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HISTOMORPHOMETRIC AGE EVALUATION USING SECONDARY DENTIN IN ADULT TEETH WITH AND WITHOUT OCCLUSAL ATTRITION

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Abstract:
This research aimed to assess the correlation between the age of both males and females and the surface area of secondary dentin (SD) in the transverse ground section of human teeth with and without occlusal attrition. Eighty extracted sound teeth were allocated into four groups: Group I, 20 teeth without occlusal attrition from male patients; Group II, 20 teeth without occlusal attrition from female patients; Group III, 20 teeth with occlusal attrition from males; Group IV, 20 teeth with occlusal attrition from females. Later, transverse sections at the cervical third of each tooth root were prepared and examined. The correlation between the surface area of SD and the actual age together with patients' sex was determined utilizing Pearson's correlation coefficient. Linear regression was also calculated to study the relationship between the SD surface areas as a predictor of patients' age. From the obtained data, the chronological age was statistically significant, with a surface area of SD among all groups. The females showed a strong correlation, and the males showed a non-significant correlation between the chronological age and SD surface area. As well as, both teeth with occlusal attrition and those without occlusal attrition demonstrated a strong correlation between surface area and chronological age. The current research has highlighted the viability of using SD for age estimation in Egyptian adults for both sexes; however, the teeth showed occlusal attrition. Also, using the regression equations developed from this study specific to the sex and the tooth condition, dental age can be estimated by measuring the SD surface area from the histologic ground section.

Keywords: Secondary dentin, age estimation, transverse ground section, light microscope, forensic dentistry.

1. Introduction: -

Forensic dentistry is an important field of forensic medicine that analyzes dental or maxillofacial evidence from which dental outcomes can be assessed [1].

Estimation of dental age has an essential role in forensic odontology. In addition, teeth may be the sole parameter for person identification when they have undergone extensive alterations that external characteristics of the person harvest no information or in the case of unknown cadavers [2]. Also, preserving human teeth for a long time after death usually occurs; thus, they serve as an essential parameter in forensic odontology [3].

Several techniques are applied for age determination from teeth, including histological, radiographic, clinical, chemical, and physical assessment [1]. The histological method was developed in 1950 by Gustafson and Malmö [4]. They presented the age estimation parameters established on macroscopic and microscopic teeth features, including attrition, periodontitis, root resorption, cementum annulations, root dentin translucency, and SD deposition.

Physiologically, SD deposition begins shortly after root formation completion and then persists throughout the life from the primary odontoblast cells, which formerly deposit the primary dentin (PD) during tooth development [5].

Histologically, the distinction between SD and primary dentin is established by a bent in the direction of dentinal tubules between the two layers [6]. This bent appears as a dark line in the histological ground section [7]. Noteworthy, SD is not accumulated equally around the pulp chamber borders, and SD is mainly on the chamber's floor and roof. Thus, this causes an asymmetrical decrease in the size and shape of the pulp (pulp recession) [7].

Many factors affect SD formation, such as occlusal attrition, caries, and osmotic pressure changes in the pulp chamber [8]. Occlusal surface attrition is produced from tooth-to-tooth contact during chewing and function. Nudel et al. [9] suggested that occlusal forces that cause tooth wear might play a significant role in SD formation.

To our knowledge, there is no data concerning age evaluation for Egyptian adults using the surface area of SD in the histologic ground section. Thus, the main goal of this research was to determine the age of both male and female Egyptian adults by histomorphometric evaluation of the surface area of SD in the ground transverse section (TS) of premolar teeth. And evaluate the accuracy of the measuring surface area of SD in determining the chronological age of premolar teeth with and without occlusal attrition.

2. Materials and Methods:

2.1. The sample:

The study was performed after receiving approval from the Research Ethics Committee (REC), Faculty of Dentistry, Tanta University. Relevant rules and provisions of Helsinki held all procedures.

Eighty extracted premolar teeth gathered from the Department of Oral and Maxillofacial Surgery in the Faculty of Dentistry at Tanta University were used in this study. Teeth were taken from adult female and male patients aged 20-70 years with recognized age. The surgical teeth extractions were for various therapeutic reasons such as malocclusion, mobility, orthodontics, or prosthodontics after getting written consent from the patients.

Inclusion criteria: premolar teeth without occlusal attrition and others with occlusal attrition.

