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DENTAL AGE ESTIMATION USING RADICULAR DENTIN TRANSLUCENCY FOR EGYPTIAN ADULTS

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ABSTRACT

Aim: to estimate the age of Egyptian adults using root dentin translucency (RDT) method (Gustafson's morpho-histologic approach). In addition, to compare the accuracy of measuring the surface area versus the length of RDT in ground section of single rooted teeth in determining the chronological age. Besides, to observe the correlation between RDT and gender.

Methods: The study sample was consisting of 50 sound teeth that had been extracted from patient ranging in age from 20 - 70 years then, divided into 5 equal experimental groups. Group I contained sound teeth extracted from patient aged 20-30 years. Group II sound teeth extracted from patient aged 31-40 years. Group III sound teeth extracted from patient aged 41-50years. Group IV sound teeth extracted from patient aged 51-60 years. Group V sound teeth extracted from patient aged 61-70 years. Stained longitudinal ground section for both groups were prepared &observed under light microscope (LM). Both surface area and length of RDT were measured on magnified images using Image-J software. After that, all data were recorded, tabulated, and statistically analyzed using software SPSS.

Results: RDT surface area and length was positively correlated with chronological age. The range of difference between chronological and estimated age by both RDT area and length was the lowest (-10.68-8.65) compared to estimated age by RDT area only (-10.89-13.47) and RDT length only (-14.46-16.74). Also, there was not a significant sex difference determined.

Conclusion: RDT is a reliable method for age estimation for permanent sound teeth. We can use specific regression formula for Egyptian population by RDT area, length, or both.

KEYWORDS: Age estimation, adult Egyptians,dentin translucency, ground section, light microscope, forensic odontology.

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INTRODUCTION

Forensic odontology is a science of forensic medicine which deals with examination of dental evidence from which a proper evaluation and presentation of dental findings can be made [1]. Dental identification of persons can be confirmed by several methods including visual identification, anatomical structures, medical devices, fingerprint, DNA, and dental comparisons. While visual methods of identification are commonly used, they should be used with caution as facial and other body characteristics can be changed due to trauma, swelling, fragmentation, plastic surgeries and decomposition [2].

When the subjects have undergone extensive changes those external characteristics yield no information, the teeth are often the only means of identification. Also, human teeth can be preserved for a long time after death without gross changes to serve as an important tool in forensic science [3]. Estimation of age is a vital aspect in identifying living individuals, cadavers and skeletal remains. It is also a vital forensic and legal issue as the age of criminal responsibility has significant implications during legal convictions and/or sentencing. Moreover, in modern multicultural societies where legal and illegal immigration is rising, an increasing demand also exists for age estimation in living persons who have no documentation for proof of identity[4].

Dental age assessment is the prediction of actual or chronological age through information obtained from the teeth. Various methods are utilized for determination of age from dentition including clinical, radiographic, histological, physical and chemical analysis [2]. Dentin forms the major bulk of the tooth. It develops uniformly from the infancy to adolescence. After adolescence, the dentin undergoes physiological changes such as sclerosis. This process of sclerosis in dentin is known as dentin translucency that gradually increases as age advances. Thus, these changes can help possibly us to estimate age [5, 6].

Gustafson (1950) depicted six parameters that could be used in age estimation of individuals. He used longitudinal ground sections of teeth and illustrated six age-progressive change including occlusal attrition, periodontitis, secondary dentin deposition, cementum apposition, root resorption and radicular dentine translucency[7].

Histologically, translucent dentin is one of the physiological forms of dentin that starts in the apical end of root and gradually extend to the crown of the tooth [8]. The appearance of translucency under LM is due to the equalization of the difference in refractive indices between intra tubular organic and extra-tubular inorganic materials after calcification of dentinal tubules with advancing age [9].

