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Estimation of Dental Age Using Root Dentine Translucency

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Abstract: Age estimation is a process of particular interest in cases of forensic interest as well as in anthropological studies. Many methods have been suggested such as cementum thickness, dental colour, tooth attrition, secondary dentine formation, periodontosis, apical resorption and many techniques have been employed but root dentine translucency remains the method of choice providing the most accurate results for age estimation. The aim of the present study is: to investigate the correlation between the percentage of root translucency in human single-rooted teeth of the lower jaw and age to compare the percentage of translucency between the roots of a molar to compare the percentage of root dentine translucency of the same root of a molar in different individuals of know age. During the study, 44 fresh extracted teeth were utilized. In particular, 17 incisors, 13 canines, 3 premolars of the upper jaw, 3 first molars of the upper jaw and 7 of the lower jaw. The teeth were extracted from 32 individuals, between 12 and 77 years. Group A was made up of the single-rooted teeth of the lower jaw, whereas group B of the molars. Segments with 250 μg thickness were scanned and saved as bitmap files. The translucency was measured with the use of Visio Professional 2002, a Microsoft product. There is a positive linear correlation between root dentine translucency and age, with a correlation coefficient of r = 0.784. The is a lack of uniformity in dentine translucency among the roots of the same molar tooth. When each root by means of its translucency is taken into account, there is a gradual increase of root dentine translucency of all roots with age, except the distal roots of 2 samples. Among the results of the roots of the upper teeth there is uniformity in the development of root dentine translucency and the palatal root presents a higher percentage than the mesiobuccal and the previous a higher than the disto-buccal.

Key words: Age estimation, root dentine translucency, scatter plot, linear regression analysis

INTRODUCTION

The major features of human dentine are dentinal tubules radiating from the pulp to the dentine-enamel or dentine-cementum junction (Kinney *et al*., 2005), providing elastic support to the enamel (Elbaum *et al*., 2007). They are tube-like channels of 1-2 μm diameter (Porter *et al*., 2005) resulting from the deposition of anorganic substance in the form of apatite crystalls on collagen-rich matric around odontoblast's prolongations. The diameter of the tubules decreases gradually from their pulpal ending to the dentine-enamel or dentine-cementum junction.

There are many morphological changes that appear during maturation such as dental wear, cementum apposition, secondary dentine apposition, gingival recession, root resorpion, root transparency, acid racemization, color change and reduction in size of the pulpal cavity (Prince and Konigsberg, 2008; Sengupta *et al*., 1999). The formation of secondary dentine and/or fibrodentine leads to changes in the dental pulp and to a restriction mostly in the mesiodistal dimension more often present after the 6th decade of life (Schroeder, 1993). Another form of dental hard tissue alteration is transparent or sclerotic dentine that forms from the root of a tooth and extends gradually to the crown of the tooth and is distinguished from pathological transparent dentine because of the absence of caries or trauma due to injury, i.e., without the influence of environ-mental factors (Kinney *et al*., 2005; Vasiliadis *et al*., 1983b).

Many variables have been used as age determinants and even dental histological techniques can contribute to age determination (Sengupta *et al*., 1999). The choice to use teeth for age determination is well accepted due to their longevity ability of being resilient to change (Prince and Konigsberg, 2008; Brkic *et al*., 2006). It is of high importance to take into account that physiological or biological aging is in many cases not related to calendar (chronological) aging. In this manner, a biological marker independent of any environmental alteration is needed to provide information about the age of an individual (Prince and Konigsberg, 2008; Sengupta *et al*., 1999;

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Amariti *et al*., 1999). Such a biomarker is root dentine translucency, supported by Gustafson (1950). It is essential to extend these previous studies on healthy dentin in order to study more efficiently the microstructure of human teeth and the effect of aging on the micromorphology of the teeth. In the analysis, which follows the important role of sclerotic dentine as an age-determinant will be advocated.

An older approach for age estimation was suggested by Gustafson (1950). It focused on 6 determinants, including attrition, periodontosis, secondary dentine, cementum apposition, apical resorpion and root translucency.

