

**Age Estimation Based on Secondary Dentin Thickness in Thai Adults****การประมาณอายุจากความหนาของเนื้อฟันทุติยภูมิในคนไทยวัยผู้ใหญ่**

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**ABSTRACT**

Age estimation is very important in forensic science. The objective of this study was to find the correlations between age and secondary dentin thickness and inclusion parameters. The data were based on 106 samples of extracted teeth (male 53; female 53) of deceased individuals ranging in age from 32 to 86 years, with an average age of  $67.19 \pm 12.42$  and  $59.53 \pm 16.77$  years for male and female, respectively. Freshly extracted maxilla and mandible of left teeth (tooth ID 3, 4 and 5) were selected for the present study. After cleaning and data collection, the teeth were imaged using Image-J<sup>®</sup> 1.46r for the analysis of secondary dentin thickness. Five equations were established for age estimation: Equations based on secondary dentin thickness for certain age ( $R^2 = 0.955$  and  $R^2 = 0.953$ ) and for age group ( $R^2 = 0.855$ ); Equations based on inclusion parameters (tooth wear and secondary dentin thickness) for certain age ( $R^2 = 0.964$ ) and for age group ( $R^2 = 0.900$ ). Sex, secondary dentin thickness and tooth wear were found to be correlated with age with the value of  $-0.223^*$ ,  $0.499^*$  and  $0.582^{**}$ , respectively. It could be concluded that secondary dentin thickness and inclusion parameters were promising for estimating age in a range of 50-80 years, while secondary dentin thickness alone was unreliable for this purpose.

**บทคัดย่อ**

การประมาณอายุเป็นหนึ่งในปัจจัยสำคัญในงานด้านนิติวิทยาศาสตร์ วัตถุประสงค์ คือ หาความสัมพันธ์ของอายุกับความหนาของเนื้อฟันทุติยภูมิและการรวมพารามิเตอร์ วัสดุและวิธีการ คือ ใช้ฟันแท้ด้านซ้ายทั้งข้างบนและล่างของผู้ใหญ่ 106 ซี่ ประกอบด้วย ฟันตำแหน่ง 3, 4, และ 5 (เป็นเพศชาย 53 ซี่ อายุเฉลี่ย  $67.19 \pm 12.42$  ปี และเพศหญิง 53 ซี่ อายุเฉลี่ย  $59.53 \pm 16.77$  ปี) มาทำความสะอาด แล้วเก็บข้อมูลฟันและนำภาพที่ได้มาวิเคราะห์ความหนาของเนื้อฟันทุติยภูมิและตติยภูมิด้วย โปรแกรม Image-J<sup>®</sup> 1.46r ผลและวิจารณ์ผล: สมการในการประมาณอายุมี 5 สมการ ได้แก่ สมการที่ประมาณอายุจากความหนาของเนื้อฟันทุติยภูมิทั้งแบบแต่ละอายุ ( $R^2=0.955$  และ  $R^2=0.953$ ) และแบบกลุ่มอายุ ( $R^2=0.855$ ) และสมการที่ประมาณอายุจากการรวมของพารามิเตอร์แบบแต่ละอายุ ( $R^2=0.964$ ) และแบบกลุ่มอายุ ( $R^2=0.900$ ) เพศ ความหนาของเนื้อฟันทุติยภูมิและความสึกของฟันมีความสัมพันธ์กับอายุที่ค่านัยสำคัญทางสถิติ  $-0.223^*$   $0.499^{**}$  และ  $0.582^{**}$  ตามลำดับ สรุปผลการศึกษา: เนื้อฟันทุติยภูมิและการรวมของพารามิเตอร์ สามารถประมาณอายุได้ในช่วงอายุ 50-80 ปี และความหนาของเนื้อฟันทุติยภูมิเพียงอย่างเดียวไม่สามารถทำให้การประมาณอายุแม่นยำได้จะต้องอาศัยหลายพารามิเตอร์ร่วมด้วย

**Keywords:** Age estimation, Secondary dentin thickness, Tooth wear**คำสำคัญ:** การประมาณอายุ ความหนาของเนื้อฟันทุติยภูมิ การสึกของฟัน

