Introduction:
Age estimation is important in forensic medicine and odontology for identification of deceased victims and also for crimes and accidents. Dental maturity has played an important role in estimating the chronological age of individuals because of the low variability of dental indicators. 1

Various methods have been constructed and tested to estimate the age of young individuals. Among them are the physical examinations using anthropometric measurements, 2, 3 skeletal maturation, 4 dental age estimation, 5 a combination of dental development and anthropometric measurements 7 and a combination of skeletal and tooth eruption. 8

Age estimation is an important step in constructing a biological profile from human skeletal remains. The goal of the forensic anthropologist is to assist medical legal officials with identification by presenting a probable age range of the deceased. In adults, this is typically done by examining various skeletal traits which have been shown to degenerate with age in a predictable manner. 9

Estimation of age at death and determination of sex of the victim or remains are important guides that help in the process of identification. Teeth are among the most reliable tools in the process of identification of age, especially in the first and second decades. The stages of development can be considered as one of the most dependable indicators in assessing the age of the victim. 10

Developmental stages of dentition and craniofacial skeleton are well established. Any disturbances during this period produce changes in these tissues and serve as lifelong permanent record. Even after the complete development of dentition and craniofacial skeleton certain physical, chemical and biological changes takes place which aid in the age estimation. 10

Dental Age Estimation Methods 11
Various methods are utilized for determination of age from dentition. Age assessment methods may be classified as:

A. According to the state of development of the dentition:
O Methods applied to the forming dentition
O Methods for the adult fully formed dentition.

B. According to the technique of investigation:
O Clinical or visual
O Radiographic
O Histological
O Physical and chemical analysis

i. Clinical or visual method: Visual observation of the stage of eruption of the teeth and evidence of changes due to function such as attrition can give an approximate estimate of age.

ii. Radiographic method: Radiography can provide the gross stage of dental development of the dentition.

iii. Histological method: Histological methods require the preparation of the tissues for detailed microscopic examination, which can determine more accurately the stage of development of the dentition. This technique is more appropriate for post-mortem situations. It is also significant in estimation of age of early development of dentition.

iv. Physical and chemical analysis: The physical and chemical analysis of dental hard tissues to determine alterations in ion levels with age have been proposed. While these techniques, as yet, are not of great value to the forensic odontologist, future developments might provide an adjunctive means of collecting evidence of value in the dental context.

Amongst the various methods used to estimate the age of an individual dental age provides more accurate indications during first 18 to 20 year of life. But both dentition and bone development are affected by genetic, environmental, nutritional, endocrinical factors. So chronological age doesn't coincide with skeletal or dental age.

Methods to Be Employed in Dental Age Estimation
Age estimation using the dentition can be grouped into 3 phases:
1. Age estimation in prenatal, neonatal and early postnatal child
2. Age estimation in children and adolescents
3. Age estimation in adults

1. AGE ESTIMATION IN PRENATAL, NEONATAL AND EARLY POSTNATAL CHILD
The primary tooth germ begins to form at seven weeks in utero (IU), and the enamel formation of all deciduous teeth is usually complete by the first year. Among the permanent teeth, the first molar shows germ formation first at about 3.5-4 months IU. Age estimation in this group of individuals can be very accurate.

Determination of age during the development of dentition can be obtained with an accuracy of “plus or minus one year” and during the early part of this period, microscopic examination of teeth may provide the age with an accuracy of “plus or minus few days”.

In case of Prenatal, natal and neonatal periods the histological methods are used to assess the stage of tooth development during the premineralization period.

Mineralization of deciduous dentition commences from two to four months in utero. Some of the histological methods can detect early mineralization 12 weeks before being detectable in the radiographs.
One of the earliest studies in this regard was done by Kraus and Jordan (1965). They studied the tooth development in 95 fetuses, and recorded the chronology of the early stages in the formation of the deciduous dentition. They provided data showing various stages of premineralization and early mineralization of various deciduous teeth and first permanent molar during intrauterine development.