Scanning Electron Microscope (SEM) is of particular value for the age estimation process and is being used in many cases of forensic interest. In particular, it does not only help the assessment of root dentine translucency but it contributes to the detection of tooth restorations on teeth leading to an easier identification of cadavers (Fairgrieve, 1994).

A question raised after the evaluation of sclerotic dentine is, except of it's value in age estimation, it's effect on the mechanical properties of dentine and the integrity of teeth during aging. It is also of importance to evaluate whether sclerotic dentine is the result of the closure of dentinal tubules or due to changes in the mineralization of intertubular dentine. New investigations need to provide evidence of the degree of interracial diversities in root dentine translucency and the accuracy of the age-estimation process when the same method is used for all nations.

The studies provide conclusive evidence that the base of each investigation is the choice of the correct age determinant and the software of statistical analysis (Prince and Konigsberg, 2008).

The aim of the present investigation is to provide conclusive evidence of the role of root dentine translucency in age estimation, the uniformity of it's distribution in the roots of a molar and among the same type of root in different molars.

MATERIALS AND METHODS

In this study, fresh extracted teeth were utilized, from 32 individuals, between 12 and 77 years. In particular, 17 incisors, 13 canines, 3 premolars of the upper jaw, 3 first molars of the upper jaw and 7 of the lower jaw (Table 1-3). The samples were devided into 2 groups. Group A was made up of the single-rooted teeth of the lower jaw, whereas group B of the molars.

The samples were kept in formalin solution 10% until they were used. Before segmenting the teeth, lines were

Table 1: Group A, sigle-rooted teeth

NR of samples	Type of tooth	Age
50	Incisor	77
51	Incisor	77
52	Incisor	77
56	Incisor	45
57	Incisor	45
58	Incisor	45
59	Incisor	45
66	Incisor	47
70	Incisor	52
71	Incisor	47
72	Incisor	58
73	Incisor	52
74	Incisor	68
75	Incisor	65
76	Incisor	68
77	Incisor	66
78	Incisor	71
42	Canine	43
43	Canine	77
45	Canine	63
44	Canine	14
60	Canine	60
65	Canine	47
79	Canine	58
80	Canine	55
81	Canine	48
82	Canine	67
83	Canine	71
84	Canine	60
85	Canine	75
4	Premolar	25
9	Premolar	63
8	Premolar	30
61	Premolar	60

Table 2: Group B, teeth of the lower jaw

NR of samples	Age
87 distal root	12
87 mesiolingual root	12
87 mesiobuccal root	12
86 distal root	13
86 mesiolingual root	13
86 mesiobuccal root	13
17 distal root	13
17 mesiolingual root	13
17 mesiobuccal root	13
33 distal root	13
33 mesiolingual root	13
33 mesiobuccal root	13
15 distal root	19
15 mesiolingual root	19
15 mesiobuccal root	19
88 distal root	47
88 mesiolingual root	47
88 mesiobuccal root	47
28 distal root	68
28 mesiolingual root	68
28 mesiobuccal root	68

Table 3: Group B, teeth of the upper jaw

NR of samples	Age
24 palatal root	35
24 mesiobuccal root	35
24 distal-buccal root	35
21 palatal root	51
21 mesiobuccal root	51
21 distal-buccal root	51
89 palatal root	48
89 mesiobuccal root	48

drawn on the distal approximal surface, one parallel to the anatomical cervix of the teeth and 2 other separating the root portion into equal parts. Before applying the technique of Allred (1958) adhesive wax of 2 mm thickness was applied in order to ensure that the blade of the microtome cuts a vertical smooth surface avoiding a slope or vibration of the blade (Allred, 1958). The utilized microtome was isomet low speed saw from Buhler and the blade from the same company, was diamond-cowered, with a diameter of 10.2 cm and a thickness of 0.3 mm. The segments were made from the mesial approximal surface towards the distal, parallel to the oblong surface of the tooth. The segments were 250 μm thick. According to Johanson (1971) and Vasiliadis (1981), it allows the accurate assessment of the margins of translucent dentine and the longevity of the segments. An electronic thickness gauge was employed for thickness measurement. Peripheral segments were excluded because they aren't displaying the apical part of the root.