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## Introduction

In forensic medicine, personal identification of personal death is very important. There is plenty of evidence for postmortem personal identification. However, for a long-time death, only human remains as bones and teeth are useful for personal identification. Teeth are resistant to degradation through the age at death (Stein, 1990) and can resist to high temperatures, closed to 1600 °C without loss of their structure (Clement and Ranson, 1998). In fact, the matrix of enamel and dentin as moisture evaporates while increasing pressure causes the extreme temperature and enamel protected within the bone so they is likely to remain (Ubelaker, 2009). Therefore, gradual changes in the structures as appeared in teeth throughout life are the key parameter for age estimation. Accordingly, the enamel, dentin or cementum can be used for age estimation (Antonio, 2003).

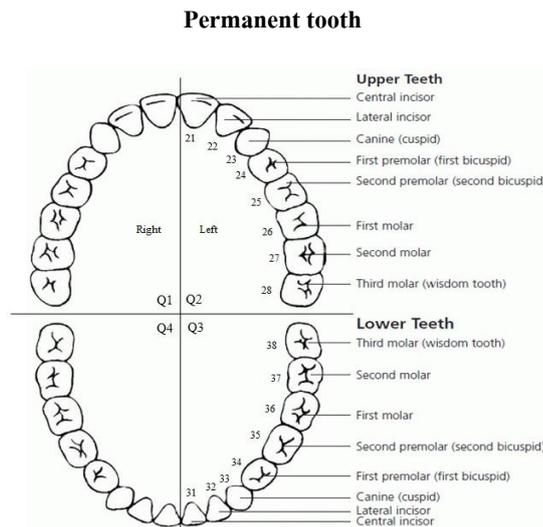
Several methods have been introduced for estimating age from teeth, including radiographic, histological and biochemical methods. Histological method is employed to prepare ground sections of teeth for microscopic examination to estimate the stages of development of tooth dentition. Histological method has been previously studied by several researchers. Gustafson (1950) studied the loosen teeth under microscopic examination for age changes appeared in dental tissues, and six parameters for attrition, periodontics, secondary dentin, cementum apposition, root resorption and transparency of root. The error of Gustafson's method used for the calculation of the age estimates was  $\pm 3.6$  years (Gustafson, 1950). In spite of its several advantages, Gustafson's method cannot be used in living person (Priyadarshini et al., 2015). Gustafson's method has been adopted widely in many studies. For instance, Dalitz (1962) used this method to re-examine xx to make 5-point grading scale system instead of 4-point grading scale system. Maples (1978) used only two (secondary dentin and root transparency) out of six parameters of Gustafson's method for estimating age. In addition, Solheim (1993) selected five out of six parameters (with the exclusion of root resorption) and added three parameters (roughness, color and sex) for age estimation. Johanson (1971) followed the method of Solhem by using the aforementioned six parameters and adding more details of root transparency. Out of the six parameters of Gustafson's method, the secondary dentin was widely studied by several researchers. The secondary dentin forms after the complete formation of tooth roots and its thickness can be used for age estimation (Star, 2010).

The age advance causes pulp cavity smaller due to the secondary dentin is continuously deposited (Morse et al., 1991). There is a strong positive correlation between the secondary dentin thickness and the advanced age (Solheim, 1992). Total pulp area decreases significantly due to the decrease of the secondary dentin when age advances (Rai et al., 2007). The secondary dentin deposition can be used to indicate age estimate, which is statistically reliable (Bhakhar et al., 2013). As age advances, the secondary dentin thickness increases as well (Priya et al., 2014). The secondary dentin thickness is one of the important pieces of evidence for age estimation. However, studies on the secondary dentin thickness in Thai adults have not yet been conducted. Thus, investigating the variations in the secondary dentin thickness in Thai adults is of great interest.

For this purpose, this study was carried out to examine the correlation between secondary dentin thickness and actual age.

