Original Article

Chronological Age Estimation Using Transparent Root Dentin: A Stereomicroscopic Study

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Abstract:
Introduction: Estimation of age in individuals has received considerable attention in the forensic scientific literature as well as in archaeological cases. Dentine undergoes measurable age dependent morphological alterations (transparency/sclerosis) that start in the root and gradually extend to the crown of the tooth. This increase in size of the apical zone of translucent dentin has been used as a method to assess the age of the individuals.
Aims & Objectives: To estimate chronological age of the individuals using apical translucent dentin. **Material & Methods:** 50 single rooted permanent teeth extracted for therapeutic reasons and immediately after extraction the tooth was preserved in 10% neutral formalin until the ground sections were prepared. Mesio-distal sections of the tooth were made up to the thickness of 0.5mm using lathe machine and Arkansas stone and stained with 1% methylene blue. The area and length of translucency were measured.
Results: In the present study the correlation of coefficient and significance correlation were calculated, which showed that the parameters were highly significant and correlated with the chronological age. Thus both the parameters were accurate. **Conclusion:** This study can be adopted in age estimation of an individual using TRDL and TRDA values in adults. This is the simplest method which can be used in the estimation of age in forensic odontology.
Key Words: TRDL (Transparent root dentin length), TRDA(Transparent root dentin area), Age estimation.

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Introduction

Forensic science is primarily concerned with the application of science in court or legal proceedings. This is an interesting, challenging and adventurous field.[1] Forensic Odontology is a major branch of forensic science, as the dental tissues are the strongest tissues in the human body and therefore their characteristics remain unchanged even after long periods of stay in extreme environments. The teeth can often survive long periods of immersion under water, burial under oil, fire, exposure to biological agents in the natural environment. Thus the importance of dental identification is increasing year by year.[1]

Age estimation is of broader importance in forensic science, not only for the identification purposes of the deceased victims, but also in connection with crimes and accidents.[2] In a developing country like India, a large number of people are illiterate and have no knowledge or records of

their date of birth which is required by law enforcing agencies in matters like criminal responsibilities, identification, judicial punishment, consent, rape, criminal abortion, employment, attainment of majority, kidnapping, and prostitution.[3] In addition chronological age is important in most societies for school attendance, social benefits, employment, and marriages.[2] The choice to use tooth for age determination is well accepted due to their longevity ability of being resilient to change.[2] Estimation of human age is a procedure adopted by anthropologist, archaeologist, and forensic scientist. The estimation of age at the time of death is often an important step in identification of human remains.[2] Once a human tooth is fully formed and has reached the occlusal plane there are major changes which are seen with increasing age. Dentin translucency is one of the changes seen with increasing age. Root translucency develops as the dentin tubules within a tooth root begin to mineralize from the root apex towards the crown. This mineralization is a result of calcium salt formation in and around the tubules.[4] Physiologic transparent root dentin as distinguished from pathologic transparency subjacent to caries appears to form without trauma. Several techniques are described in literature that addresses age estimation in adults. In general methods are divided into three categories: morphological, radiological, and biochemical methods. Which are all based on degenerative processes, observed in the dental structure.[5] The present study is carried out to estimate the chronological age using transparent root dentin in a rural population.

Material & Methods:

Source of Data: 50 single rooted human permanent teeth extracted for therapeutic reasons in department of Oral and Maxillofacial Surgery, Rural Dental College, Loni.

Inclusion criteria: Single rooted human permanent teeth extracted for therapeutic reasons (malocclusion/ orthodontic treatment, prosthetic and periodontal disease) from the individuals of age ranging from 15-75 years.

Exclusion criteria: Orthodontically treated teeth, Root canal treated teeth, Teeth with history of trauma, Teeth associated with any pathology and Permanent molars were excluded

Armamentarium used:

Sterile disposable gloves, 10% formalin, Digital vernier caliper, Carborundum stone, Lathe wheel machine, Microscopic glass slide, DPX(Disterene dibutly phthalate Xylene), Stereomicroscope, Graph paper

Method of Collection of Data:

Freshly extracted permanent teeth for valid clinical reasons (malocclusion/ orthodontic treatment, prosthetic and periodontal disease) from the individuals of age ranging from 15-75 years were used. The age and sex of the patients from whom teeth were extracted was recorded along with date and reason for extraction, and consent from patients was taken. Immediately after extraction the tooth was rinsed in normal saline solution and preserved in 10% neutral formalin until the ground sections are prepared.