The segments were observed under dry environment in order to allow air to enter the non-sclerotic tubules. Each sample was scanned with a Hewlett Packard scanner and the pictures were saved as bitmap files. The computer utilized was an Acer product, 1353 LM. The area of the root surface up to the anatomical cervix, without cementum, the area of the root canal and of sclerotic and semi sclerotic dentine were evaluated with the utilization of the program Visio Professional 2002, a Microsoft product. The same product was used for adaptation of the brightness and contrast. The areas were evaluated by drawing the perimeter of the parameter being assessed and with the command Shape area and perimeter from the menu line Tools>Macros>Visio Extras>Shape area and Perimeter. For the statistical analysis of the data after the observation of the samples of Group A, SPSS v.12. package was employed. The scope of the investigation was to evaluate the correlation between root dentine translucency and age. Regression analysis was applied to express the correlation between the two variables. The one being dependent and represented at the y axis and the 2nd being independent represented at the x axis.

RESULTS

According to the present study, there is gradual increase in translucency of root dentine with age, with a correlation coefficient of 0.784. There is a positive linear correlation between the 2 variables.

Group A: During the observation of the segments of single-rooted teeth from young individuals, dentine was translucent only at the apical part at the dentinecementum junction. In contrast, in older individuals dentine translucency was present at the hole apical part of the root extending towards the root canal and the crown of the tooth. The line between translucent and non-translucent dentine has a spiral form following the course of the dentinal tubules (Fig. 1) (Table 1 and 4).

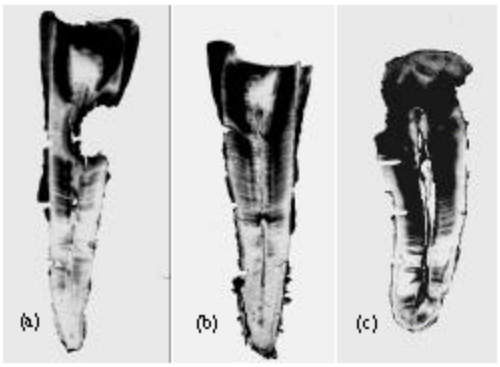

Fig. 1: a) sample 50, 77 years, b) sample 52, 77 years, c) sample 61, 60 years

Table 4: Group A, results of single-rooted teeth

	Group A results		
NR of samples	Real age	Translucent dentine (%)	Type of tooth
50	77	55.37	Incisor
51	77	50.76	Incisor
52	77	43.59	Incisor
56	45	31.37	Incisor
57	45	30.86	Incisor
58	45	31.82	Incisor
59	45	33.90	Incisor
66	47	36.12	Incisor
70	52	31.33	Incisor
71	47	33.72	Incisor
72	58	40.00	Incisor
73	52	36.72	Incisor
74	68	45.49	Incisor
75	65	43.65	Incisor
76	68	44.15	Incisor
77	66	40.67	Incisor
78	71	52.67	Incisor
42	43	28.14	Canine
43	77	53.89	Canine
45	63	40.86	Canine
44	14	00.00	Canine
60	60	33.86	Canine
65	47	23.96	Canine
79	58	39.58	Canine
80	55	37.63	Canine
81	48	25.68	Canine
82	67	48.25	Canine
83	71	50.21	Canine
84	60	44.73	Canine
85	75	55.32	Canine
4	25	10.00	Premolar
9	63	40.90	Premolar
8	30	22.52	Premolar
61	60	44.54	Premolar

Group B: When assessing the percentage of translucent dentine among molar's roots there is less uniformity. In particular, only in one molar of the lower jaw, sample 87, the percentage of translucent dentine in the distal root was equal with that in the mesiolingual and mesiobuccal root. In the samples 86 and 17, the percentage of root dentine translucency in the distal root was equal with that in the mesiobuccal root and less than than in the mesiolingual root (Fig. 2 and 3, Table 2 and 5). In the samples 33 and 15, the percentage of root dentine translucency of the distal root was less than that of the mesiobuccal root and the previous less than that of the mesiolingual root. Only in one sample, 28, the percentage of root dentine translucency of the distal root was higher than that of the mesiobuccal and less of that of the mesiolingual root. In the sample 88 the persentage of translucency of the distal root was higher of that of the mesiobuccal and the previous was higher of that of the mesiolingual root. When assessing root dentine translucency in molar roots of the lower jaw there is high discrepancy among the roots of the same tooth. In the upper jaw the percentage of root dentine translucency in the palatal root was in general higher of that of the mesiobuccal root and the previous was higher of that of the distal-buccal (Table 6). As in group A, in segments from young individuals dentine was translucent only at the apical part, at the dentine-cementum junction. In contrast, in older individual's dentine translucency was present at the whole apical part of the root extending towards the root canal and the crown of the tooth.