### Objective of the study

1. To examine the correlation between secondary dentin thickness and chronological age in Thai adults.
2. To examine the correlation between inclusion parameters and chronological age in Thai adults.
3. To examine tertiary dentin thickness, tooth wear, number of tooth root, number of tooth canal and odontometry.



**Figure 1** Permanent tooth (From <http://goo.gl/bJ2TJv> and <http://goo.gl/YtyQYj>)

### Dentin

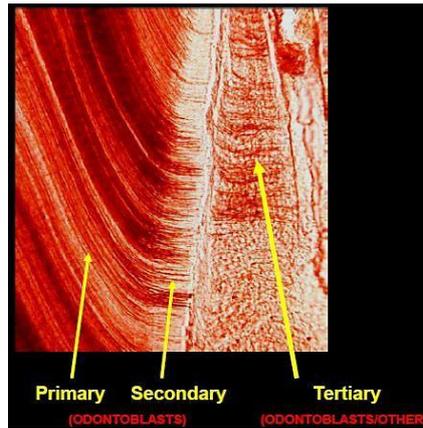
Dentin was the hard tissue and compound were organic and water (35% such as collagen fiber and proteoglycan), inorganic (65% such as hydroxyapatite crystallite). Dentin was part of the pulp-dentin complex and formed the bulk of the tooth. Dentin was a bonelike matrix characterized by multiple closely packed dentinal tubules that traverse its whole thickness and contain the cytoplasmic extensions of odontoblaste that once formed the dentin and maintain it (Antonio, 2003).

Types of dentin: were three types such as primary dentin, secondary dentin and tertiary dentin; (Figure 2)

- Primary dentin was outline the pulp chamber and was referred to circumpulpal dentin and the most of tooth was formed primary dentin.

- Secondary dentin was developed after root formed has been completed and represents the continuing in throughout life but very slower than deposition. Also, use to for age estimation.

- Tertiary dentin was reaction to various stimuli such as attrition, caries, or restorative dental procedure as unlike primary or secondary dentin that formed along the entire pulp-dentin border.



**Figure 2** Types of dentin as formed according to deposited (From <https://goo.gl/lkr2E7>)

### Materials and methods

The data were based on 106 samples of extracted teeth (male = 53; female = 53) of deceased individuals ranging in age from 32 to 86 years. The samples were collected from the Department of Anatomy of the Faculty of medicine of Khon Kaen University, Thailand. Freshly extracted maxilla and mandible of left teeth were selected for the present study. Prior to sample collection, permission of institutional ethics committee (Office of the Khon Kaen University Ethics Committee in Human Research (No. HE 591215)) was taken. The teeth were divided into four age groups with 10 years of interval, i.e. 50-59 years, 60-69 years, 70-79 and 80-89 years. After cleaning and storage in 10% hydrogen peroxide for 7 days, the teeth were washed in running tap water and air-dried for approx. 1-2 days at room temperature.

Before longitudinal buccolingual section, the teeth were subjected to odontometric analysis using vernier caliper and Image-J<sup>®</sup> 1.46r program and were examine for the number of tooth roots and canals, and tooth wear. After fixation, the teeth were embedded in resin block for 3 days

After that, fixed teeth embedded in resin block for 3 days. All cutting teeth into resin block for running water were longitudinal buccolingual section by Buehler Isomet 1000<sup>®</sup> in 0.8 mm. Ground thickness and speed 350 rpm. Next, wet rubbed all teeth with sand paper and measured thickness by digital micrometer as 80-100  $\mu$ m. Then, lay sections in one corner and mounted on a standard microscope slide and covered with cover slip. The slides were viewed under a light microscope with magnification at 100X and images captured by using digital camera. All image's data were measured for tooth secondary dentin and tertiary dentin thickness with Image-J<sup>®</sup> 1.46r software and all data were analyzed correlation with the SPSS statistical program.