Exclusion criteria: Teeth with root resorption, developmental anomalies, endodontic problems, periapical pathologies, periodontal diseases, or severe caries. Teeth samples were allocated into four groups as follows:

	Number of teeth in each group	Teeth status	Age range	Gender
Group I	20 teeth	without attrition	20-70 years	Male
Group II		without attrition		Female
Group III		with attrition		Male
Group IV		with attrition		Female

2.2. Histological section preparation:

After extraction, teeth were washed with saline and kept in 10% formalin. Ground TS of the cervical third of the root was prepared according to Maat et al. [10]. First, the cervical third of the root was sawed transversally using low-speed contra (Champion-Korea), followed by manual milling with micro-motor (Champion-Korea). Hand grinding was then done by Arkansas Stone (Arkansas Stone-West Monroe) to achieve a proper thickness of 100-150 µm. They were washed with water, dried out, and mounted on a microscopic glass slide using Canada balsam. Also, they were coded for examination under a light microscope (LM). Afterward, all microscopic images were taken for each slide at magnification x 40.

2.3. Image-J analysis and measurement:

SD surface area was measured on the captured images by analysis system (Image-J) (National Institutes of Health in the USA) as follows: SD appears at the pulpal surface of dentin with the course of dentinal tubules differ from that of PD, and a separating line could be detected between SD and PD (Fig.1). Surface area of SD was determined using some tools available on Image-J software: on the scale bar analyze portion was selected. Then, the free hand tool was activated to outline the SD (Fig. 2,3,4,5). To assess potential interobserver variation, the second observer again measured the SD surface area on all group sections, and the average SD area was recalculated.

2.4. Statistical analysis:

The assembled data were analyzed using SPSS (version 20) (Statistical Package for Social Studies). For statistical values, the mean and standard deviations were determined. The statistical differences between the two mean values were used using the student's T-test. Also, the correlation between sex, occlusal attrition, and age was calculated using Pearson's correlation coefficient. A simple linear regression equation was calculated to study the relationship between the SD surface areas as a predictor of chronological age. The point of significance was adopted at $P<0.05$.

3. Results: -

3.1. Histological Results Figures:

3.2. Statistical Results:

Table (1) demonstrates Pearson's correlation between chronological age and surface area of SD relative to attrition and gender. The chronological age was significantly correlated with a surface area of SD among all groups. The females showed a strong correlation, and the males showed a non-significant correlation between the chronological age and SD surface area. As well as both teeth with attrition and teeth without attrition demonstrated a strong statistical correlation between the chronological age and SD surface area.

Table (2) reveals the mean and standard deviation of chronological age in years and the surface area of SD in mm. The mean and standard deviation of the age for all teeth without attrition (groups I & II) were 6.33+1.06, and for all teeth with attrition (groups III & IV), were 8.85+1.12 while the mean and standard deviation of surface area for group I were 6.34+0.88. For group II, there were 6.31+1.27. For group III, they were 9.31+1.16, and for group IV, 8.40+0.93.

Table (3) shows the mean and standard deviation of SD surface area about teeth attrition and gender. Both the mean and standard deviation of SD surface area for all teeth without attrition (groups I & II) were 45.75+14.13, and for all teeth with attrition (groups III & IV), were 46.00+14.15 while the mean and standard deviation of surface area for group I were 45.90+14.97. For group II, there were 45.60+14.05. For group III, it was 46.00+14.75, and for group IV, 46.00+14.31.

Table (4) exhibits linear regression analysis between the chronological age and SD surface area SD, which was statistically significant in all groups. This creates the following equation to determine age in years using the surface area of SD. Chronological age = 4.763 + 0.062 (SD surface area)
Linear regression analysis between chronological age and surface area of SD was statistically highly significant in all teeth without attrition (groups I & II). This gives the following equation to determine age in years established on the surface area of SD. Chronological age = 3.540 + 0.061 (SD surface area)
In all teeth with attrition (groups III & IV), the linear regression analysis between chronological age and surface area of SD was highly significant, with the following equation determining the age in years established on the surface area of SD. Chronological age = 6.063 + 0.061 (SD surface area)
In all teeth related to males (in groups II & IV), the linear regression analysis between chronological age and surface area of SD was highly significant, with the following equation determining the age in years established on the surface area of SD. Chronological age = 5.356 + 0.054 (SD surface area)
In all teeth related to females (in groups I & III), the linear regression between chronological age and surface area of SD was highly significant, with the following equation determining the age in years established on the surface area of SD. Chronological age = 5.356 + 0.054 (SD surface area)