Lower jaw: There is low uniformity in the results of root dentine translucency in the lower jaw (Table 5). In 2 samples of distal roots, the percentage of translucency was higher than that of the mesiobuccal root (samples 28, 88) and only in one (sample 88) it was higher of that of the mesiolingual root. When comparing the results among the mesial roots of a molar, only in one sample (88) the percentage of translucency of the mesiobuccal root was higher for about 4, 05% of that of the mesiolingual.

In all other samples (86, 17, 33, 15, 28) translucency of the mesiobuccal root was lower compared to the mesiolingual. In 3 of the previously mentioned samples (86, 17, 33), from young individuals (13 years) there is only a minimal difference in translucency when comparing the mesiobuccal root with the mesiolingual. In particular, it ranges from 8.53% in sample 33-10.94% in sample 17. In sample 28, in an older individual this difference is 45.6% for the mesiolingual root. The difference of translucency formation between mesiolingual and distal roots is only 3.09%. The analysis of the percentage of translucency for each root of the teeth of the lower jaw leads to the

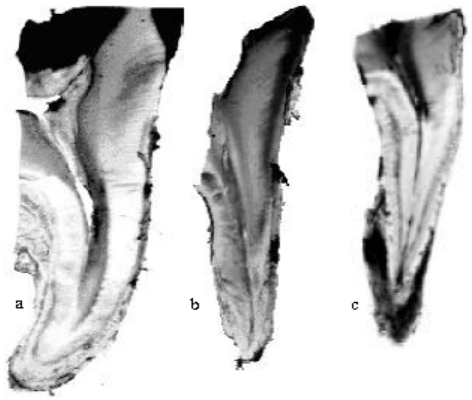

Fig. 2: a) sample 88, mesiolingual root, 47 years, b) sample 17, mesiolingual root, 13 years, c) sample 28, mesiobuccal root, 68 years

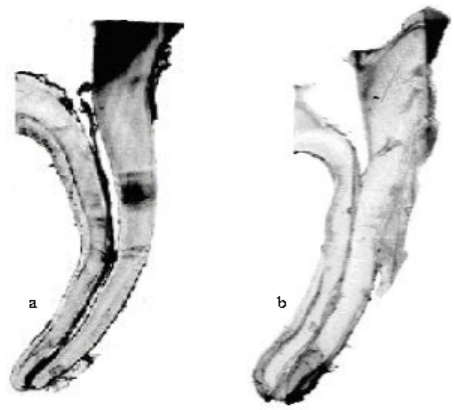

Fig. 3: a) sample 33, distal root, 13 years, b) sample 86, distal root, 13 years

Table 5: Group B, results of the teeth of the lower jaw

NR of samples	Age	Translucent dentine (%)
87 Distal root	12	0.00
87 Mesiolingual root	12	0.00
87 Mesiobuccal root	12	0.00
86 Distal root	13	0.00
86 Mesiolingual root	13	9.35
86 Mesiobuccal root	13	0.00
17 Distal root	13	0.00
17 Mesiolingual root	13	10.94
17 Mesiobuccal root	13	0.00
33 Distal root	13	0.00
33 Mesiolingual root	13	12.18
33 Mesiobuccal root	13	3.65
15 Distal root	19	8.53
15 Mesiolingual root	19	18.13
15 Mesiobuccal root	19	15.04
88 Distal root	47	70.72
88 Mesiolingual root	47	27.52
88 Mesiobuccal root	47	31.57
28 Distal root	68	59.39
28 Mesiolingual root	68	77.70
28 Mesiobuccal root	68	32.10

Tablconclusion that there is a gradual increase of translucency in the all roots with age. There's only one sample, 88, from an individual aged 47 years, where the percentage of translucent dentine in the distal root (70.72%) is higher than that of the sample 28 (59.93%) of an 68 years old individual.