### Examination for measurement of secondary dentin and tertiary dentin

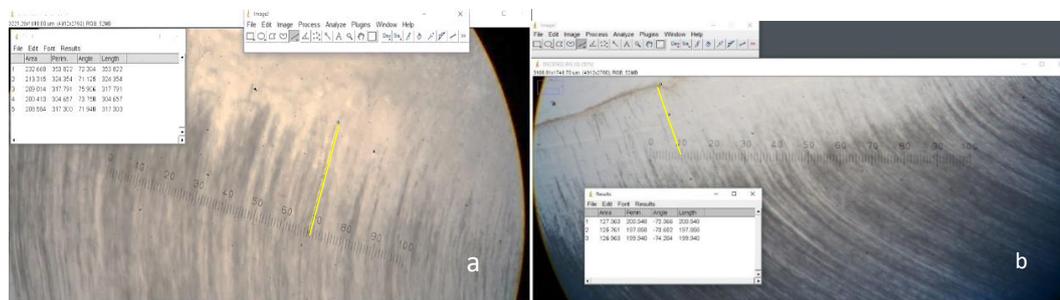
Before, bring to all images of secondary dentin and tertiary dentin were captured under light microscope for measurement by Image-J<sup>®</sup> 1.46r program must be calibration between ocular micrometer and stage micrometer.

### Collecting data for secondary dentin and tertiary dentin thickness

All secondary dentin and tertiary dentin thickness were measurement by Image-J<sup>®</sup> 1.46r program. The measure process was repeated for two times in another day.



**Figure 3** Set scale was known distance.



**Figure 4** (a) Measure for secondary dentin thickness, (b) Measure for tertiary dentin thickness.

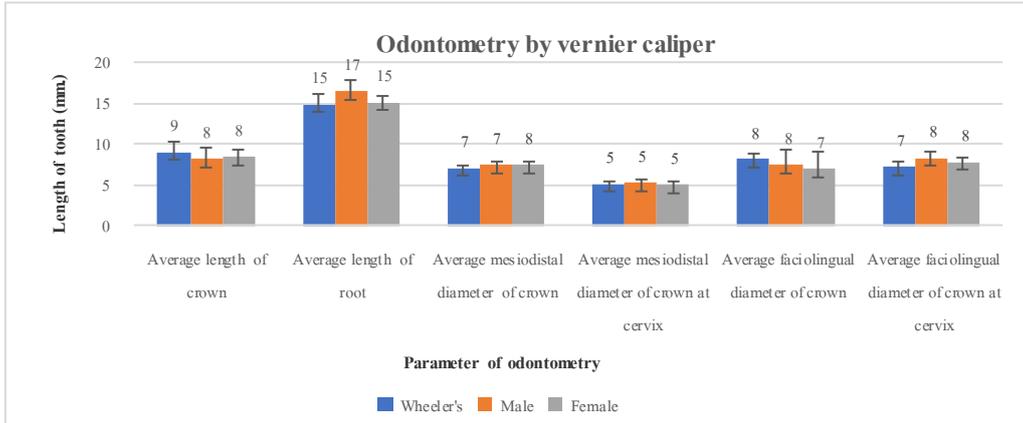
### Results and Discussion

There were tooth ID 3,4,5 with in examination for odontometry, number of tooth root, number of root canal, and tooth wear. And the 106 teeth (males and females were each 53 teeth) were tooth ID 3,4,5 for measured secondary dentin thickness and tertiary dentin thickness.

Inclusion 106 teeth in this study for measured secondary dentin thickness and tertiary dentin thickness, age range 32-86 years, tooth ID 23, 24, 25, 33, 34 and 35. Average ages were  $63.81 \pm 14.93$  years. Divide were 53 males with  $67.19 \pm 12.42$  years and 53 females with  $59.53 \pm 16.77$  years.

