنان، شفافية عاج الجذر، وخطوط تطبق الملاط في معرفة العمر الزمني. تم تعديل دقة النتائج باستخدام (معادلة الانحدار الخطي المتعدد) من خلال القياسات التي تم الحصول عليها من نسبة حجم اللب إلى حجم السن وشفافية عاج الجذر.

الأدوات والطرق المستخدمة: تم فحص مائه وخمسون من الضواحك الأولي المخلوعة باستخدام تصوير الأشعة المخروطية المقطعية وتطبيق برنامج التصوير ميمكس، ثم تم إعداد مقاطع منحوتة لقياس الطول الرأسي لشفافية عاج الجذر باستخدام المجهر الضوئي المستقطب. لقياس أعداد خطوط تطبق الملاط تم استخدام مجهر تباين الطور. تم إجراء مقارنة بين الأساليب الثلاثة والعمر الزمني للحصول على الأسلوب الأكثر دقة.

النتائج: لم تكن معادلة الانحدار الخطي التي تنبأت بالعمر باستخدام حجم اللب إلى حجم السن ذات دلالة إحصائية في حين أن استخدام معادلة شفافية عاج الجذر و خطوط تطبق الملاط كانت ذات دلالة إحصائية (p=0.709). أظهر نموذج الانحدار الخطي المتعدد تأثيرا كبيرا على التنبؤ بالعمر. (P<0.001).

الاستنتاج: استنتجت دراستنا المقارنة أن خطوط تطبق الملاط باستخدام مجهر تباين الطور أعطي أفضل وأكثر النتائج دقة ارتباطا بالعمر الزمني (متوسط قيمة ٠,٠١-) يليه شفافية عاج الجدر باستخدام المجهر الضوئي المستقطب (متوسط قيمة ٠,٠١٨-). نتائج نسبة حجم اللب الي حجم السن باستخدام تصوير الأشعة المخروطية المقطعية وتطبيق ميمكس أظهرت النتائج الأكثر ضعفاً بالنسبة للعمر الزمني. ولذلك فان نموذج الانحدار الخطي المتعدد بخطوط تطبق الملاط وشفافية عاج الجذر يمكن استخدامه لزيادة دقة النتائج التي تم الحصول عليها.