Discussion:
The present research focused on estimating the age of both male and female Egyptian adults by measuring the surface area of SD on the transverse ground section of premolar teeth, one of the six parameters for age estimation discovered by Gustafson and Malmö [4]. We also tried to evaluate measuring surface area of SD in determining the chronological age of premolar teeth with/without occlusal attrition. Occlusal attrition is another parameter of the six criteria for age estimation developed by Gustafson and Malmö [4].
Dentine formation is a dynamic process throughout the tooth life, even after tooth eruption and root completion. Secondary dentin is considered a physiological age-related change as it is deposited in a slow and regular form, and it is not affected by caries, periodontal diseases, or other oral diseases [11]. Previous studies stated that dentin formation can appear normally as a feature of the aging process due to different physiological and pathological spurs that cause pulp cavity size reduction [12]. Thus, it is used as an important age estimation tool, and in some cases, it is considered the only parameter for age estimation in forensic odontology [13,14]. Bermudez and Nicolas [15] confirmed that teeth size and morphology differ according to several factors, such as sex, environment, and dietary habits. However, Dempsey et al.[16] stated that genetic factors are responsible for the varying morphology of the teeth. It has been well-known for a long time that the physiological age changes on teeth, such as occlusal attrition and cementum deposition, can be measured macroscopically or microscopically, while SD deposition can be assessed under a light microscope using the ground section [17]. Histologically, SD was first used in the histological section in 1950 by Gustafson and Malmö [4]. This study used SD as a single histological variable for developing the age estimation regression formula in teeth with and without occlusal attrition. Also, Benzer [18] stated that a cap of clear irregular SD is produced on the pulp chamber floor, followed by SD deposition along the root canals and on the roof of the pulp chamber. Thus, in this research, the transverse ground sections for all teeth were conducted from the cervical third of the root for the ease and clear estimation of SD surface area. During the microscopic examination of SD, the ground sections of the older teeth showed clearer and easier determination than those of young age. This might be attributed to an increase in mineralization of SD by age [19].
From the statistical results, the chronological age was significantly correlated with a surface area of SD among all groups, with a correlation coefficient of 0.514. This was lower than the value of previous studies, which measured the thickness of SD. They recorded 0.76 [20], 0.664 [14], and 0.96 [11]. Recently, Pandji et al. [21] realized that the correlation between the chronological age and SD of maxillary canine on CBCT radiographs was not significant enough to be applied as a single tool for age estimation in males and females. They found the correlation coefficient was 0.270 for males and 0.427 for females. However, Bhakhar et al. [22] concluded that the SD

thickness parameter cannot estimate age alone but only permits classifying individuals into a range of age or age categories.

Interestingly, females showed a strong correlation, but males showed a non-significant correlation between the chronological age and surface area of SD. This was in agreement with Woods et al. [23], as they found that females have a higher rate of SD formation than males, especially after the fourth decade of life when they measured the dimensional changes in the pulp cavity, not the surface area of dentin. However, Zilberman and Smith [24] found that girls had a lower degree of SD deposition than boys when they measured the SD thickness on the pulp chamber floor in children. They attributed this issue to the possible sex differences in the amount of SD formed, and they stated that the dimorphism could be associated with the distinct developmental path of odontoblasts in the crown that differentiate earlier than the radicular odontoblasts. Dentin formation in the root is more complicated than in the crown, with formation than the epithelial sheath of Hertwig and the differentiation of cementoblasts. Recently, Arthanari and Mohamed [25] measured the pulpal ratio in sex determination, and they found that SD was a valuable tool for determining the sex of the Indian population. Also, García-Campos et al. [26] concluded that the SD proportion of the adult teeth differed between males and females and suggested that there were intersexual variations. Nevertheless, Pandji et al. [21] found that SD volume was higher in the male sample than in the female one, and they attributed this result to sexual dimorphism in female and male teeth.

Substantially, sexual variation in the dental hard tissue volume can be exhibited because of the X and Y chromosomes and the amelogenin gene that is found on the X chromosome. Thus, the X chromosome can affect enamel formation. It was found that X chromosomes affect the formation of enamel only, while Y chromosomes affect enamel and dentin formation [26]. Another recent study by Pentinpuro et al. [27] found that females with chromosome (47, XXX) have an increase in SD deposition in the mandibular teeth roots than in males with chromosome (47, XXY) which is reflected in the crown height. This was inconsistent with the results of the current research.

Both teeth with/without attrition demonstrated a strong correlation between the chronological age and SD surface area; this was in agreement with previous studies [8,28] but in contrast with Nudel et al. [9], who found that in teeth with severe attrition, age estimation might be overestimated due to increased SD formation. They attributed this result to the increased occlusal force that causes odontoblastic activation and SD deposition as a compensatory mechanism for occlusal attrition.

