



## Tooth pulpal surface area as an indicator of age – A computer aided trial study

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**ABSTRACT** Age estimation of skeletal remains is an art and not a precise science. Due to inherent challenges in aging, age estimation of the individual whether living or dead is an intimidating task in forensic investigations. The present study was undertaken to estimate the age by evaluating the Pulpal Surface Area (PSA) of ground sections of 30 extracted unaffected lower first premolar teeth of 10 different age groups in horizontal cross section at cervical line as constant with the aid of computerised digital scanner and an image analysing software “ImageJ” which is a cost-effective alternative for image analysis and a welcome tool for pathologists and researchers. The results were statistically analysed using one-way Anova and student's 't'-test. The difference in PSA among different age groups was found to be highly significant.

**KEYWORDS :** age estimation, pulpal surface area, cervical line

### Introduction:

Age estimation of skeletal remains is an art and not a precise science. Next to sex, age is an essential basic biological parameter which facilitates the identification of human remains in both forensic and archaeological contexts. Due to inherent challenges in aging, age estimation of the individual whether living or dead is an intimidating task in forensic investigations.<sup>20</sup>

Age estimation helps in both civil and criminal cases such as kidnapping, rape, marriage, employment, identification, senior citizen concession, retirement benefits, old age pension cases and in question of age fitness or unfitness.<sup>17</sup>

Developmental indicators most commonly used for this purpose are bone maturation, secondary sexual characteristics, height and weight. More recently, the dental maturation indicator system has been described as another useful index for age estimation.<sup>10</sup>

Teeth, the hardest structures in the body can act as biomarker of aging.<sup>12</sup> The reasons for this are, they remain intact even when other components of the skeleton have disintegrated and are known to have high resistance to severe insults such as cold, heat, fire and chemicals. Also, it has been further observed that the incremental pattern of tooth development or formation is not markedly affected by diseases, drug intake, endocrine status etc., as compared to bone mineralization, making them the favourite tissue in forensic investigations.<sup>13,21</sup>

There are several biological age indicators in tooth; one of the major and reliable indicators is the pulp cavity which gets reduced in diameter as the age advances. The reason for this is due to more amount of secondary dentin deposition as age advances.

### Materials and methods:

Method of Collection of samples:

30 Freshly extracted mandibular 1st premolar teeth from the individuals of age ranging from 16–60 years and above were collected from the department of Oral and Maxillofacial surgery, Mahatma Gandhi Post Graduate Institute of Dental Sciences and Hospital, Puducherry after obtaining prior informed written consent from patients.

30 samples fulfilling inclusion and exclusion criteria were selected and divided into 10 groups with an age interval of 5 years and 3 samples were kept under each group.

### Preparation of Ground Section of teeth:

After cleaning the tooth, it was marked at cervical line and sectioned with the help of a micromotor and carborundum disk to obtain a horizontal section in mesio–distal direction exactly 1mm above and 1mm below the cervical line. Manual grinding was done using fine carborundum stone till the section of 0.25mm thickness was obtained. The thickness was checked using Vernier calliper and Screw Gauge.

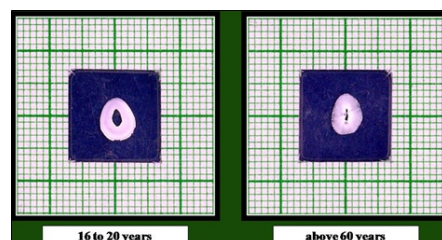
### Measurement of surface area of pulp chamber:

A computerized digital scanning method and software named “ImageJ” which is the world's fastest pure Java image processing programme was adopted for measuring the pulpal surface area of each tooth sections.<sup>6,8</sup>

### Preparation of graph paper background:

Before scanning of each ground section was being done, there was a need of a 'known measure' in the usage of “ImageJ” software. To accomplish that a graph paper background (of size 4x4cm) was prepared using standard graph paper which was made to have a square shaped slit in the centre (1.5x1.5cm).

