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SECTION AGE ESTIMATION

A Comparative Study of Efficacy of Single Rooted and Double Rooted Teeth in Age Estimation Using Dentin Translucency

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ABSTRACT
BACKGROUND: Among various methods of age estimation using dental tissues, measurement of root dentin translucency (RDT) is said to be the most accurate. Numerous studies have estimated age by measuring RDT in single and double rooted teeth and have shown conflicting results. Only few studies have compared efficacy of using single and double rooted teeth for RDT measurement in age estimation.
AIM: To analyze the efficacy of single rooted teeth (SRT) and double rooted teeth (DRT) for measurement of sclerotic dentin (SD) and age estimation.
METHOD: Study was conducted on 120 freshly extracted SR and DR teeth with 60 teeth in each group. Ground sections of 150 µ thickness were observed under stereomicroscope and photographed. The sclerotic dentin length was measured on the images captured using image analysis software.
RESULTS AND CONCLUSION: The present study did not reveal significant difference (p = 0.012) between SRT and DRT when RDT was used for age estimation. However DRT provided more accurate age estimation than SRT with less mean age difference.

KEYWORDS: translucent dentin, single rooted teeth, double rooted teeth, age estimation.

INTRODUCTION

Age estimation has prime importance in reconstructive human identification especially when there is a lack of ante-mortem data. Dental identification assumes a key role when the deceased person is skeletonized, decomposed, burned, or dismembered". Unlike soft tissues, teeth are extremely resilient and are often well preserved after death without significant loss of micro-structure even after being subjected to temperatures as high as 1600°C.[1]

Various age estimation techniques have been proposed using different skeletal and dental tissues. These methods are either based on the well-ordered cascade of changes that occur during the formation and eruption of teeth or rely on continuous processes that alter the nature of the dental tissues even when individual growth is completed.[2]

Dentine is one of the dental hard substances that is continuously synthesized, maintained and repaired throughout life. Dentine is considered to be an ideal tissue for the study of aging process.[2]

Many methods of dental age estimation have been suggested using parameters such as cementum thickness, dental colour, tooth attrition, secondary dentine formation, and apical resorption. Root dentine translucency has been considered as one of the methods providing accurate results.[3]

Although many studies have been conducted regarding age estimation using root dentine translucency, conflicting results are presented in respect of the selection of teeth used in the studies. Some studies have suggested that the measurement of root dentine translucency in single-rooted teeth is the only technique to give accurate results[2,4]. Other studies have demonstrated that double-rooted teeth showed significant correlation with the known age in relation to the determined age compared to results obtained when single-rooted teeth were used.[5]

Therefore, the aim of the present study was to compare the accuracy of age estimation by the measurement of root dentine translucency using both double and single-rooted tooth.

MATERIALS AND METHODS

120 freshly extracted teeth with an equal number of single-rooted teeth (SRT) and double-rooted teeth (DRT) were collected from individuals between 20 -50 years of age. Following explanation of the study design, written consent and a detailed case history was recorded from each individual. Institutional ethical clearance was requested and granted.

Single-rooted and double-rooted teeth (maxillary first premolars, mandibular molars) extracted for therapeutic reasons were included in the study. Endodontically treated teeth, teeth with history of trauma, teeth associated with cysts and tumors and impacted and non-vital teeth were excluded. All the teeth were fully erupted.

Each study group was further subdivided depending on age as 21-30 years (S1 & D1), 31-40 years (S2 & D2), and 41-50 years (S3 & D3) in which S denotes single rooted and D denotes double rooted teeth.

Immediately after extraction, the teeth were thoroughly rinsed in normal saline solution and preserved in 10% neutral buffered formalin until ground sections were prepared. Each dried tooth was embedded in autopolymerizing acrylic resin and longitudinal sections of 150 µ thickness in the axio-bucco-lingual plane were obtained using a hard-tissue microtome (LEICA. SP1600, Germany) (Fig 1). The ground sections were mounted on a glass slide using DPX mounting media and a coverslip was placed over them. All slides were coded to ensure blinding and viewed using a stereomicroscope (Olympus SZx 12 Japan). The photomicrographs of ground

sections of teeth were captured using stereomicroscope attached to 3 chip CCD camera (Proview, media Cybernetics USA) (0.5 x magnifications) under 5x magnification objective and stored in the computer (17" monitor and Intel Pentium III processor windows 95/ NT/ 98, media cybernetics, USA) for further processing. Following calibration the length of the apical translucent dentine was measured in millimetres using image analysis software (Image Proplus version 4.1, media cybernetics, USA. The measurements were stored in Microsoft Excel for further statistical analysis.