Up-till-now, there is no information regarding age estimation for Egyptian adults using radicular dentine translucency parameter. Thus, the aim of this study was to estimate the age of both female and male Egyptian adults using RDT method. In addition, to compare the accuracy of measuring the surface area versus the length of the translucency in longitudinal ground section of single rooted teeth in determining the chronological age.

MATERIALS AND METHODS

The sample

Fifty extracted teeth were used in our study. They were obtained from the Oral Surgery and Maxillofacial Department in Faculty of Dentistry at Tanta University. They were extracted from patients ranging from 20-70 years old of both sexes with well-known chronological age. The teeth extractions were performed as a part of essential clinical care. Written and oral consents were obtained from the patients**.** Also, the study conducted after getting permission from Faculty of Dentistry, Tanta University, Research Ethics Committee (REC).

Single-rooted permanent teeth extracted for various therapeutic reasonsuch as orthodontic

treatment, malocclusion, mobility, or prosthetic purpose were taken for the study. On other hand, badly decayed teeth, impacted teeth, teeth with severe attrition, abrasion, and erosion, teeth with external and internal root resorption, and multirooted teeth were excluded from the study.Age and sex of the patients were recorded, and all the corresponding teeth were given code numbers. Afterward, the selected teeth were divided into five experimental groups each one consists of 10 teeth.

	Age range (years)	Gender
Group I	20<30	
Group II	30<40	5 Male
Group III	40<50	5 Female
Group IV	50<60	
Group V	60<70	

Histological section preparation

The extracted teeth were preserved in 10% buffered formalin then they were washed under running water before preparing thin histological ground sections that were be longitudinal (LS) to allow better viewing of the whole root surface (**Aggarwal et al., 2008).** The LS was performed according to (**Maat et al., 2001)** schedule. First, the tooth was sawed longitudinally from its midline into two halves using low speed contra. Then, manual grinding was done with micro motor and then hand grinding using Arkansas stone for each half. Grinding was done till the section reach its final thickness (100-150 μm). The ground sections were then washed and stained with 1% methylene blue for 30 seconds. (figure 1). Then the section was dried and mounted on a glass microscopic slide using Canada balsam. All specimens were labeled and stored for recordpurpose.

After excluding unbeneficial sections, the ground sections in each group were examined by LM. Afterward, images were taken for each section at magnification x 40 for apical root areas.

Image-J analysis and measurement:

Both surface area and the length of RDT were measured on magnified images by Image-Janalysis system as follow:

RDT appears as transparent region on the image of the tooth ground section when compared with the other structures. Translucency was measured using number of tools available on Image-J software.