Each section was placed on glass plate of the scanner above which the graph paper background was placed in such a way that the graph should face the glass plate of the scanner and the cut clear zone must show proper positioning of the ground section. Prior to scanning, the scanner setting was verified to be 100% of the original to ensure life-size scanned images. Subsequently, an image of 600 dpi resolution of the section with graph paper background was obtained. Then the scanning was performed one by one and scanned images of ground sections of each groups of this study were saved as high resolution JPEG files on a desk top computer. (Figure.1)



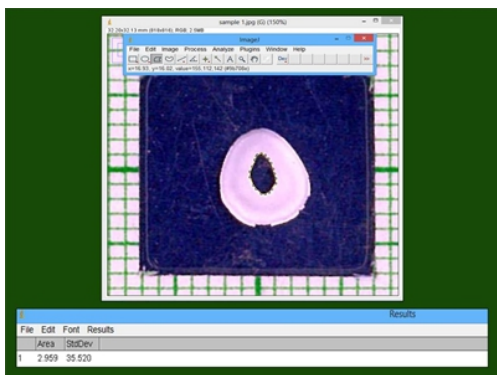
**Figure.1** Scanned images of teeth sections of two different group exhibiting difference in size of pulp cavity

**Method of using “Image J” in measuring Pulpal Surface Area (PSA):**

For the purpose of measuring the pulpal surface area of individual sample, the respective digital image was imported to "ImageJ".

With the help of graph paper background in the scanned image of each sample, a known distance value was established by drawing a straight line of 10mm length with 'straight line' tool of 'ImageJ' software. After precisely selecting pulpal surface using 'Polygon selection' tool strictly following the outline of pulpal area of respective sample image, clicking 'Analyze' menu and then selecting option 'Measure' (Ctrl+M) displayed the pulpal surface area of that particular sample in millimeter at 'Result' page.

The same method was followed individually for all the samples of 10 different age groups each containing 3 samples and respective displayed values were noted down by the investigator and two independent observers separately to minimize the inter-observer bias. The collected data were analyzed and correlated with suitable statistical methods.



**Figure.2** Measuring PSA of tooth section using software “ImageJ”

**Results:**

The data was collected and subjected to statistical analysis using one-way ANOVA and student's t-test. The comparison of Mean Pulpal Area using One-way ANOVA among all the groups revealed very high level of statistical difference in PSA (p-value < 0.01) (Table 2). Student's 't' test was applied to compare the Mean Pulpal Area between any two groups of our study and found that there was statistically significant difference in Mean PSA between most of the groups.

Age Group (in Years)	Mean	Standard Deviation
16 – 20	2.9060	0.42
21 – 25	2.1883	0.27
26 – 30	1.6010	0.09
31 – 35	1.1003	0.10
36 – 40	1.2660	0.31
41 – 45	1.5267	0.13
46 – 50	1.4250	0.16
51 – 55	1.2543	0.14
56 – 60	0.8760	0.11
Above 60	0.5077	0.54

**Table.1** Age Group wise Mean Pulpal Surface Area (PSA)

	Sum of Squares	df	Mean Square	F	p Value
Between Groups	12.31	9	1.37	18.85**	0.000
Within Groups	1.45	20	0.07		
Total	13.76	29			

**Table.2** Results of One Way ANOVA comparing Mean PSA by Age Groups

\*\*Significant at 1% level (p < 0.01). F value is significant, in turn exhibiting the truth that surface area differ significantly by age levels of the cases.

**Discussion:**

Age estimation to establish the identity of a person for ethical,

humanitarian and official records particularly in the legal and criminal investigations in the field of forensic science is of paramount importance. Various modalities are available in the assessment of age of a person. But this particular fact is gaining momentum in forensic dentistry by studying the tooth retrieved from the person.

Dental structures are more and more useful in morphological method because of their resistance to physical and chemical agents. There are several biological age indicators in tooth; one of the major and reliable indicators is the pulp cavity which gets reduced in diameter as the age advances. The reason for this is due to more amount of secondary dentin deposition as age advances.