Age Assessment from the Neonatal line:
The neonatal line is considered as an indicator of birth. Neonatal lines are present in both enamel and dentine of deciduous teeth and permanent first molars which indicate the development during the transitional period between intrauterine and extrauterine environments. So the neonatal line can be used to assess the amount of pre- and postnatal enamel formation.

Age assessment based on thickness of enamel and dentin from the neonatal line:
Miles (1959) determined age in death by measuring the thickness of enamel and dentin from the neonatal line and divided it by the appropriate daily rate of formation. To give the age at time of death, the measured distance is divided by the appropriate daily rate of formation taken from tables published by Massler and Schour (1941).

Age Assessment from the Incremental lines:
In the dentine incremental lines of Von Ebner and contour lines of Owen are present. These lines are used to estimate age of the neonate or fetus at death.

Incremental lines of Retzius are caused by variation in the rhythmic mineralization of enamel prisms. This rhythmic pattern may be altered by various external factors such as metabolic disturbances so that the lines may appear closer or the rest periods may be prolonged. (Teivens A., Mornstad H, Noren)

Age Assessment from the Weight of the Development Dentine:
On occasions, forensic investigators may require age estimation of skeletal remains. Histological sections and radiography in such cases is not practical. An alternative is to measure the dry weight of the mineralized tooth cusps developed by Stack.

2. AGE ESTIMATION OF CHILDREN AND ADOLESCENTS:

1. Odontological age estimation of children and adolescents depends on the eruption of teeth which can be either visual or through radiographic methods. Another event that may be used to measure dental age in children and adolescents is tooth calcification.

AGE ESTIMATION USING CHARTS PREPARED FROM POPULATION SURVEYS:
There are numerous studies in this subject which are conducted on different populations. Data derived from these studies are given in the form of charts based on eruption of deciduous teeth, shedding of deciduous teeth, and eruption of permanent teeth. Radiographical evidence of formation of crowns and root completion has been utilized for the same purpose.

These charts are based on dental surveys of cross sections of the population and show the progressive states of dental development for each year of age.

AGE ASSESSMENT BY EXAMINATION OF THE INCREMENTAL PATTERN OF TOOTH FORMATION:
The accuracy of age determination during the early part of the dental developmental period may be increased beyond that available from comparison with standard charts, by the use of a method described by Boyd (1963). The method involves a microscopic examination of the incremental markings found in longitudinal ground sections of the teeth, and relies on the identification of the neonatal line in teeth forming at birth. Starting from the neonatal line, the numbers of small incremental lines that cross the enamel prisms are counted up to the edge of the forming enamel front. Making the assumption that each increment represents one day’s addition of enamel, the number of increments is taken to represent the number of days of age.

If the tooth exhibiting the neonatal line has ceased enamel formation (i.e., has no forming enamel front) it is impossible to tell how many days have passed since the completion of enamel formation. Hence the direct age count is not applicable.

However, it may be possible to transfer the counting procedure to another tooth still undergoing enamel formation at the time of death. The technique assumes that the stimulus causing the formation of a prominent incremental line (as demonstrated in dentine) acts on all teeth forming at the time the stimulus occurs. Thus the position of a particular stria will represent a layer of enamel formed at the same time in different teeth.

Once the stria common to several teeth have been identified, the age estimation is carried out in the following manner. A section from a tooth, which was forming at birth, is selected, and the number of daily increments is counted between the neonatal line and a particular stria. Transferring to a section from a later forming tooth the daily increment count is continued from the same specific stria as before, until the forming enamel front is reached. The total number of daily increments will represent the age in days.

RADIOLOGICALLY DETERMINED DMF INDEX VARIATIONS FOR FORENSIC AGE ESTIMATION OF YOUNG ADULTS
Olze A et al (2006) radiologically determined DMF index variations for forensic age estimation in young adults. The variables examined include the DMFT index of all permanent teeth, the DMTF index of all permanent teeth excluding third molars and the DFT index of third molars projecting beyond the occlusal plane. They concluded based on orthopantomograms, of variations on the DMF index appears to provide a unsuitable additional criterion for forensic age estimation of young adults. By contrast, the evaluation of single variables does not yield sufficient data to determine with the accuracy required in criminal proceedings whether a person has attained 21 years of age.