Upper jaw: The results of the upper jaw had uniformity (Table 6). The percentage of root dentine translucency in the palatal root was higher than that of the mesiobuccal and the previous of that of the distobuccal. The difference in root translucency between the palatal and the mesiobuccal root ranged from 19.52% (sample 24) to 28.69% (sample 21). With increasing age the divergence increases. There is no correlation between the translucency of the palatal and distobuccal root. In sample 24.35 years old, the mesiobuccal root's translucency was 49.53% higher, in sample 89.48 years, the difference in translucency among the 2 roots was 26.87% and in sample 21.51 years it was 29.92%. When comparing the buccal roots the results have a low uniformity. In sample 24, the mesiobuccal root shows 30.01% more translucency than the distobuccal root. In samples 21 and 89 the divergence is 1.23 and 4.47%, respectively. If we take into account the whole results, no differences are seen among the roots of a molar in root translucency. There is no correlation between a particular root of a molar and a higher root dentine translucency. Attention should be payed on the limited amount of samples. The results cannot be generalized.

Statistical analysis: The results can been seen on Table 7. It is to be assumed that there is an approximate linear relationship between the 2 variables. This correlation is mathematically expressed via the angle between the line of maximum adaptation on the scatter plot and the vertical y-line (Fig. 4).

The correlation coefficient between the two variables is 0.784, indicating a high positive correlation between age and translucent dentine. The 95% confidence interval ranges between 0.771 and 1.385. The inclination of B Line is 0.962. The constant is 15.515. The divergence between the estimated and the real age is assumed to be ±4.1 years. In the scatter plot, the dispersion of the spots is relative low, indicating a high correlation. The inclination of the dispersion represents a line, denoting that there is a linear

Table 6: Group B, results of the teeth of the upper jaw

NR of samples	Age	Translucent dentine (%)
24 Palatal root	35	49.53
24 Mesiobuccal root	35	30.01
24 Distal-buccal root	35	0.00
21 Palatal root	51	39.40
21 Mesiobuccal root	51	10.72
21 Distal-buccal root	51	9.48
89 Palatal root	48	37.13
89 Mesiobuccal root	48	14.73

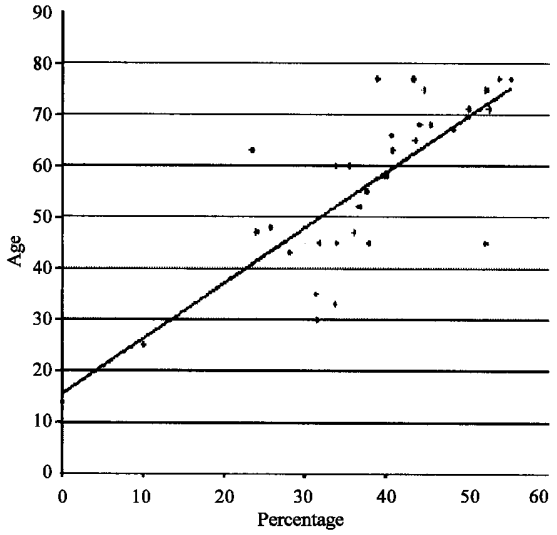

Fig. 4: The extent of root transparency at various ages in intact roots

Table 7: Results of the statistical analysis

	Variables[b]			Model summary			
Model	Entered	Removed	Method	R	R^2	Adjusted R^2	SE of the estimate
1	VAR00003[a]	-	Enter	0.784[c]	0.615	0.603	10.36733

	Coefficients[b]						
	Unstandardized		Standardized			95% confidence interval for B	
Model	B	SE	β	t	Sig.	Lower bound	Upper bound
1 (constant)	15.515	5.940	-	2.612	0.014	3.415	27.615
Var00003	1.078	0.151	0.784	7.148	0	0.771	1.385

[a]All requested variables entered; [b]Dependent var00002; [c]Predictors (constant), var00003