Estimation of age using apical translucent dentine:

Preparation of Ground Section of teeth:

Manual grinding of Mesio-distal sections of the tooth were made up to the thickness of 1mm using lathe machine first and later using Arkansas stone until the thickness of 0.5mm(Figure-1) and which is measured using vernier caliper (Figure-

Figure 1: Ground section prepared from 50 extracted teeth

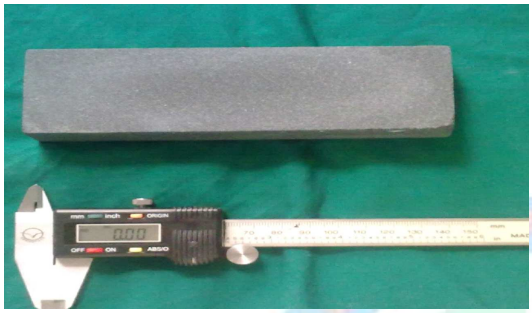


Figure 2: Digital vernier caliper and Arkansan stone

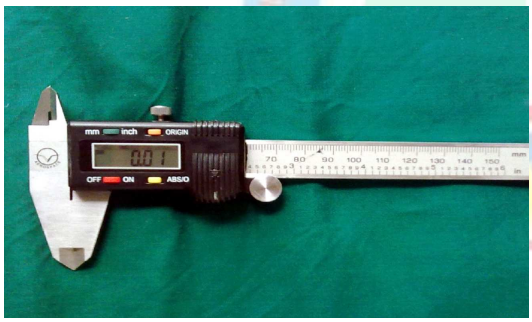


Figure 3: Digital vernier caliper with a resolution of 0.01mm

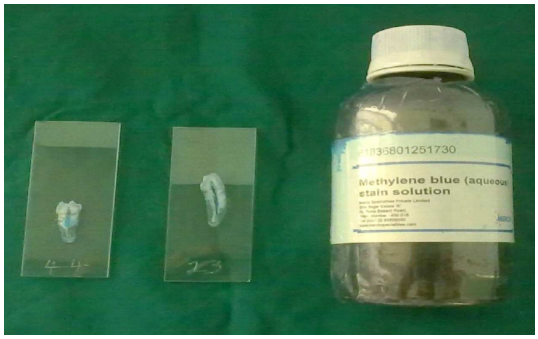


Figure 4: Methylene blue stain solution bottle and ground section stained with it

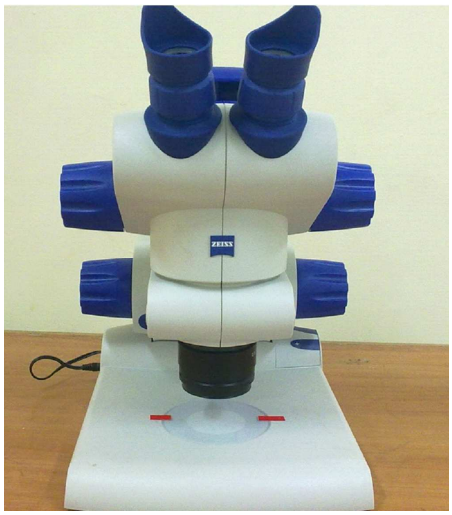


Figure 5: Stereomicroscope

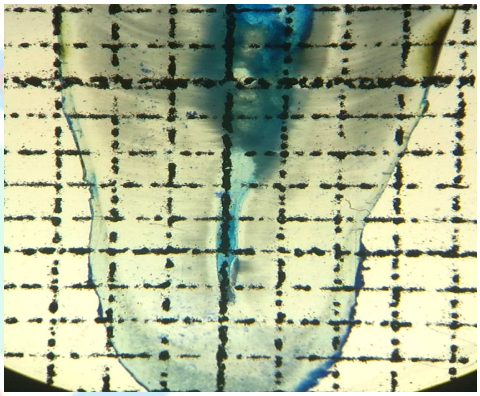


Figure 6: Ground section under stereomicroscope with a transparent graph superimposed on it to measure transparent root dentin area (TRDA)