In single-rooted tooth, the root dentine translucency was measured on the side of the root that showed the greatest translucency length apico-coronally (Fig 1). In double-rooted tooth, the root dentine translucency was measured from both the roots and mean of these measurements was calculated (Fig 2).

The modified Bang & Ramm's formula[6] for an Indian population is detailed below. By input of the measurements for each individual their age was estimated.

- Linear regression (if $T \geq 9mm$): $Age = 35.5619 + (3.4828 \times T)$
- Quadratic regression (if $T \leq 9mm$): $Age = 29.9074 + (7.4507 \times T) + (-0.4369 \times T^2)$

Where, T = Length of apical translucent dentin.

All of the data obtained were recorded in tabulated format and subject to statistical analysis using regression analysis and unpaired t-test for comparison with the study group. Student unpaired 't' test was used for the inter-group comparison and Tukey post-hoc test was used for the pair wise intra-group comparisons. Repeat measurements of the length of root dentine translucency were recorded by two observers and the measurements were subjected to paired t-test to assess potential intra- and inter-observer error.

RESULTS

The mean and standard error of estimated age using root dentine translucency in single-rooted and double-rooted teeth was 7.53±2.4 and 6.05±1.4 respectively. The mean age difference on inter-group comparison was statistically insignificant (p= 0.012). However, double-rooted tooth showed less mean age difference when compared to single-rooted teeth (Table 3).

The mean age difference between known age and estimated age showed a gradual increase with advancing age in both the study groups (Table 1 and 2).

Intra-group comparison in single-rooted teeth revealed statistically insignificant results similar to double-rooted teeth. However there was an exception between D3 and D1 where a statistically significant difference was observed (p=0.004) (Table 1 and 2).

Age wise intra-group comparison of single-rooted and double-rooted teeth revealed statistically insignificant results. However there was an exception between S1 and D1 where a statistically significant difference was observed (p=0.003) (Table 4).

Correlation analysis of single and double-rooted teeth was positive with a percentage correlation of 85% and 88% respectively and a standard error of 2.4 yrs and 1.4 yrs respectively (Table 5). A positive correlation was also found between root dentine translucency and advancing age (Table 6).

Inter-observer variability (Table 7) and intra-observer variability (Table 8) regarding the measurement of root dentine translucency in single and double-rooted teeth revealed there was no statistically significant difference.

Table 1 - Intra-group measurement and comparison of translucent dentin length in single rooted teeth

Single rooted teeth	Age groups (in years)	Mean value of transparent dentine in length (mm)	Difference between chronological age and estimated age (M±SE)	P value of ANOVA	Post Hoc Tukey test (Intra-group comparison)
S1	21-30	0.15	6.55±0.344	0.208 (NS)	S1 and S2 – 0.542(NS)
S2	31-40	2.04	7.65±0.822		S2 and S3 – 0.751(NS)
S3	41-50	4.66	8.40±0.913		S3 and S1 – 0.184(NS)

NS- Not Significant, mm-millimeter, M- Mean, SE- Standard Error.

Table 2 - Intra-group measurement and comparison of translucent dentin length in double rooted teeth

Double rooted teeth	Age groups (in yrs)	Mean value of transparent dentine in length (mm)	Difference between chronological age and estimated age (M±SE)	P value of ANOVA	Post Hoc Tukey test (Intra-group comparison)
D1	21-30	0.05	4.50±0.550	0.006(NS)	D1 and D2 - 0.144(NS)
D2	31-40	2.06	6.20±0.894		D2 and D3 – 0.244(NS)
D3	41-50	4.45	7.45±0.366		D3 and D1 – 0.004(S)*

S*- Significant, NS- Not Significant, mm-millimeter , M- Mean, SE- Standard Error

Table 3 - Intergroup comparison of translucent dentin length between single rooted and double rooted teeth

Study groups	Estimated age (M±SE)	Mean value of transparent dentine in length (mm)	Un-paired t test
Group 1- Single rooted teeth	7.55±2.4	2.37	0.012(NS)
Group 2 - Double rooted teeth	6.02±1.4	2.35	