THIRD MOLARS IN AGE ESTIMATION
Although the third molar is a valuable indicator of age in the age-group 16-23 years when all other teeth have completely developed, its accuracy in age estimation is questionable due to their great variation in genesis, position, morphology and time of formation.

3. ESTIMATION OF AGE IN ADULTS (ABOVE 20 YEARS):
At the end of the period of tooth formation, usually about 20-22 years of age, the methods employing the stages of tooth development, as age landmarks are no longer applicable. It becomes
difficult to estimate age using teeth.

In the adult dentition age estimation techniques are limited to the assessment of the progression of ‘wear’ and ‘age’ changes in the teeth. So most of the methods used in adults use various regressive changes of hard and soft tissues of the teeth.

Compared with age estimation in children using development of dentition, age estimation with the use of teeth is less accurate.

**GUSTAFSON’S METHOD (1950):**
In 1950, Gosta Gustafson developed a method for age estimation based on morphological and histological changes of the teeth. This assessed regressive changes such as:
- Amount of occlusal attrition (A)
- Coronal secondary dentine deposition (S)
- Loss of periodontal attachment (P)
- Cementum apposition at the root apex (C)
- Root resorption at the apex (R)
- Root dentine translucency (T).

**AGE ESTIMATION USING ATTRITION: 20**
Different types of scoring methods are used by different researchers to estimate age in adults using attrition of the teeth but all of them agreed that it is not a reliable index when it is used alone as it is affected by food habits, para functional habits, missing opposing tooth etc.

**MILE’S METHOD OF AGE ESTIMATION (1963):**
When considering dentitions from unsophisticated societies an approximate guide to the age of an individual may be obtained by assessing the relative ‘wear’ of the three molar teeth (Miles, 1963).

**SCOTT SYSTEM FOR SCORING SURFACE WEAR IN MOLARS (1979):**
Score 0: No information available (tooth not occluding, unerupted, ante mortem or postmortem loss, etc.)

Score 1: Wear facets invisible or very small

Score 2: Wear facets large, but large cusps still present and surface features (crenulations, non carious pits) very evident. It is possible to have pinprick size dentine exposures or dots which should be ignored. This is a quadrant with much enamel.

Score 3: Any cusp in this quadrant is rounded rather than being clearly defined as in 2. The cusp is becoming obliterated but is not yet worn flat.

Score 4: Quadrant area is worn flat (horizontal) but there is no dentine exposure other than a possible pinprick sized dot.

Score 5: Quadrant is flat, with dentine exposure one-fourth of quadrant or less. (Be careful not to confuse noncarious pits with dentine exposure.)

Score 6: Dentine exposure greater; more than one-fourth of quadrant area is involved, but there is still much enamel present. If the quadrant is visualized as having three sides, the dentine patch is still surrounded on all three sides by a ring of enamel.

Score 7: Enamel is found on only two sides of the quadrant.

Score 8: Enamel on only one side (usually outer rim) but the enamel is thick to medium on this edge.

Score 9: Enamel on only one side as in 8, but the enamel is very thin—just a strip. Part of the edge may be worn through at one or more places.

Score 10: No enamel on any part of the quadrant—dentine exposure complete. Wear is extended below the cervicoenamel junction into the root.

**MICROMETRIC MEASUREMENTS BY SCANNING ELECTRON MICROSCOPE (SEM) FOR DENTAL AGE ESTIMATION IN ADULTS**
Sema Kedici P et al (2000) obtained 20 measurements of different variables in incisor teeth using a SEM micrometric scaler and the results were statistically correlated with age by the multiple regression method. A formula was then derived from the calculations for age estimation which gave statistically acceptable results. Gender differences were also investigated and when separated delivered even stronger correlation.

**AGE ESTIMATION USING DEPOSITION OF SECONDARY DENTINE**
Size of the pulp chamber indicates the amount of secondary dentine formation.