The Egyptian population-specific regression equation was obtained for all groups; Age (y) = 4.763 + 0.062 (SD surface area), for all teeth without attrition (group I & II); Age (y) = 3.540 + 0.061 (SD surface area), for all teeth with attrition (group III & IV); Age (y) = 6.063 + 0.061 (SD surface area), for all males; Age (y) = 5.356 + 0.054 (SD surface area), and for all females; Age (y) = 4.145 + 0.070 (SD surface area). Age was assessed with the mean absolute error ranging from 3.540 to 6.063 years between the groups.

Conclusion:

The current research highlighted the viability of using secondary dentin as a single parameter for age estimation in Egyptian adults for both sexes; however, the teeth show occlusal attrition. Also, using the regression equations developed from this study specific to the sex and the tooth condition, dental age can be estimated by measuring secondary dentin surface area from the histologic ground section.

Acknowledgments:

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Figures:

Fig.1:

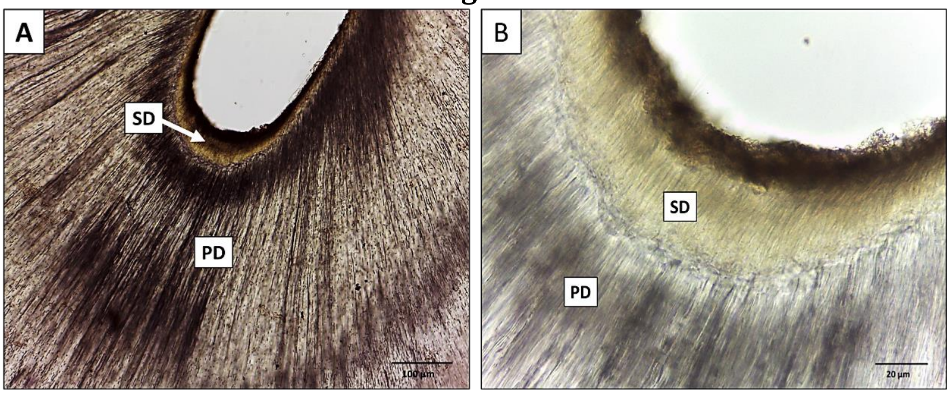


Fig.1: Micrograph of lower right canine taken by LM illustrates the ease of detection of the secondary dentine (SD) from the primary dentin (PD) in the transverse ground section (T.S., Orig. mag. AX 100, BX400).

Fig. 2:

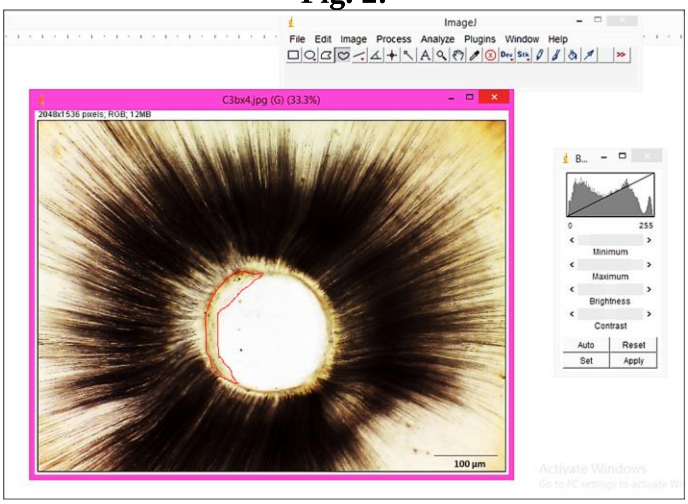


Fig. 2: Micrograph of TS ground section lower first premolar without attrition (**Group I**) related to a male patient aged 35 years selected on Image-J software for measuring the surface area of SD (T.S., Orig. mag. X40).

Fig. 3:

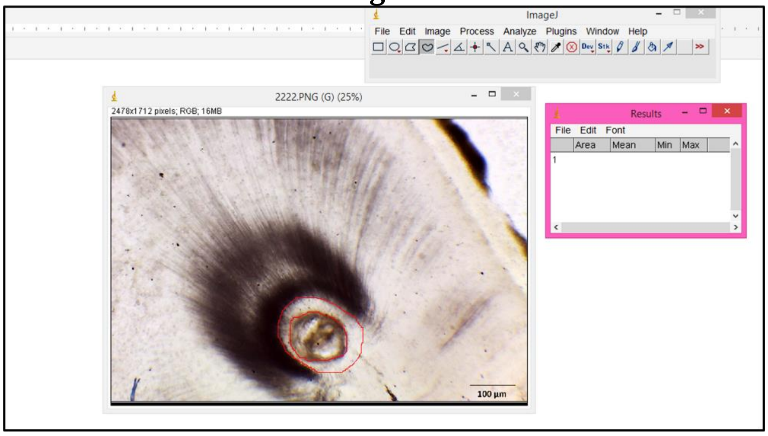


Fig. 3: Micrograph of TS ground section of lower first premolar without attrition related to a female patient aged 57 years (**Group II**) selected on Image-J software for measuring the surface area of SD (T.S., Orig. mag. X40).