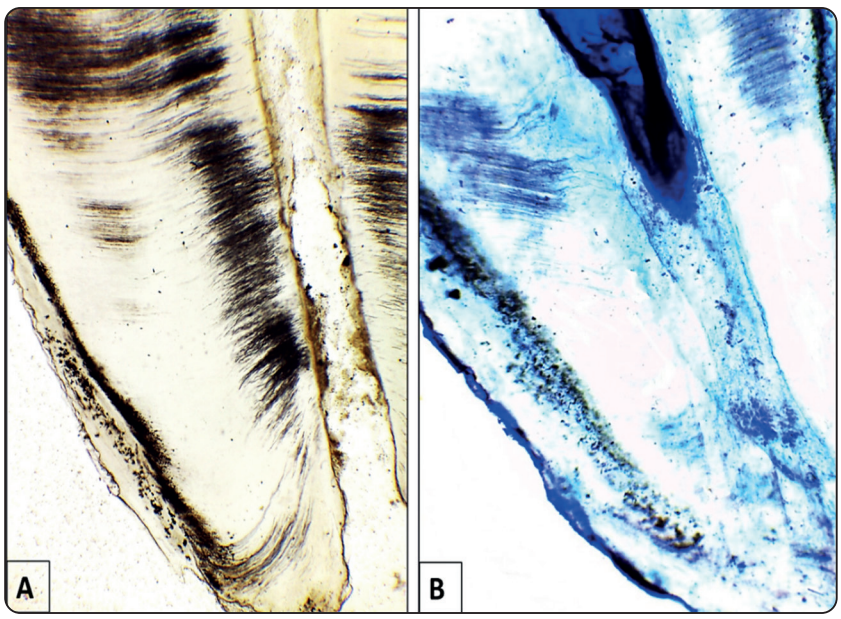

Fig. (1): Micrograph of lower right central incisortaken by LM depicts the ease determination of RDT in the ground section stained with methylene blue at (B) more than in the unstained ground section at (A). (L.S., orig. mag..A& B X 40)

For measuring surface area of RDT, analyze portion on the scale bar was activated, then the proper scale for the captured image was determined. After that, the freehand tool was selected to outline the RDT for measurement in mmon both sides of the tooth midline and the total area of both sides was taken (figure. 2).

Similarly, for measuring the average length of RDT, analyze portion on the scale bar was activated, then the proper scale for the captured image was determined. After that, the straight-line toolwas selected to outline the length/distance from the apical to coronal extent of RDT of (the highest ones) both sides of the tooth midline (figure. 2).

To evaluate possible inter observer variation, both RDT area and length were again measured by the second observer on all teeth sections and RDT was recalculated.

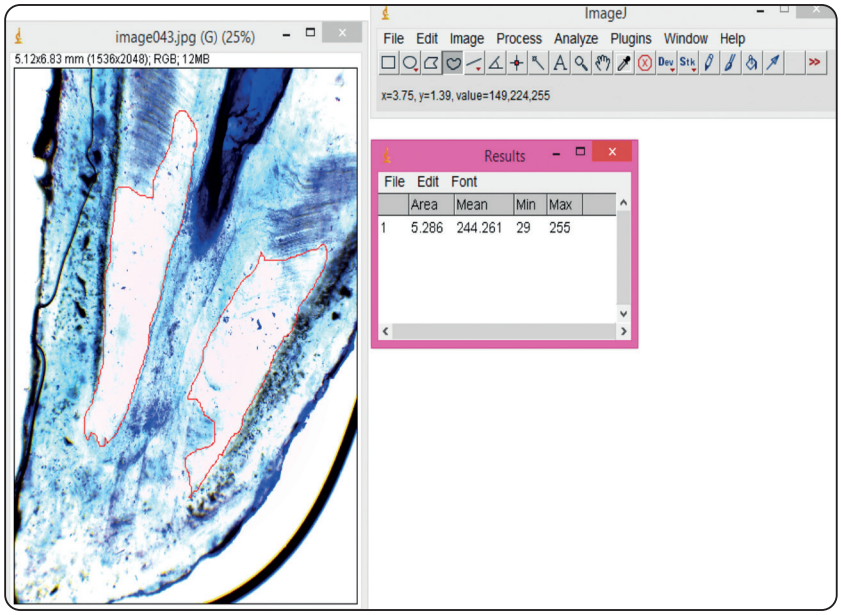

Fig. (2): Light microscopic image of the tooth section selected on Image-J software for measuring the surface area of RDT. Note the separated areas of RDT were measured on both sides of tooth midline.