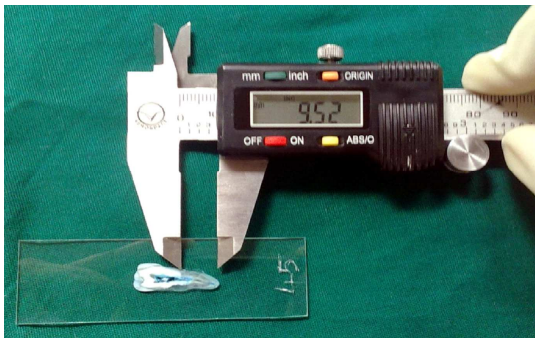


Figure 7: Measurement of transparent root dentin length using digital vernier caliper

2). The digital vernier caliper has measurements from 0-150mm (0-6 inches) with the resolution of 0.01mm and accuracy of 0.02mm (Figure 3). The section is then stained with 1% methylene blue (Figure-4).All the prepared sections were mounted on slide using DPX and viewed under stereomicroscope for calculating the area. Area of the ground section is measured under stereomicroscope (Figure 5). Over the ground section, a marking was made with a lead pencil at the cementoenamel junction (CEJ) and graph sheet is superimposed on the ground section and observed under stereomicroscope. The number of squares in translucent apical zone is counted (Figure 6). The total number of squares in translucent zone area will give the area of translucent zone. The translucent zone length was measured using venire caliper (Figure 7 & 8).

The Scoring Criteria for Transparent Root Dentin Area and length:

The ground section was observed under stereomicroscope to measure the area. Over the ground section graph paper was superimposed. The number of squares was counted in the translucent apical zone. Counting was done according to the following method: one completely filled square was taken as 1 mm^2, more than half filled square was also taken as 1mm2, and less than half filled square was not counted. The total number of squares in the translucent zone gave the area of the translucent zone. The translucent zone length was measured using digital venire caliper. After obtaining the scores and values, the data was used for statistical analysis.

Results:

Statistical analysis was done. Mean, Standard deviation, Correlation of Coefficient , Student ‘t’ test , multiple regression equations were calculated for Transparent root dentin length(TRDL), Transparent root dentin area(TRDA).

Regression equations for estimation of age from above two parameters are as follows:

1. Transparent root dentin length (mm) (TRDL)

$Y = 4.37X + 24.49$ (Y – Estimated Age, X -TRDL value)

2. Transparent root dentin area (mm) 2(TRDA)

$Y = 0.93X + 27.24$ (Y- Estimated Age, X- TRDA value)

Discussion:

Age estimation can prove to be a critical part in victim identification process. In case of unidentified dead bodies, age estimation becomes necessary if there is no ante mortem information available [3].Translucency of dentin is the optical manifestation of changes in the dentin. The only proof of age estimation is the optical translucency that is optically recognized. As it has been shown the dentin in the area of high mineralization allows the light to pass through it and does not reflect and scatter light as normal dentin does. Thus the dentin root translucency becomes an important process in age estimation. The reason the root dentin appears translucent is the occlusion of the tubules by calcified material which has a refractive index, very close to that of the rest of the dentin.[6] The relationship between translucency of the root dentin and increasing age was shown during the 1940’s and used as one of the criteria for age estimation from human teeth by Gustafson in 1950; Bang and Ramm, 1970; Johanson, 1971; Azaz et.al., 1977. This led to the belief that the root dentin translucency is not only associated with age but arises strictly as a result of increasing age.[7]

Transparent root dentin has been studied in the intact tooth in order to correlate with the age of the person as done by earlier reports of Solheim. However, this is a three dimensional phenomenon which encounters difficulties in the measurement procedures. Alternatively transparent dentin can also be studied in different thickness of sectioned tooth. To be more accurate in the measurement of transparent dentin, the procedure of dye imbibition was adopted by using 1% methylene blue. The sclerosed dentin remained colorless, normal dentin stained blue, and cementum took up dark blue in color. This was possible because the transparent dentin is made up of completely mineralized tubules that did not permit the entry of dye into this zone.[8]