Secondary dentin may be defined as the dentin formed continuously throughout life after the crown is fully formed.<sup>14</sup> Formation of secondary dentin starts at the side of the pulp where the antagonist meets the tooth during mastication and seems to be mainly related to age.<sup>9</sup> According to **Gustafson**, secondary dentin deposition was used as one of the parameters for age estimation.<sup>7</sup>

Studies with respect to role of pulp cavity in age estimation have also shown that with advancing age, the size of the dental pulp cavity is reduced as a result of secondary dentin deposition which is a purely a physiological phenomenon. Thus the measurement of **Pulpal Surface Area (PSA)** in ground sections prepared in horizontal direction at the level of cervical line of a tooth was used as a single reliable indicator of age in our study.

Lower first premolars were selected for this study as they are less readily damaged by direct heat or traumatic force than incisors and canines due to the reason of being covered by the soft tissue of the cheek, and are not as easily lost in the dry skull material as single-rooted anterior teeth.<sup>1</sup>

Lower premolars also suffer minimal wear due to particular work and have simple, less diverse root morphology than that exhibited by molar teeth led us to conclude that premolar teeth should have a high potential as a forensic sample for age estimation.<sup>3</sup> Premolars were selected for this study also because these are known to give best correlation coefficient as compared to other teeth as per the study of **Kvaal and Solheim**.<sup>11</sup>

While performing age estimation, accuracy and precision are of utmost importance. Horizontal ground sections were prepared in mesio-distal direction at the level of cervical line consistently for all samples.<sup>16</sup> **Soomer H et al.** found that the data derived from direct sectioned methods had a trend towards higher accuracy and precision as compared with intact methods or indirectly from dental radiographs.<sup>20</sup>

In contrast to most of the age estimation studies based histological changes in teeth, longitudinal ground section of teeth were prepared either mesio-distally or bucco-lingually. In this present study, horizontal ground sections were prepared at the level of cervical line at where **Pulpal Surface Area** was measured. This is supported by **Solheim T (1992)** who studied age estimation using secondary dentin as a parameter and found that pulp widths were most strongly correlated with age in the cervical area and the correlation decreased towards the apex.<sup>18</sup>

If overall reduction in pulp cavity occurs along with age, the decrease in Pulpal Surface Area (PSA) at the level of cervical line can be also used as a reliable indicator. This is in accordance with **Cameriere et al. (2004)** and they studied age estimation utilizing pulp/root ratio, root length, pulp/tooth length ratio, pulp/tooth area and pulp/tooth width ratio at three different levels of 100 single rooted maxillary right canines and found that Pearson correlation coefficient for the ratio of pulp/tooth area was correlated best with age (r = 0.85).<sup>3</sup>

Keeping in mind that accuracy and precision are the main requirements for age estimation, we have adopted a computer aided digitalized scanning method and an image analysing tool “**ImageJ**” which is a cost free and easily downloadable software from web.<sup>6,8</sup>

In our study the comparison of Mean Pulpal Surface Area (Table 2) studied among 10 groups and observed that PSA with different age groups was found to be statistically significant (p < 0.001). Our result is in concordance with studies of **Solheim et al**,<sup>19</sup> **Kvaal et al**,<sup>11</sup> **Bosman et al**,<sup>2</sup> **Cameriere et al**.<sup>3</sup> They suggested that the width of pulp

is a better indicator of age.

In the due course of study, we have also found that computer-aided<sup>46</sup> forensic odontology gives rapid and reproducible result as compared to routine radiographic method because when the curve arch of the jaw is projected on to a flat film, there will always be a certain amount of distortion while measuring the image presented there.<sup>45</sup> Our this observation is in accordance with the study of **Singaraju S et al.**<sup>15</sup>

Our present trial study confirms that as age advances there is a gradual reduction of pulp cavity which in turn leads to decrease in the pulpal surface area (PSA) due to continuous deposition of secondary dentin which is mostly a physiological phenomenon. The results of our study recognize the use of secondary dentin deposition as a parameter in age estimation studies seems to be a more reliable method in forensic aspect and also reveals that secondary dentin formation is closely related to chronological age.

The outcome of the current study provides hope that utilization of our novel method can be a useful tool for age estimation in the field of forensic dentistry.

### Conclusion:

Our study indicates that increase in age results in decrease of Pulpal Surface Area due to continuous deposition of secondary dentin which can be used to assess the size of an individual. The present study also gives a scope for future studies on larger sample size with an adequate representation of samples from different age groups and sex distribution.

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