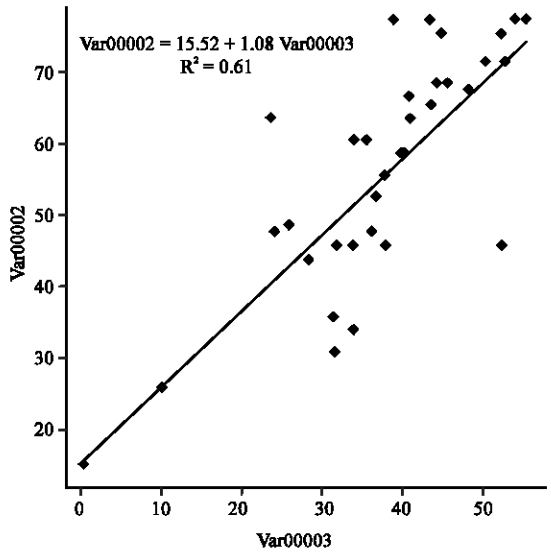

Fig. 5: Linear regression

correlation (Fig. 5). It is feasible to estimate the age via the assessment of root dentine translucency. The equation is:

$$Y_{age} = 15.52 + 1.08 \times \text{translucent dentine (\%)}$$

DISCUSSION

Many approaches have been suggested for the age estimation process by means of direct and indirect observation of teeth with the use of microscope or even the science of histology. In Lamendin *et al.* (1992) supported the method of the assessment of the area of translucent dentine at the root of a tooth and the periodontal regression measured from the enamel-cementum junction to the gingival margin (Prince and Konigsberg, 2008; Lamendin *et al.*, 1992). In particular, the values are measured in relation to the root height, which according to Vasiliadis *et al.* (1983a) influences the formation of root dentine translucency. In the present study however, only the area of root dentine translucency was assessed without mentioning the root length as a variable which influences translucency formation.

Other researchers stressed that employing the periodontal regression in age estimation leads to inaccurate results because it is influenced by intrinsic and extrinsic factors (Prince and Konigsberg, 2008). Brkic *et al.* (2006), advocate that the method suggested by Gustafson (1950), employing 6 variables for age estimation, present a higher correlation coefficient with age than the method of root dentine translucency or the observation of the root and root canal via X-rays (Brkic *et al.*, 2006). On the other hand, Amariti *et al.* (1999) stressed that dentinal tubule density/sclerosis provides more accurate results for age estimation than secondary dentine assessment and cementum thickness. Other researchers like Schmeling *et al.* (2007) suggest as a method of choice for age estimation the recemization of aspartic acid in dental ranks without mentioning sclerotic dentine. It is a matter of fact that in the elderly there aren't any morphological characteristics that can give accurate results and only biochemical methods can contribute to age estimation (Ritz-Timme *et al.*, 2000).

Sclerotic dentine although seems to provide the most accurate age estimation results compared to the other methods. Sclerotic dentine is the result of a diminished function of odontoblasts (Tziafas, 1999). It must be distinguished between sclerotic dentine and reparative. The later is translucent but not necessary sclerotic. The translucency is related to the absence of dentinal tubules from the start (Vasiliadis *et al.*, 1989). The formation of sclerotic dentine isn't only present in older individuals but also in younger aging between 12-19 (samples 86, 17, 15, 33). The evaluation of the initiation of sclerotic dentine formation and the differences between this altered form of dentine and normal dentine will contribute to the understanding of the effect of the aging process on human teeth. There is high divergence between the estimated age among the suggested methods. Kashyap and Rao (1990), advocate that they are able to estimate the age of the individual with a variation of 2 years. Kinney *et al.* (2005) investigated that the formation of sclerotic dentine is close related to a change in the mineralization process. In particular, transparent dentine was higher mineralized than normal dentine and at the pulpal region the mineralization was 40% higher than in the normal dentine that has a higher amount of mineral content on the outer layers of dentine. The mineral content increases with incremental transparency.