In the present study, premolars and incisors were selected as these are known to give best coefficient as compared to other teeth. Molars on the other hand show greater effect of diet as they are used in mastication of food but they are very strong and hard and it is difficult to make their ground section. Also first molars were found to have attritional very early.[3] In the present study 50 extracted teeth were taken out of which 28 extracted teeth were from males and 22 extracted teeth were from females between the age ranges of 15-75 years, and values of TRDL, TRDA, were measured. Individual values of these two parameters were plotted against the actual age in a scatter plot and coefficient of correlation; regression formulae for each parameter were derived using regression analysis.

In the present study regression line for TRDL was obtained by plotting the values of TRDL against the actual age. This regression line was used to derive the regression formulae which was Y=4.37X+24.29 (Y-estimated age, X-TRDL value) and the Regression line for TRDA was obtained by plotting the values of TRDA against the actual age. This regression line was used to derive the regression formulae which was Y=0.93 X+27.24 (Y-estimated age, X-TRDA value).

In the present study 50 cases were divided into 6 age groups it was observed that maximum number of cases in the study belonged to the age group of 45-54 years.

In our study the mean total score were given to individual age groups using TRDL and TRDA value, and the total scores were calculated. It was observed that mean total score for TRDL increased with increasing age. It was 0 for age group of 15-24, 1.95±0.35 for the 25 to 34 age group, 3.44±0.66 for 35-44 age groups, 5.61±0.08 for age group 45-54, 8.05±0.49 for age group of 65-75.

By using TRDA value, it was observed that mean total score for TRDA increased with increasing age. It was 0 for the age group of 15-24 years, 6.43±3.15 for 25 to 34 years of age group, 13.44±2.87 for 35-44 years of age group, 23.42±6.16 for 45-54 years of age group, 37±1.41 for the age group of 55-64, 42 ±8.68 for the age group of 65-75 years. This strongly correlates to the study by Singhal A et al.[8]The comparison of the mean actual age and mean estimated age, using the TRDL and TRDA values and newly derived regression equation, it was found that mean calculated age for TRDL(44.88±10.93),

TRDA(44.86±8.26) was slightly higher as compared to the mean actual age (44.68±10.81) but statistically this difference was not significant.

In the present study the percentage of variation in chronological age was 24.19% and in estimated age using TRDL value it was 24.35% & in estimated age using TRDA value it

was 18.41%. So this indicates that value of percentage variation obtained by TRDL was more close to the percentage variation of chronological age than that of the TRDA.

It was observed that the correlation coefficient(r) for TRDL was 0.9745 which shows that TRDL was highly correlated with age as compared to TRDA, which is having correlation coefficient(r) of 0.9451. It was compared with Anita Singhal et.al.[8]who scored correlation coefficient(r) for TRDL was 0.81 and for TRDA 0.70 which were highly correlated with chronological age. These correlation coefficient values are slightly lower than the present study.

In the present study by applying student 't' test, the p value was less than 0.001($p<0.05$), so there was highly statistical significance between chronological age and estimated age using TRDL and TRDA, which was supported by Anita Singhal et.al.[8] In the present study TRDL and TRDA were used for age estimation which has highly significant correlation with chronological age. It strongly correlated by Singhal A et al.[8]

Summary & Conclusion:

Many variables have been used as age determinants. The choice to use teeth for age determination is well accepted due to their longevity ability of being resilient to change. 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 individuals such a biomarker is root dentin translucency, supported by Gustafson (1950). The transparent root dentin is one of the most important parameter of age estimation, as it is more reliable compared to other parameters. In the present study 50 extracted teeth were taken by using two different parameters

1) By measuring TRDL
2) By measuring TRDA

The correlation of coefficient and significance correlation were calculated, which showed that the parameters were highly significant and correlated with the chronological age. Thus both the parameters were accurate.

The correlation of coefficient using TRDL value was 0.9745 and by using TRDA value was 0.9451. So the estimated age using TRDL value was more accurate than TRDA value.