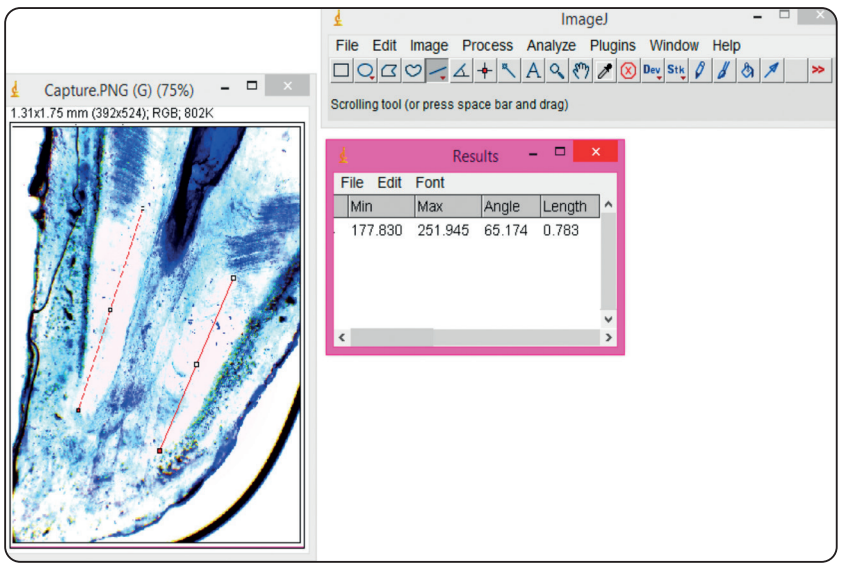

Fig. (3): Light microscopic image of the tooth section selected on Image-J software for measuring the average length of RDT on both sides of the root midline.

Statistical analysis

The collected data were organized, tabulated and statistically analyzed using SPSS version 19 (Statistical Package for Social Studies) created by IBM, Illinois, Chicago, USA. For numerical variables, the range mean, and standard deviations (SD) were calculated. For comparison of mean age, RDT area and length in relation to gender, the normal distribution was not guaranteed so Mann-Whitney test was used. The correlation between two variables was calculated using Pearson's correlation coefficient. Linear regression analysis was performed to identify the formula that can be used for estimation of age. Comparison between chronological age and estimated age was done using paired t test. The level of significant was adopted at $p<0.05$.

RESULTS

Table (1) demonstrate the mean and SD of chronological age in years, RDT lengths and areas in mm. The mean and SD of age for males were 53.50±11.36 and for females were 48.38±13.80 while the mean and SD of RDT area for males were 14.19±1.56 and for females 13.12±2.44 regarding RDT length for males 3.03±0.88 and for females 2.62±0.99. Totally, there was not a significant sex difference determined

TABLE (1): Comparison of chronological age, area, and length of RDTin relation to gender.

Variables	Males	Females
Age in yeas		
Range	33-70	20-70
Mean±SD	53.50±11.36	48.38±13.80
Median	54.0	48.50
RDTarea		
Range	11.8-16.9	7.7-15.9
Mean±SD	14.19±1.56	13.12±2.44
Median	14.41	13.75
RDTlength		
Range	1.2-4.5	1.2-4.2
Mean±SD	3.03±0.88	2.62±0.99
Median	3.20	2.63

Table (2) displays the correlation between chronological age, RDT area and length. Results indicated that, significant difference were found between RDT area, length and chronological age of all age groups ($p<0.05$).

TABLE (2) Correlation between chronological age in years, RDT area and length

Variables	RDTarea		RDTlength	
	r	p	r	p
Chronological age	0.903	<0.001	0.908	<0.001
RDTarea			0.817	<0.001

Table (V-3) demonstrates linear regression analysis of the correlation between chronological age and RDT area which was highly significant. This brings the following equation to calculate age in years-based RDT area:

Dental age = -25.924 + 5.621 (RDT area)

TABLE (3) Linear regression analysis of the correlation between chronological age and RDT area.

Variables	B	t	p
Constant	-25.924	4.301	<0.001
RDTArea	5.621	12.970	<0.001

Regression equation: **Dental age = -25.924 + 5.621 (RDT area)**

Table (4) show linear regression analysis of the correlation between chronological age and RDT length which was highly significant. This brings the following equation to calculate age in years-based RDT length.

Dental age = 16.796 + 12.088 (RDT length)

TABLE (4) Linear regression analysis of the correlation between chronological age and dentine length

Variables	B	t	p
Constant	16.796	6.161	<0.001
Length	12.088	13.357	<0.001

Regression equation: **Chronological age = 16.796 + 12.088 (RDT length)**.