NS- Not Significant, M- Mean, SE- Standard Error, mm-millimeter

Table 4 - Age wise intergroup measurement and comparison of translucent dentin length between single and double rooted teeth

Groups 1 versus Group 2	Age groups (years)	Un paired t test
S1 vs D1	21-30 yrs	0.003(S)*
S2 vs D2	31-40 yrs	0.229(NS)
S3 vs D3	41-50 yrs	0.340(NS)

S*- Significant, NS- Not Significant

Table 5 - Correlation between chronological age and estimated age of single and double rooted teeth

		r	p	r^2	SE
Correlation between	Chronological Age and Estimated age using single rooted teeth	0.92	<0.000	85%	2.4 yrs
	Chronological Age and Estimated age using double rooted teeth	0.94	<0.000	88%	1.4 yrs

$p < 0.000$ HS, r – Correlation coefficient, r^2 – Percentage correlation, SE- Standard error

Table 6 - Correlation between root dentine translucency with increasing age

Correlation of dentin translucency with increasing age	r	p	r^2	SE
	0.91	0.000	83%	0.015

$p < 0.000$ HS, r – Correlation coefficient, r^2 – Percentage correlation, SE- Standard error

Table 7 - Inter-observer variation in measuring root dentine translucency length among single and double rooted teeth

	Study groups	Number of teeth	p-value	R
Inter-observer variability	Single rooted teeth	60	0.437(NS)	0.98
	Double rooted teeth	60	0.999(NS)	0.97

NS: Not Significant, r – Correlation coefficient

Table 8 - Intra-observer variation in measuring root dentine translucency length among single and double rooted teeth

	Study groups	Number of teeth	p-value	R
Intra-observer variability	Single rooted teeth	60	0.254(NS)	0.99
	Double rooted teeth	60	0.765(NS)	0.99

NS: Not Significant, r – Correlation coefficient

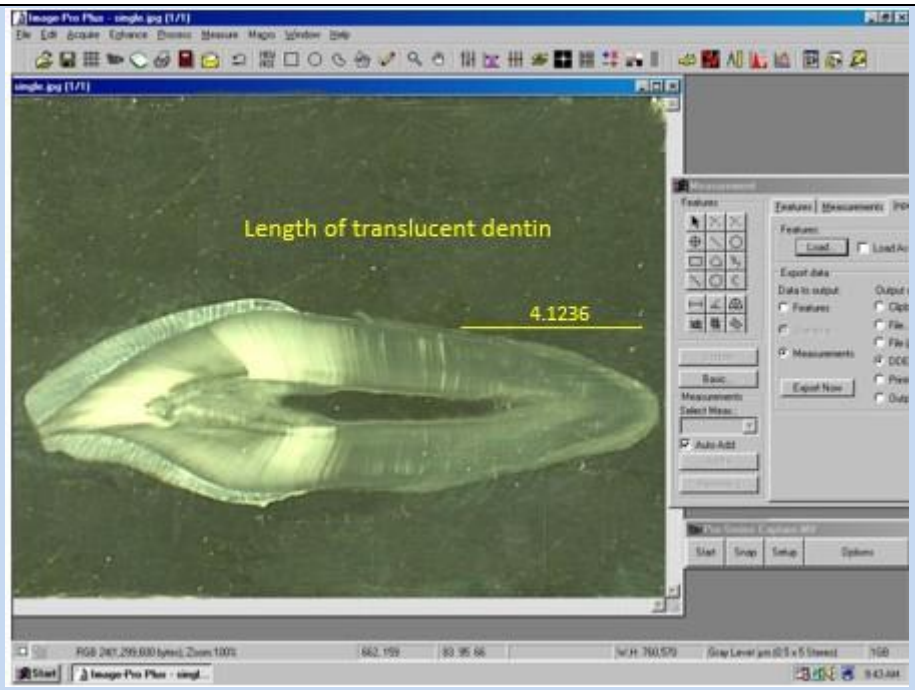

Fig.1: Photomicrograph showing measurement of translucent dentin length in single rooted tooth using image analysis software (5x magnification)