This study can be adopted in age estimation of an individual using TRDL and TRDA values in adults. This is the simplest method which can be used in the estimation of age in forensic odontology.

Table 1: Sex wise distribution of case

Sex	No of cases
Male	28
Female	22
Total	50

Out of 50 cases 28 were males and 22 were females.

Table 2: Distribution of cases in various age groups (Graph-1)

Group	Age Group	No. of cases
I	15-24	01
II	25-34	07
III	35-44	18
IV	45-54	19
V	55-64	02
VI	65-75	03

50 cases were divided in 6 different groups with maximum number of cases between the age group of 45-54 years. (Table 2) total score 3.44±0.66, age

group 45-54 had mean total score 5.61±0.08, age group 55-64 had mean

Table 3: Mean total score and standard deviation calculated from transparent root dentin length (TRDL) (Graph-2)

Group	Age Group	Mean total score	S.D
I	15-24	0	0
II	25-34	1.95	0.35
III	35-44	3.44	0.66
IV	45-54	5.61	0.08
V	55-64	8.05	0.49
VI	65-75	10.34	1.65

total score 8.05±0.49, age group 65-75 had mean total score 10.34±1.65. It was observed that score was significantly increasing with the increasing age. (Table 3)

	Correlation coefficient (r)
Transparent root dentin length (TRDL)	0.9745

Table 3b: Karl Pearson's co-relation coefficient between Chronological age and TRDL

***Highly significant, p<0.01**

By applying student't' test there is a highly significant correlation between age and TRDL, that is p<0.01. It also indicates that there is a high positive relationship between age and TRDL. (Table 3 b)

Age group 15-24 had mean total score o, age group 25-34 had mean total score 6.43±3.15, age group 35-44 had mean total score 13.44±2.87, age group 45-54 had mean total score 23.42±6.16, age group 55-64 had mean total score 37±1.41, age group 65-75 had mean total score 42±8.68. It was observed that score was significantly increasing with the increasing age. (Table 4 a)

Table 4(a): Mean total score and standard deviation calculated from transparent root dentin Area (TRDA) (Graph no-3)

Group	Age Group	Mean total score	Standard deviation
I	15-24	0	0
II	25-34	6.43	3.15
III	35-44	13.44	2.87
IV	45-54	23.42	6.16
V	55-64	37.00	1.41
VI	65-75	42.00	8.68

Table 4b: Karl Pearson's correlation coefficient between Chronological age and TRDA

	Correlation coefficient (r)
Transparent root dentin area (TRDA)	0.9451*

***Highly significant, p<0.01**

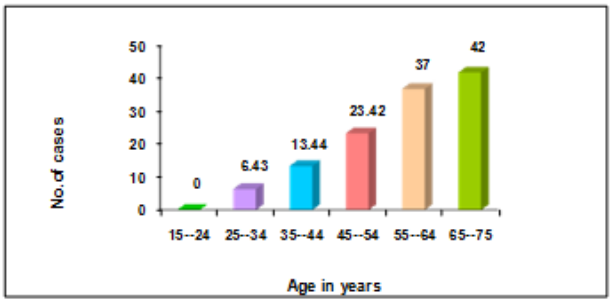
By applying student't' test there is a highly significant correlation between age and TRDA, that is p<0.01. It also indicates that there is a high positive relationship between age and TRDA.

Table 5: Values of Karl Pearson's correlation coefficients between age and various measurements (n=50)

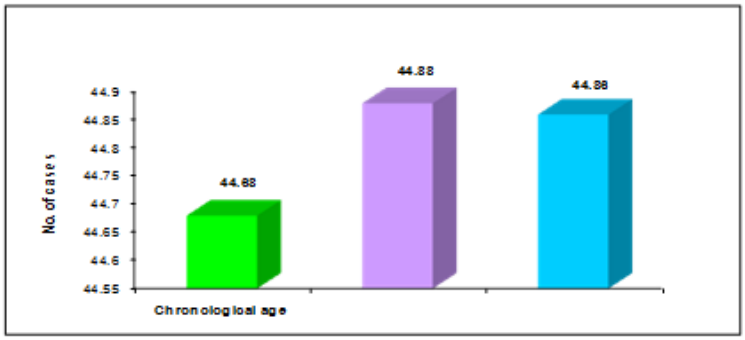
	Karl Pearson's correlation Coefficient (r)
Transparent root dentin length (mm) (TRDL)	0.9745*
Transparent root dentin area (mm square) (TRDA)	0.9451*