Fig. 4:

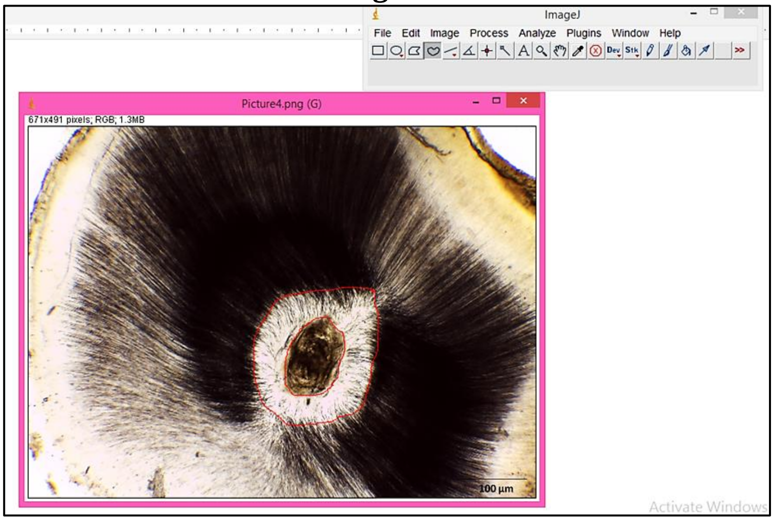


Fig. 4: Micrograph of TS ground section of upper second premolar with attrition related to a male patient aged 73 years (**Group III**) selected on Image-J software to measure the surface area of SD (T.S., Orig. mag. X40).

Fig. 5:

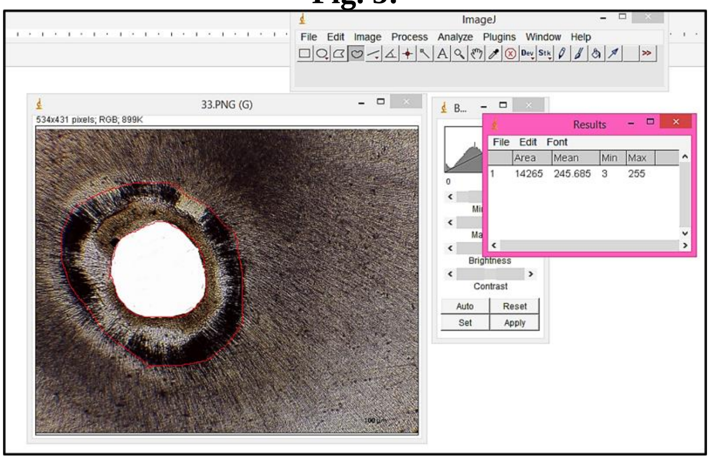


Fig. 5: Micrograph of TS ground section of lower second premolar with attrition related to a female patient aged 35 years (**Group IV**) selected on Image-J software to measure the surface area of SD (T.S., Orig. mag. X40).

Fig. 6:

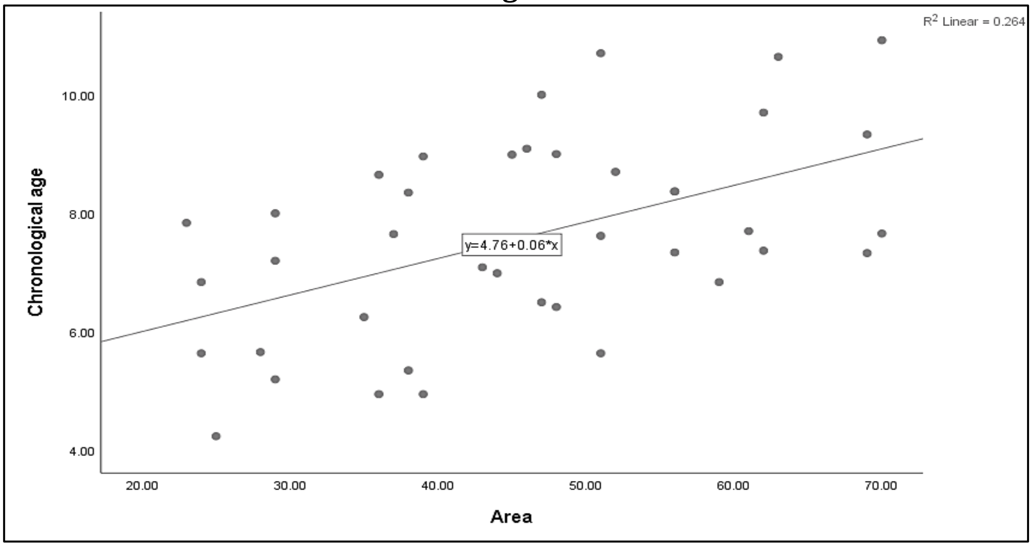


Fig. 7:

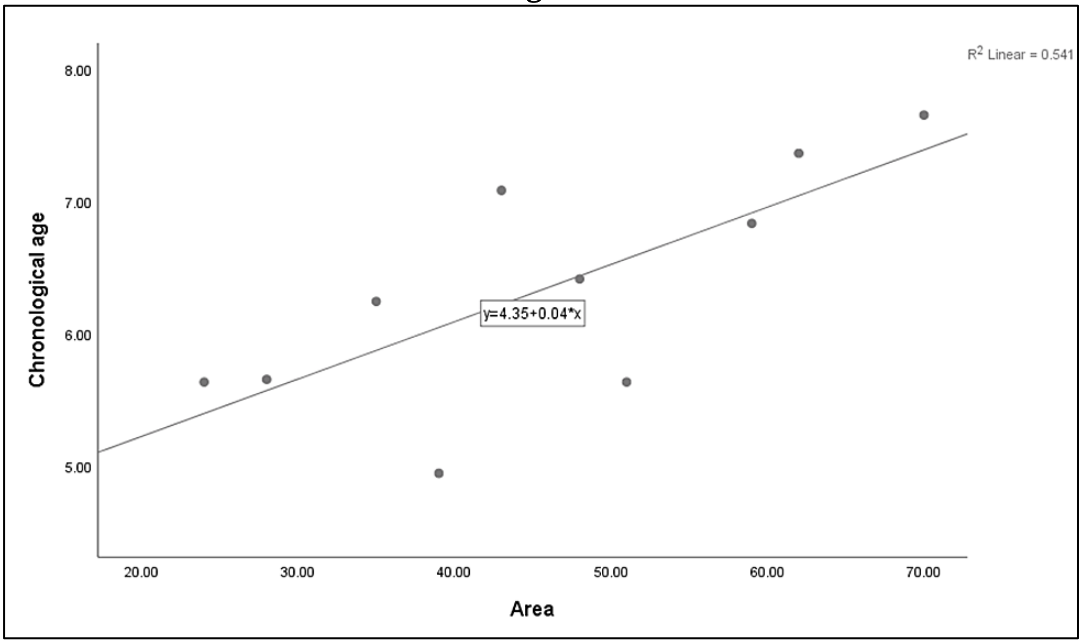


Fig. 8:

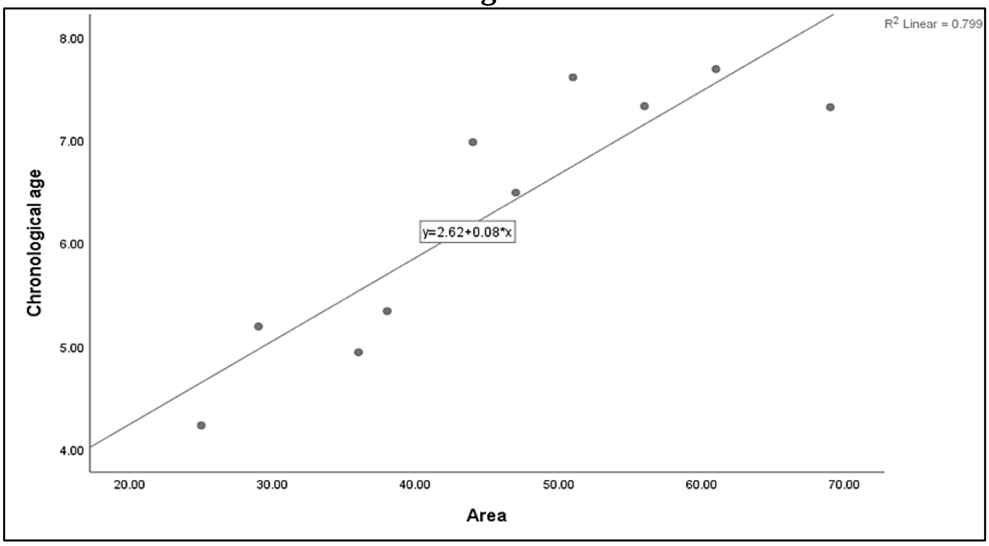


Fig. 9:

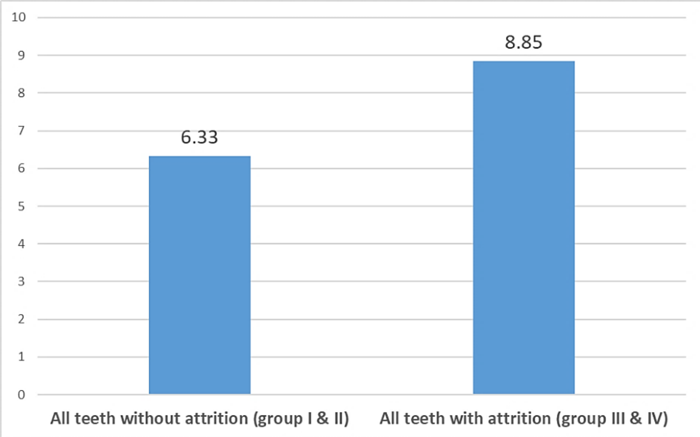
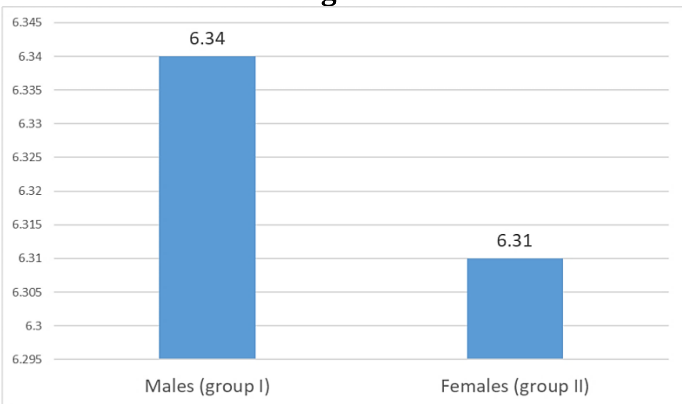


Fig. 10:



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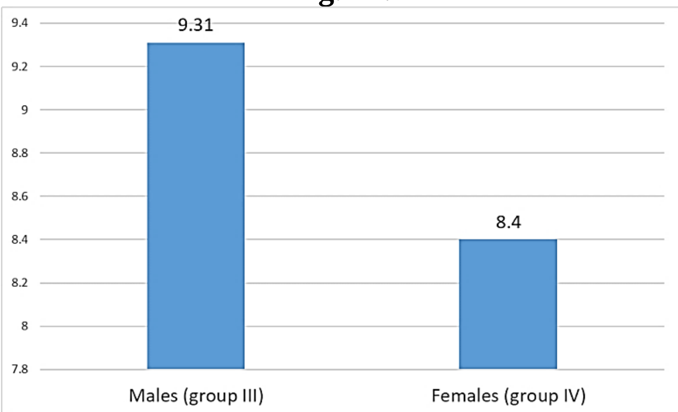

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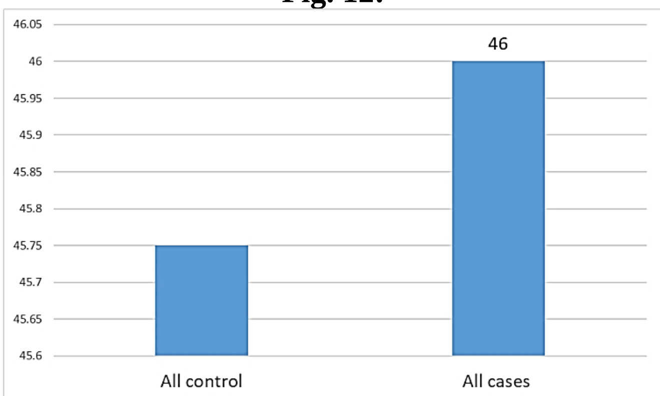


Fig. 13:

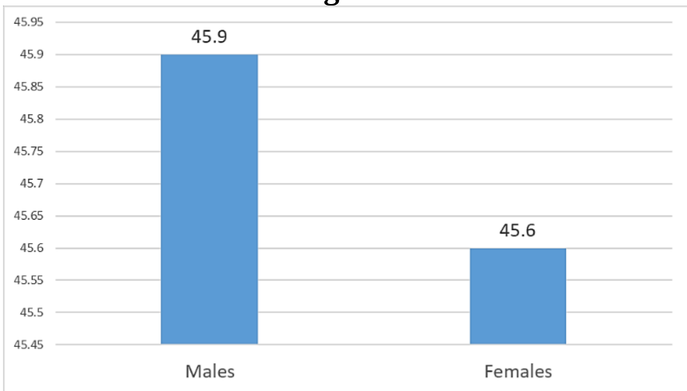
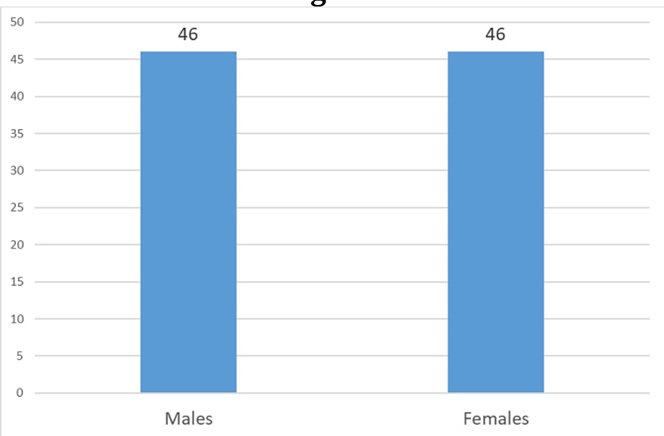


Fig. 14:



Tables:

Table (1): Correlation between chronological age and surface area of secondary dentin about attrition and gender.

Surface area of secondary dentine	Chronological age in years	
	r	*P*
Among all groups	0.514	0.001*
Among males (in groups I & III)	0.426	0.061
Among females (in groups II & IV)	0.635	0.003*
Among teeth with attrition (in groups III & IV)	0.764	<0.001*
Among teeth without attrition (in groups I & II)	0.809	<0.001*

(*) means significant correlation.

Table (2): Comparison of the chronological age of teeth attrition and gender

Chronological age	Range	Mean $\pm$ standard deviation	t	p
All teeth without attrition (groups I & II)	4.2-7.7	6.33$\pm$1.06	7.301	<0.001*
All teeth with attrition (groups III & IV)	6.8-10.9	8.85$\pm$1.12		
Teeth without attrition Males (group I) Females (group II)	 4.9-7.6 4.2-7.7	 6.34$\pm$0.88 6.31$\pm$1.27	0.061	0.952
Teeth with attrition Males (group III) Females (group IV)	 7.8-10.9 6.8-9.7	 9.31$\pm$1.16 8.40$\pm$0.93	1.921	0.071

(*) means significant difference.

Table (3): Comparison of SD surface area about teeth attrition and gender

Area	Range	Mean ± SD	t	p
All teeth without attrition (groups I & II)	24-70	45.75±14.13	0.056	0.956
All teeth with attrition (groups III & IV)	23-70	46.00±14.15		
Control group			0.046	0.964
Males (group I)	24-70	45.90±14.97		
Females (group II)	25-69	45.60±14.05		
Teeth with attrition			0.001	1.000
Males (group III)	23-70	46.00±14.75		
Females (group IV)	24-69	46.00±14.31		

Table (4): Linear regression of the relationship between chronological age and SD surface area

Variables	B	t	p
All groups:			
Constant	4.763	5.951	<0.001*
Area	0.062	3.689	<0.001*
	Age (y) = 4.763 ± 0.062 (SD surface area) R^2 = 0.264, P <0.001		
All teeth without attrition (groups I & II)			
Constant	3.540	7.088	<0.001*
Area	0.061	5.830	<0.001*
	Age (y) = 3.540 ± 0.061 (SD surface area) R^2 = 0.654, P <0.001		
All teeth with attrition (groups III & IV)			
Constant	6.063	10.445	<0.001*
Area	0.061	5.021	<0.001*
	Age (y) = 6.063 ± 0.061 (SD surface area) R^2 = 0.583, P <0.001		
All males			
Constant	5.356	4.149	0.001*
Area	0.054	2.000	0.061
	Age (y) = 5.356 ± 0.054 (SD surface area) R^2 = 0.182, P =0.061		
All females			
Constant	4.145	4.317	<0.001*
Area	0.070	3.487	0.003*
	Age (y) = 4.145 ± 0.070 (SD surface area) R^2 = 0.403, P =0.003*		