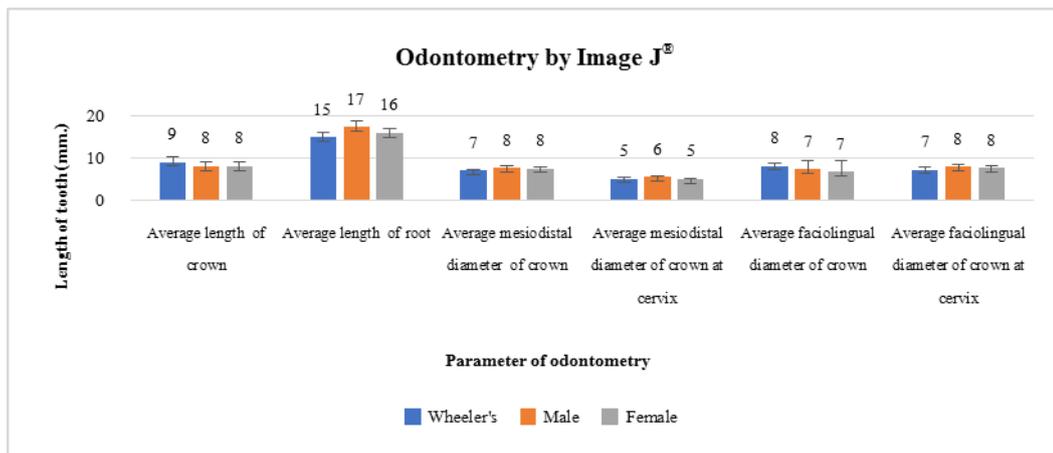
**Odontometry or dental measurement**

**Measured by vernier caliper compare with Wheeler's 1984**



**Figure 5** Odontometry by vernier caliper

**Measured by Image-J<sup>®</sup> 1.46r program compare with Wheeler's 1984**



**Figure 6** Odontometry by program Image J<sup>®</sup>

**Number of tooth root and number of root canal**

**Table 1** Number of tooth root and number of root canal compare with Wheeler's 2010

Tooth ID	Wheeler's (root)	Number of tooth root (root)	The most of number of root tooth (root)	Wheeler's (canal)	Number of root canal (canal)	The most of number of root canal (canal)
23	1	1	1	1	1 or 2	1
24	1 or 2	1 or 2	1	2	1 or 2	2
25	1	1 or 2	1	1 or 2	1 or 2	2
33	1	1	1	1	1 or 2	2
34	1	1 or 2	1	1 or 2	1 or 2	2
35	1	1	1	1	1 or 2	2

**Note:** 23= canine maxillary left, 24= first premolar maxillary left, 25= second premolar maxillary left,

33= canine mandibular left, 34= first premolar mandibular left, 35= second premolar mandibular left

**Tooth Wear**

Tooth wear 120 teeth were recorded. The score of tooth wear was used to Smith and Knight (Smith, Knight, 1984) they use tooth wear index by score system to four surface such as buccal, lingual, cervical, occlusal or incisal as score were 0-4 score

The conclusion score of tooth wear in this study were Score 0, Score 1, Score 2, Score 3 and Score 4 (6, 33, 31, 26 and 24 teeth) respectively. In group age were six groups age such as 30-39, 40-49, 50-59, 60-69, 70-79, 80-89. The most frequency tooth wear of each group age such as group age 30-39 were Score 1 and Score 2, group age 40-49 were Score 0 and Score 2, group age 50-59 were Score 3, group age 60-69 were Score 2, group age 70-79 were Score 4, and group age 80-89 were Score 4.

Conclusion, in this study was found group age 50-59 years were began very tooth wear and can found very tooth wear in group age 70-79 years and 80-89 years as corresponding with in Spijker et al. Spijker et al. (2009) they found percentage of tooth wear with in adult patients. In the age of 20 years found tooth wear was increased 3% and age of 70 years found tooth wear was increased 17%. Smith, Robb (1996) they study with in tooth wear in English patients were 1,007 patients and they found tooth wear in the group age 15-26 years was 5.73% , group age 56-65 years was 8.19% and group age 65 years above was 8.84% and the percentage were shown relation tooth wear and advance age. Smith, Knight (1984) they study with in 100 patients for tooth wear index and result was the score increase with in advance age (46 years above was beginning high tooth wear). Jarvinen et al. (1992) they found palatal

of maxillary incisor was position very tooth wear from food and acid beverage because it was the first position contract with food while the food into the mouth and tongue will be push the food contact with palatal of maxillary incisor. Smith, Knight (1984) they found all position of tooth can tooth wear from the food but very tooth wear can have found with in buccal at cervix, labial of maxillary tooth and occulsal/incisal of mandibular tooth. The tooth wear has not one cause and depend duration and individual. The vegetarian's food was the most consist vegetable and fruit, spicy food and pickle food them can make tooth wear.