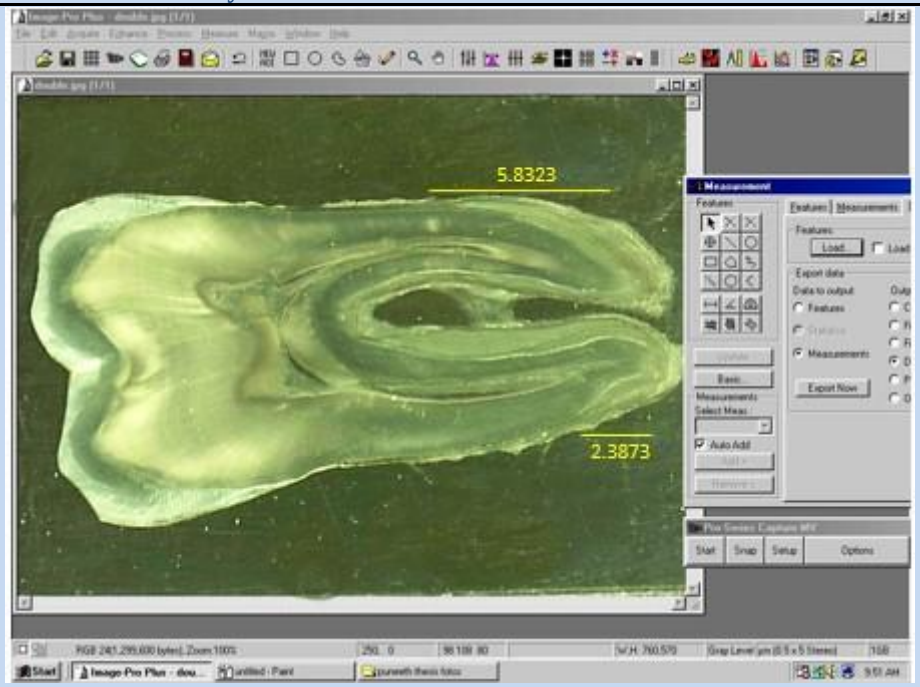

Fig.2: Photomicrograph showing measurement of translucent dentin length in double rooted tooth using image analysis software (5x magnification)

DISCUSSION

Age estimation is of great importance for the identification of unknown victims or skeletal remains in cases where trauma, crime or disaster are a feature of the case.[7] Teeth are the most durable structures in the human body. In many archaeological sites or forensic cases, the teeth are the only human remains.[8]

Gustafson's seminal work sowed the seed for future generations of researchers to investigate the changes that occur in teeth that could be used for purposes of age estimation. Among the six variables suggested by Gustafson, root dentine translucency was found to be the easiest of the six variables to assess. Gustafson (1950) first observed that root dentine becomes transparent with age, with the process beginning at the apex of the root and proceeding towards the crown of the tooth [9]. Root dentine translucency is considered to be least affected by external stimuli and most suitable for the purpose of age estimation.[10]

In the present study, a strong correlation between the length of translucent dentine and advancing years of age was found. A definite and gradual increase in the extent of the root dentine translucency with advancing years of age was noted. Previous studies have demonstrated that the extent of the root dentine translucency can be a reliable indicator of individual's age.[9, 11, 12]

In the present study the mean age difference between chronological and estimated age for the whole sample using the root dentine translucency method was 6.7 years. A value <±10 years is considered as "acceptable" in forensic age estimation, and this mean difference of estimated age in the present study was less than that reported by Acharya et al (8.3 yrs), [6] Bang and Ramm et al (11.2 yrs),[11] Meinl A et al (9.9 yrs),[2] Singhal et al (15.6 yrs),[13] and Lamendin et al (8.4yrs).[4]

It should be noted that there are some studies where the reported a mean age difference is less than that reported in the present study with mean difference of 3.5-6.5 yrs.[14, 15]

Observer variability:

Measurements were carried on 60 sections of single and 60 sections of double-rooted teeth by two observers. Inter-observer variability showed no statistical significant difference in both single-rooted (p= 0.437) and double-rooted teeth (p= 0.999) with Pearson's correlation coefficient of 0.98 and 0.97 respectively (Table 7). Intra-observer variability did not reveal statistical significant differences in both single-rooted (p= 0.254) and double-rooted teeth (p= 0.765) with Pearson's correlation coefficient of 0.99 and 0.99 respectively. The insignificant variations observed between the examiners indicated that measurement of root dentine translucency produces consistent results when undertaken by different examiners. These results were in accordance with the study carried out by Ashith and Vimi.[6]

Age estimation using root dentine translucency in single rooted teeth:

The mean age difference of 6.55, 7.65 and 8.40 between the estimated age and chronological age in single-rooted teeth was not statistically significant (p= 0.574) in the age groups of 21-30 yrs, 31-40 yrs and 41-50 yrs suggesting that value considered is acceptable for age estimation in all of these age groups.