***Highly significant, p<0.01**

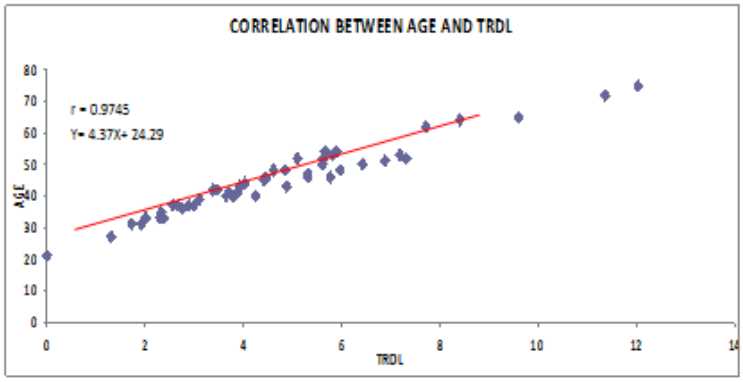
Graph 1: Mean total score calculated from transparent root dentin Area (TRDA)



Graph 2: Variation of chronological age and estimated age using TRDL and TRDA – By regression analysis



Graph 3: Scatter plot for transparent root dentin length value with chronological age



Graph 4: Scatter plot for transparent root dentin area value with chronological age

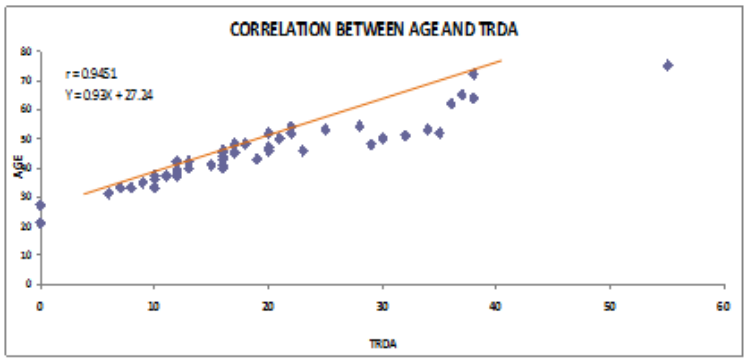


Table 6: Variation of chronological age and estimated age using TRDL and TRDA by regression analysis (Graph 4)

Method	Range	Mean	SD	CV %
Chronological age	21-75	44.68	10.8	24.1
Estimated age using TRDL value	24-78	44.88	10.9	24.3
Estimated age using TRDA value	31-72	44.86	8.2	18.4

Table 7: Comparison of chronological age estimated using TRDL and TRDA value

Method	Pearson's correlation (r)	P value	Sig.
Actual age and estimated age using TRDL value	0.9745	0.001	Highly sig.
Actual age and estimated age using TRDA value	0.9451	0.001	Highly sig.

applying Student's't' test there is a highly significant correlation between age and Transparent root dentin length (TRDL), age and Transparent root dentin area (TRDA), (i.e. $p<0.01$). Also, it is seen that there is a positive correlation between age and transparent root dentin length (TRDL), age and Transparent root dentin area (TRDA). From the above correlation value transparent root dentin length (TRDL) was highly correlated with age compared to Transparent root dentin area (TRDA). (table 5)

The percentage of variation in chronological age was 24.19% and in estimated age using TRDL value, the percentage of variation was 24.35% and estimated age using TRDA value, the percentage of variation was 18.41%. This indicates that value of percentage variation obtained by TRDL was more close to the percentage variation of chronological age than that of TRDA. (Table 6)

The value of Pearson's correlation of coefficient(r) for estimated age using TRDL and TRDA was 0.9745, 0.9451 and P value was 0.001($p<0.05$) so there was significant correlation between chronological age and TRDL, chronological age and TRDA value. (Table 7)

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