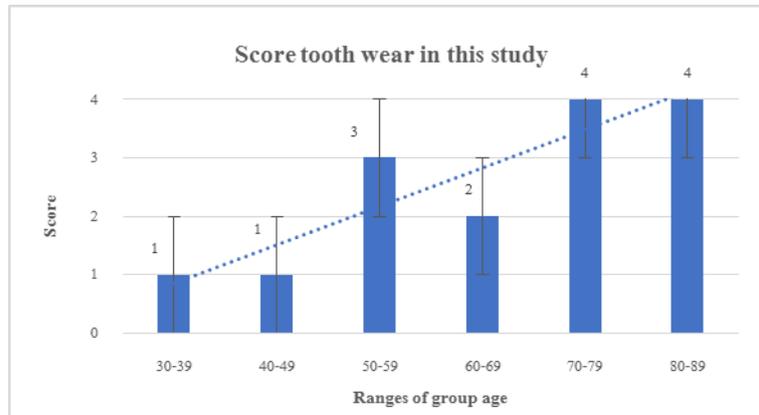


Figure 7 Score tooth wear in this study

In this study, five equations were established for age estimation: Equations based on secondary dentin thickness for certain age ( $R^2 = 0.955$  and  $R^2 = 0.953$ ) and age group ( $R^2 = 0.855$ ); Equations based in inclusion parameters (tooth wear and secondary dentin thickness) for certain age ( $R^2 = 0.964$ ) and age group ( $R^2=0.900$ ). Sex, secondary dentin thickness and tooth wear were found to be correlated with age with the value of  $-0.223^*$ ,  $0.499^{**}$  and  $0.582^{**}$ , respectively. Our results showed that secondary dentin thickness increase with increasing age, which was well supported by Piya et al. (2014). Moreover, Rai, Anand (2006) revealed that the pulp area of clinical crown, total pulp area and width at cervical pulp exhibited a significant decrease with age advance ( $p<0.01$ ) and pulp area decreased due to secondary dentin formation.

When age increase, tooth wear was increase too at significant 0.01 level corresponding with Smith and Knight (Smith, Knight, 1984).

Spijker et al. (2009) they found percentage of tooth wear with in adult patients. In the age of 20 years found tooth wear was increased 3% and age of 70 years found tooth wear was increased 17%.

#### Age estimation from the secondary dentin thickness

#### Age estimation from secondary dentin thickness with in each age

$$Y = [-6.354(x_1)] + [0.100(x_2)], R^2 = 0.955 \text{ -----Equation 1}$$

Y = Age,  $x_1$  = Sex (Male=0, Female=1),  $x_2$  = Very thickness of 2° dentin

**Ex.**

$$Y = [-6.354(x_1)] + [0.100(x_2)]$$

$$Y = [-6.354(0)] + [0.100(741.88)]$$

$$Y = 74 \text{ years}$$

Also, age estimation is 74 years and actual age is 80 years

$$Y = 0.095(x_1), R^2 = 0.953 \text{ -----Equation 2}$$

$$Y = \text{Age}, x_1 = \text{Very thickness of } 2^\circ \text{ dentin}$$

**Ex.**

$$Y = 0.095(x_1)$$

$$Y = 0.095(741.88)$$

$$Y = 70 \text{ years}$$

Also, age estimation is 70 years and actual age is 80 years

**Age estimation from the secondary dentin thickness in group age with range 10 years**

$$Y = 0.006(x_1), R^2 = 0.855 \text{ -----Equation 3}$$

$$Y = \text{Group age}, x_1 = \text{Very thickness of } 2^\circ \text{ dentin}$$

**Ex.**

$$Y = 0.006(x_1)$$

$$Y = 0.006(726.27)$$

$$Y = 4 \text{ ranges years or } 60\text{-}69 \text{ years}$$

Also, age estimation is 60-69 years and actual age is 80-89 years

**Age estimation from inclusion parameters**

**Age estimation from each age**

$$Y = [5.840(x_1)] + [0.075(x_2)], R^2 = 0.964 \text{ -----Equation 4}$$

$$Y = \text{Age}, x_1 = \text{Tooth wear}, x_2 = \text{Very thickness of } 2^\circ \text{ dentin}$$