Table (5) demonstrate linear regression analysis of the correlation between chronological age, RDT length and area which was highly significant. This brings the following equation to calculate age in years based on RDTlength and area.

Chronological age = -9.661 + 6.811(RDT **length) + 3.021** (RDT **area)**

TABLE (5): Linear regression analysis of the correlation between chronological age, RDT area and length

Variables	B	t	p
Constant	-9.661	1.838	0.074
Length	6.811	5.765	<0.001
Area	3.021	5.470	<0.001

Regression equation: Chronological age = -9.661 + 6.811(RDTlength) + 3.021(RDTarea)

Table (6): shows comparison between chronological age and estimated age by different methods. Concerning all age groups, the differences between the estimated mean age and chronological age were found statistically not significant. The range of difference between chronological and estimated age by both RDTarea and length was the lowest (-10.68-8.65) compared to estimated age by RDT area (-10.89-13.47) and RDT length (-14.46-16.74).

For age groups 30-39 and 40-49, the differences between chronological and estimated ages were not statistically significant. Concerning differences between estimated and chronological age in these two age groups, the lowest range of difference was that of estimated age by both RDT area and length.

TABLE (6): Comparison of chronological age with estimated age

Age groups	Mean ± SD (Range) (Range of difference)			
	Chronological age	Estimated age by RDTarea	Estimatedage by RDTlength	Estimated by both
20-39	32.86±6.34 (20-38)	35.43±11.67 (17.5-46.5) (-10.17-13.47)	37.57±3.61 (30.8-41.1) (-7.18-16.74)	35.02±7.13 (24.9-41.6) (-4.58-8.65)
Z		0.924	1.784	1.193
p		0.391	0.125	0.278
40-49	44.60±3.63 (40-49)	45.12±3.39 (40.5-52.1) (-4.94-9.10)	42.83±6.59 (31.5-50.8) (-14.46-4.80)	43.19±4.05 (35.3-48.7) (-10.68-2.89)
Z		0.374	0.942	1.163
p		0.717	0.371	0.275
50-59	53.73±3.16 (50-59)	56.19±4.15 (50.2-63.6) (-0.67-7.62)	54.53±4.67 (42.4-58.7) (-7.58-7.74)	55.73±4.40 (45.7-61.0) (-4.23-6.69)
Z		3.019	0.679	2.170
p		0.013*	0.513	0.055
60-70	93.90±4.92 (60-70)	61.73±3.73 (54.8-69.1) (-10.89-4.13)	63.90±4.92 (53.7-70.8) (-6.34-1.36)	63.99±4.05 (55.8-67.6) (-6.59-2.65)
Z		3.250	3.010	2.417
p		0.008*	0.012*	0.034*
All ages	51.45±12.48 (20-70)	51.45±11.27 (17.5-69.1) (-10.89-13.47)	51.45±11.33 (30.8-70.8) (-14.46-16.74)	51.45±11.86 (24.9-67.6) (-10.68-8.65)
95% CI	----------	-1.72-1.71	-1.67-1.67	-1.24-1.24
t	----------	0.001	0.001	0.001
p	----------	0.999	0.999	0.999

For age group 50-59, the difference of mean chronological and estimated age by RDT area was found statistically significant (p=0.013). Mean while, differences between chronological age and estimated age by RDT length and both RDT area and length were not statistically significant.

Among age group 60-70, differences between chronological and estimated age by different methods were all statically significant. The lowest age difference in the group of 60-70 was that estimated by RDT length and area that were more or less equal.

DISCUSSION

The need for identification of an unknown person can be social, emotional or legal. Verification regarding identity of the deceased is needed for obtaining a death certificate and for claims of insurance as well as to carry out the will. Additionally, identification of an unknown dead body is indispensable to carry out the investigation of offences like murder or abuse. Age determination plays an important role in forensic medicine, not only in identification of unknown individuals, but also in judgment of crimes[10]. In air crashes and mass disasters, when skeletal remains are in poor conditions, dentition is most frequently used for identification and age estimation of the victims [11,12].