**Moore** used pulp diameter to crown diameter ratio for calculating age.

**AGE ESTIMATION USING DEPOSIT OF PERITUBULAR DENTINE**
Peritubular dentine is a mineralised deposit formed centripetally in the dentine tubules with advancing age, so that the tubular diameter is smaller in teeth from older persons.
Peritubular dentin deposition

Kvaal S.I, Koppang H.S and Solheim T (1994) investigated the relationship between age in humans and the amount of peritubular dentine and the extent of the consequent obliteration of the tubules. They also investigated whether this relationship was strong enough to be used as a parameter for age estimation.

HUMAN DENTINAL STRUCTURE AS AN INDICATOR OF AGE

Microscopically observed aging changes are seen in the shrinkage of pulp tissue, the presence of a predentin layer or in the dense dentinal tissue which can be observed by scanning electron microscopy (SEM). The origin of these changes may usually be found in the structural changes of dentine. Kosa F, Antal A, Farkas I, (1990)

AGE ESTIMATION FROM INCREMENTAL LINES OF CEMENTUM

Cementum is continuously deposited at the root end and seen as incremental lines. Many researchers have used cemental annulations to determine age of adults. At present there is controversy using this method because different studies show vast discrepancies in the results. Saglam S, Atsu K, Goldemir and Kedici PS. (1998)

Incremental lines in cementum

Kagerer P and Grupe G (2000) suggested the possibility of age estimation from acellular cementum incremental lines. This made use of mineralized, unstained cross-sections of teeth, preferably mandibular central incisors and third molars. The authors claimed an accuracy of within two to three years of the actual chronologic age. However, the pathologic state of the periodontium and/or desmodontium may compromise the precision of ageing.

In addition to age, hypomineralized bands in these incremental lines gave an indication of events such as pregnancies, skeletal trauma, and renal disorders, which could be accurately dated to an individual’s life-history, thus facilitating identification.

Wittwer-Backofen U, Gampe J, Vaupel JW. In 2004 also conclude that the tooth cementum annulations (TCA) technique is a reliable method for estimating a subject’s age from cementum annulations. Indicators like sex differences, intra individual correlations, and the effects of periodontal disease did not have a quantitative effect on the number of TCA bands.

A major disadvantage of the above methods of estimating age in adults is the necessity to extract and/or section the teeth. While this is possible in the dead, it is not practical among living adults.

AGE ESTIMATION USING COLOR OF THE TEETH

Age estimation from changes in tooth color has been suggested by many; however the use of dental color for age estimation in forensic odontology has been limited due to the difficulty of measuring color objectively.

Martin-de las Heras (2002) and coworkers, who have proposed the use of spectroradiometry as an objective method for dentine color measurements for estimation of age.

Dentine color in 250 teeth from patients ranging in age from 10 to 89 years was determined by spectroradiometry. Color measurements were performed as suggested in the CIE 1931 (International Commission on Illumination). Chromaticity coordinates (x, y, z), luminance (Y), whiteness index (WIC, %W, WIC) and yellowness index (YI) were obtained. Correlations between these colorimetric variables and aging were established by linear regression analyses. All the variables fit the mathematical model with correlation coefficients ranging from 0.53 to 0.75. This method of color measurement produced an expected associated error of calibration averaging 13.7 years about the mean estimated values, at a 70% level of confidence. Two different multiple regression models for dental age estimation were tested and variables that made the greatest contributions to age calculation were identified.

To determine the effect of postmortem interval on tooth color
and its influence in age estimation, 37 teeth obtained from human skeletal remains buried during an interval ranging from 21 to 37 years were also studied. In this material, the correlation between age and dental color measured by spectroradiometry was weaker than in fresh extracted teeth.

They found that the dentinal colors white, cream, and yellow were associated with age 12-37 years, while dark yellow and brown were associated with the age-group 55-64 years.

It is concluded that determination of dentine color by spectroradiometry is a potentially useful objective method to estimate age in forensic studies in combination with other methods.