**Ex.**

$$Y = [5.840(x_1)] + [0.075(x_2)]$$

$$Y = [5.840(4)] + [0.075(741.88)]$$

$$Y = 79 \text{ years}$$

Also, age estimation is 79 years and actual age is 80 years

#### 4.7.2 Age estimation from group age with range 10 years

$$Y = [0.741(x_1)] + [0.003(x_2)], R^2 = 0.900 \text{ -----Equation 5}$$

Y = Group age,  $x_1$  = Tooth wear,  $x_2$  = Very thickness of 2° dentin

**Ex.**

$$Y = [0.741(x_1)] + [0.003(x_2)]$$

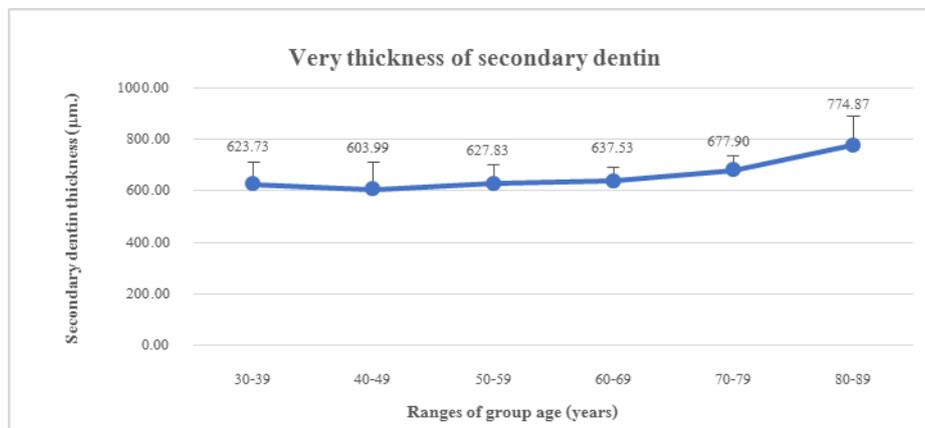
$$Y = [0.741(4)] + [0.003(726.27)]$$

$$Y = 5 \text{ ranges years or } 70\text{-}79 \text{ years}$$

Also, age estimation is 70-79 years and actual age is 80-89 years

#### Age estimation from secondary dentin thickness in this study

Correlation between each age and 2° dentin thickness has correlation with in age at significant 0.01 level (0.499\*\*) and correlation with in group age at significant 0.01 level (0.491\*\*)



**Figure 8** Very thickness of secondary dentin and ranges of group age

#### Age estimation from inclusion parameter in this study

Correlation between each age and inclusion parameter were sex, very thickness of 2° dentin and tooth wear have correlation with in age at significant 0.05 level (-0.223\*), 0.01 level (0.499\*\*) and 0.01 level (0.582\*\*) respectively and correlation with in group age were very thickness of 2° dentin and tooth wear have correlation at significant 0.01 level (0.491\*\*) and (0.627\*\*) respectively.

#### Conclusions

This research has highlighted the feasibility of using dentin thickness and inclusion parameters for age estimation in deceased individuals ranging from 50 to 59, from 60 to 69 and from 70 to 79 years of age. The results clearly demonstrated that secondary dentin thickness alone was unreliable for age estimation. Meanwhile, other parameters, such as six parameters of Gustafson's method, pulp width at cervical line and total pulp area, were

promising for this purpose with high accuracy. The findings obtained from this study should be useful in forensic science for the estimation of the age at death of unidentified bodies or long post-mortem decomposition of the body and skeleton remains.

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