Two previous studies showed a greater mean age difference between estimated and chronological age compared to the present study. Meinl A et al[2] showed a mean difference ranging between 10.5-17.3 yrs for the three age groups (10.5, 12.3, 17.3 yrs) and Lamendin H et al.[4] showed a mean age difference ranging between 3.3-13.3 years in four study groups (30-39, 40-49, 50-59, 60-69 yrs). This difference may be explained by the use of population specific formulae in this study which was specific to an Indian population.

When intra-group comparison of the mean age difference between chronological age and estimated age was carried out in single-rooted teeth the difference between the age of S1 and S2, S2 and S3 and S3 and S1 groups was not statistically significant (p= 0.542, p= 0.751, p= 0.184 respectively)

Formation of translucent root dentine usually begins in the 3rd decade (21-30 yrs), [2, 11] and this feature was also observed in five samples of the present study. Few studies have reported the formation of translucent root dentine below 20 years of age and the reason for the formation of translucent root dentine translucency in this age group is still unexplained.[3], [16] It should be noted that in our study no subjects were below 20 years of age.

In the present study, a strong correlation of 0.92 was observed between estimated age and actual age of single rooted teeth similar to other studies with different values (r=0.80, r=0.81 and r=0.78 respectively). [5, 11, 13]

Age estimation using root dentine translucency in double rooted teeth:

The rationale for selecting double-rooted teeth was that the measurement of more than one root would produce more accurate results. Three-rooted teeth were not included in the study because of difficulty in sectioning three-rooted teeth and also because of the limited availability of three-rooted teeth.

The mean age difference of 4.50, 6.20 and 7.45 between the estimated age and chronological age in double-rooted teeth was not statistically significant (p= 0.006) in age groups 21-30 yrs, 31-40 yrs and 41-50 yrs respectively suggesting that the values could be acceptable for age estimation in these age groups. These values could not be compared to any other studies since no study has been carried out

in double rooted teeth in different age groups.

When intra-group comparison of mean age difference was carried out in double-rooted teeth, the mean age difference between the D1 and D2 and D2 and D3 groups was not statistically significant (p= 0.144, p= 0.244 respectively), whereas the mean age difference between D3 and D1 was statistically significant (p= 0.004). This difference could be as a result of more subjects in group D3 who were more than 45 years of age.

In the present study, a strong correlation of 0.94 was obtained between estimated and actual age of double-rooted age, which was similar to study done by Brkic H et al.[5]

Inter-group comparison of mean age difference between single and double-rooted teeth:

In the present study comparison of the overall mean age difference of single-rooted teeth with that of double-rooted teeth demonstrated that there was no statistically significant difference (p=0.012) with a mean and standard error 7.53±2.4, 6.05±1.4 respectively. This implies that both types of teeth i.e. single and double-rooted teeth can be used for purposes of age estimation. However double-rooted teeth showed less mean age difference when compared with single-rooted teeth.

Age wise inter-group comparison of the mean age difference between single-rooted teeth and double-rooted teeth demonstrated a statistically significant difference between S1 and D1 (21-30 yrs) (p=0.003). An explanation for this difference could be that teeth in this age group showed minimal or no formation of root dentine translucency. Additionally this difference could be attributed to the age distribution of subjects in the groups S1 and D1. Only 4 subjects in group S1 were more than 25 years of age while in group D1 9 subjects were over 25 years of age. It should be noted that the differences between the other groups S2 and D2 (31-40 yrs) and groups S3 and D3 (41-50 yrs) were not statistically significant (p= 0.229, p= 0.340 respectively).

The correlation coefficients of estimated age with the actual age using root dentine translucency of single and double-rooted teeth was 0.92 and 0.94 respectively, indicating that both type of teeth can be used for estimating age.