It is of high interest that the differences in mineralization between normal and sclerotic dentine diminish towards the root of the tooth but sclerotic dentine has always a higher mineral content (Kinney *et al.*, 2005). These results are consistent with the assessment that the density of dentinal tubules decreases towards the root of the tooth (Kinney *et al.*, 2005; Schilke *et al.*, 2000). Sclerotic dentin appears to be the result of the occlusion of dentinal tubules due to the apposition of minerals into the lumen of the tubules. According to Vasiliadis *et al.* (1983b), the apposition of the minerals start from individual tubules or groups of tubules and transparency appears before the competition of the occlusion. The process of mineralization is more like that of *in-vitro* studies than of that of peritubular dentine mineralization. Microscopic observation of tooth samples

in the study of Kinney *et al.* (2005) failed to identify changes in mineral network of intertubular dentine, thus, suggesting 2 possible mechanisms of transparent dentine formation: either via the dissolution of crystallites forming smaller structures or via the passive precipitation of new crystallites into the tubules from the intertubular dentine (Kinney *et al.*, 2005). Whittaker and Bakri (1996), argue for the hypothesis that the minerals occluding the dentinal tubules are of salivary origin.

The process of sclerotic dentine formation is accompanied by the absence of a pre-dentine layer (Vasiliasis *et al.*, 1983b; Stavrianos *et al.*, 2008). There is no change in the elastic properties of dentine but a reduction in the fracture toughness of about 1/3 compared to normal dentine when mastication forces of 20 Mpa are calculated.

Regional and population differences seem not to have an impact on the accuracy of age estimation (Ubelaker and Parra, 2008). Colmenares *et al.* (2007) although, advocate that because of differences in morphology, tooth eruption and tooth size, ancestry variations should be taken into account. It appears like men have a higher ratio of root dentine translucency formation than women. This should be taken into account and the choice of the right coefficient in order to minimize errors is of importance (Lorentsen and Solheim, 1989).

Studies have proven evidence of the fact that the age estimation is more accurate when the transparent dentine is measured as the percentage of the root affected (Sengupta *et al.*, 1999). Thus, length of translucency has a lower correlation coefficient with age than percentage of root affected. It is of high value to mention the slower formation of root translucency in older patients, which leads to underestimation of age, but this does not influence the fact that there is a strong correlation between root translucency and age. It is also noteworthy that direct observation without tooth fracturing provides as accurate results as indirect techniques (Colmenares *et al.*, 2007). The fact that post-depositional changes may occur should be taken into account when estimating the age of an individual. The effect of collagenases and organic acids derived from microorganisms affecting the tooth substance can dissolve the organic and anorganic substrate, respectively (Sengupta *et al.*, 1999). Thus, secondary dentine and even sclerotic dentine can be affected and the age-estimation process will be biased. Prince and Konigsberg (2008) suggest that employing dental metric variables by means of a continuous variable such as root dentine translucency instead of a phase-oriented method may help overcoming the subjectivity of the observer and the influence of the post-depositional alterations on age-estimation (Prince and Konigsberg, 2008). Valenzuela *et al.* (2002), mention that the post-depositional interval can also influence age estimation and the right method should be chosen according to these parameters. In particular, cementum apposition, pulp length via image-analysis, dental attrition, root translucency and dental color were the variables that had the highest correlation coefficient with age in cases of human skeletal remains. In fresh extracted teeth dental attrition, translucency width measured via image-analysis and dental color were the variables most contributing to age estimation (Valenzuela *et al.*, 2002). The presence of changes in dentinal features alone can not support the age-estimation because these changes can be a sign of early toothwear as mentioned by Hojo (1990) in his study that referes to Japanese population. Employing a dental method in age estimation is of particular interest because it can be applied to individuals of all ages (Colmenares *et al.*, 2007). Other methods employing non dental variables set an age limit of 45 years in order to overcome pathological changes on the skeleton, which influence the estimation process.

In the present study, it is stressed that root dentine translucency is the only parameter providing accurate results for age estimation. The quantification of other variables does not lead to more accurate results. Although, age is correlated with an increase in root dentine translucency no clear results were obtained when comparing the roots of the same molar. The distribution of translucency in the roots of molars must be further investigated.

CONCLUSION

Root dentine translucency of single-rooted teeth is the only parameter giving accurate results for age estimation. The distribution of translucency in the roots of molars must be further investigated.

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