The teeth are not only the hardest part of the body that are preserved for a long time after death without gross changes, but also, they can display a number of observable age-related variables. Teeth constantly undergo changes either due to aging or due to environmental factors. These factors aid in appreciable changes such as attrition, secondary dentin deposition, RDT, cementum apposition, root resorption, color changes, and an increase in root roughness. By considering these age changes, various studies highlight the importance of the teeth for age assessment of the individuals [13].

There is increasingly strong evidence that RDT is directly proportional to the increase in age thus it would serve as a reliable tool to aid the purpose of age estimation in both living and the dead. The measurement of RDTin both intact and sectioned tooth with different thickness levels has been reported by Miles, [14] Bang and Ramm, [15] and Thomas et al [16].

In this study the apical part of the root was used due to the following: it had been reported that RDT starts from the apical region then extends coronally[17]. Nalabandian et al.,1960 [18] stated that RDT was first marked in the apical part of the tooth, and they clarified that it was because of the lesser diameter of dentinal tubules in apical root dentin compared to that of the coronal part and the lesser number of dentinal tubules per unit area in the apical part. In addition, the appearance of translucency under LM is due to the equalization of the difference in refractive indices between intra tubular organic and extra-tubular inorganic materials after calcification of dentinal tubules with advancing age [9]. This alteration is believed to be due to occlusion of dentinal tubules by increased intratubular calcification[19].

In the current study, the sample of the extracted teeth were collected from patients ranging in age from 20 to 70 years to assure that dental development and growth has been completed. Moreover, the teeth were evenly distributed according to gender and different age groups to observe hormonal or aging effect on accuracy of RDT.

Multirooted teeth were not included in this study because they are very strong and hard, and it is difficult to make ground section. Also, teeth with severe attrition, abrasion, erosion, and grossly decayed teeth were excluded from the study because these pathologic conditions stimulate the increase in RDT of teeth [20, 21].

In this study, manual method for sectioning of teeth was used due to availability of diamond disc and Arkansas stone rather than hard-tissue microtome. Acharya attempted a similar study

where in the teeth were sectioned using hard-tissue microtome [22,23]. Moreover, the ground sections were stained with methylene blue 1 % that stains entire teeth blue except the RDT, thus make it easy to be visualizedas adopted by Thomas et al. in 1994[24].

Singh et al. (2013) stated that there is simple digital method to measure RDT on sectioned teeth. They made unstained thin longitudinal section and measured the amount of RDT using Adobe Photoshop. They found that the correlation coefficient of RDT and age is statistically significant. Similarly, **Loganathan et al. (2019)** concluded that RDT noted in apical root portion could be used for age estimation of an individual. They calculated the ratio of RDTlength to total root length in ground section stained with 1% methylene blue by using the graph sheet [1,25].

The results of the present study revealed that high correlation between chronological age and RDT area and length. This result come in a line with **KavitaaNedunchezhian et al., 2018** who reported a strong positive correlation between age and area of RDTand the age was estimated with an accuracy of ±5 years (61.4%) and ±10 years (12.9%). Similarly, **Jigna S. et al., 2020** revealed, a linear relationship between RDT and age in the regression analysis. Pearson's correlation analysis showed that there was positive correlation ($r = 0.93$, $P = 0.001$) between RDT and age, and the difference between the chronologic age and real age is ± 4.88 years.

In this study the range of difference between chronological and estimated age by both RDT area and length was the lowest (-10.68-8.65) compared to estimated age by RDTarea (-10.89-13.47) and RDT length (-14.46-16.74). This result is in accordance with **KiranKumar, et al., 2014** who made Multilinear regressive analysis to calculate age by both area and length and showed a high coefficient of regression ($R = 0.7797$)[26].

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