Previous studies have reported a variety of correlation coefficients for the extent of root dentin translucency with age; r= 0.73 (Miles, 1983); r= 0.65-0.83 (Bang and Ramm, 1970); r= 0.86 (Johanson, 1971); r=0.75 (Azaz, 1977); r=0.87 (Vasiliadis et al., 1983)} [16].

The results of the present study also fall within these previously published figures i.e. r= 0.83, with the exception of Chinese and Malays where root dentine translucency did not correlate highly with age.[16]

FUTURE SCOPE

Although the formation of root translucent dentine is said to be an age-related change, few studies have reported root translucent dentine to be present in subjects below 20 years of age. The reason for this anomaly is not explained. For this reason the present study did not include subjects below 20 years of age. Further studies including subjects from both the age groups 11-20 yrs and 21-30 yrs should provide an insight into the process involved in the formation of root translucent dentine. The formation of translucent root dentine is also thought to be affected by other factors including sex, type of tooth and dental arch. These

variables could also be included in future studies.

CONCLUSION

Both single-rooted and double-rooted teeth showed high correlation for purposes of age estimation. However double-rooted teeth showed less mean age difference and standard error when compared with single-rooted teeth suggesting that double-rooted teeth provide a more accurate method of age estimation than single-rooted teeth. The minimum age that can be calculated using the modified Bang and Ramm formula is 29.9 years and this precludes any application for use with younger age groups. Use of the modified Bang and Ramm formula in studying samples obtained for subjects less than 30 years is inappropriate and this fact should be considered in the future studies where translucent root dentine is used for purposes of age estimation.

REFERENCES

1. Verma AK, Kumar S, Rathore S, Pandey A. *Role of dental expert in forensic odontology. Natl J Maxillofac Surg* 2014; 5(1): 2-5.
2. Meinl A, Huber CD, Tangl S, Gruber GM, Teschler M, Watzek G. *Comparison of the validity of three dental methods for the estimation of age at death. Forensic Sci Int* 2008; 178: 96-105.
3. Metska E, Stavrianos C, Vasiliadis L. *Estimation of dental age using root dentine translucency. Surg Journal* 2009; 4(2): 21-28.
4. Lamendin H, Baccino E, Humbert JF, Tavernier JC, Nossintchouk RM, Zerilli AJ. *A simple technique for age estimation in adult corpses: the two criteria dental method. Forensic Sci* 1992; 37(5): 1373-9.
5. Brkic H, Milicevic M, Petrovecki M. *Age estimation methods using anthropological parameters on human teeth. Forensic science international* 2006; 162: 13-16.
6. Ashith BA, Vimi S. *Effectiveness of Bang and Ramm's formulae in age assessment of Indians from dentin translucency length. Int J Legal Med* 2009; 123(6): 483-488.
7. Anita Singhal, V Ramesh, PD Balamurali. *A comparative analysis of root dentin transparency with known age. J Forensic Dent Sci* 2010; 2:18-21.
8. Schmeling A, Geserick G, Reisinger W, Olze A. Age estimation. Forensic Science international 2007; 165: 178-81.
9. Maples WR. *An improved technique using dental histology for estimation of adult age. J Forensic Sci* 1978; 23: 764-70.
10. Vasiliadis L, Stravrianos C, Kafas P. *Assessment of dentin translucency in estimating age: A research. Int J Orofac Sci* 2008; 1(4): 1-3
11. Bang G, Ramm E. *Determination of age in humans from root dentin transparency. Acta Odontol Scand* 1970; 28(1): 3-35.
12. Solheim T. *Dental root translucency as an indicator of age. Scand J Dent Res* 1989; 97: 189-97.
13. Anita Singhal, Ramesh V, Balamurali PD. *A comparative analysis of root dentin transparency with known age. J Forensic Dent Sci* 2010; 2:18-21.
14. Andrea D, Irene C, Antonina V. *Root Dentine Transparency: Age Determination of Human Teeth Using Computerized Densitometric Analysis. Am J Phys Anthropol* 1991: 85: 25-30.
15. Mandojana JM, Martin S, Valenzuela M, Luna JD. D*ifferences in morphological age – related dental changes depending on post-mortem interval. J Forensic Sci* 2001; 46(4): 889–92.
16. Vasiliadis L, Darling AI, Levers BGH. *Histology of Sclerotic Human Root Dentin. Archs Oral Biol* 1983; 28(8): 693-700.

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