AGE ESTIMATION USING FLUORESCENCE FROM DENTINE AND CEMENTUM

It has been proposed that color changes in the dentine and cementum are caused by infusion of decomposition products from erythrocytes such as porphyrines, which fluoresce. Griffin. R.C, H.Moody, K.E.H. Penkman, M.J. Collins. (2008)

AGE ESTIMATION IN DENTAL PULP DNA BASED ON HUMAN TELOMERE SHORTENING

Tomoya Takasaki et al (2003) conducted a study for the estimation of age based on evidence found in teeth has received considerable attention within the field of forensic science. They determined the terminal restriction fragment (TRF) length, as telomere length, to estimate age. Using dental pulp DNA they found that the average TRF length showed a tendency to shortening with aging. Their findings show that telomere shortening, based on dental pulp DNA is a new and useful approach to estimate age of the subject at the time of death. Takasaki T, Tsuji A, Ikeda N and Ohishi M. (2003)

Telomere shortening during aging, TRF length clearly showed a tendency to shortening with aging. Picture of analysis by Quantity One showed the average TRF length. M: Molecular weight marker, lane 1: 0 year old, lane 2: 14 years old, lane 3: 26 years old, lane 4: 36 years old, lane 5: 46 years old, lane 6: 55 years old, lane 7: 63 years old, lane 8: 71 years old, lane 9: 85 years old.

PROSTHETIC RESTORATIONS, DENTAL ROOT FILLINGS AND PERIODONTAL BONE RESORPTIONS AS A FORENSIC-ODONTOLOGIC AID FOR DETERMINING THE AGE

Dental treatment often causes permanent alteration of the teeth, which is visible on radiographs.

Friedrich RE, v Maydell LA, Ulbricht C, Scheuern HA.(2005) conducted a study in which the aim was to determine whether the evaluation of such findings on radiographs correlates with the chronological age in teenagers and young adults.

The dental X-rays (OPG) of 1053 outpatients (age: 14 to 24 years) were evaluated. The values "prosthetically restored", "filled dental root", or "periodontal bone loss" were recorded for each tooth present. The data were then evaluated using statistical tools.

The correlation between the number of prosthetically restored teeth and the chronological age is high. The positive predictive value of determining an age of at least 18 years proved to be very high. However, these findings are rarely present in this age group. The number of endodontically treated teeth correlated less exactly with age. On the other hand, the determination of periodontal bone loss gave reasonable positive predictive values for the threshold value "chronological age of 18 years or more".

AGE ESTIMATION BY USING PERI-APICAL X-RAYS

Morse et al (1994) have described a method for estimating the age in the older age group. They take the periapical radiographs of these subjects and derive six parameters from them:

1. Coronal length (A): distance from incisal edge to top of pulp chamber
2. Apical length (B): distance from root apex to apical end of root canal
3. Root canal length (C): tooth length minus coronal length minus apical length
4. Cervical width (D): width of root canal at the cervix
5. Mid root width (E): width of root canal at its halfway point
6. Apical width (F): width of root canal at its most apical point.

They have found that with age these parameters change, and they have been able to find statistically significant differences between various age groups. They have come to the conclusion that vertical root canal shrinkage occurs at the rate of 0.32 mm/year; horizontal root canal shrinkage at the rate of 0.07 mm/year; and combined root canal shrinkage at the rate of 0.39 mm/year.

Table showing dimensions of various tooth parameters measured radiographically in a 23 year old and 45 year old subject

<table>
<thead>
<tr>
<th>Parameter</th>
<th>23 years (mm)</th>
<th>45 years (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronal length (A)</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Apical length (B)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Root canal length (C)</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Cervical width (D)</td>
<td>1.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Mid root width (E)</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Apical width (F)</td>
<td>0.5</td>
<td>0</td>
</tr>
</tbody>
</table>

Age Estimation by Pulp/Tooth Ratio in Canines by Periapical X-Rays

Roberto Cameriere (2007) conducted an investigation to examine the possible application of the pulp/tooth area ratio by periapical images as an indicator of age at death. A total of 200 peri-apical X-rays of upper and lower canines were assembled from 57 male and 43 female skeletons of Caucasian origin, aged between 20 and 79 years. They belong to the Frasetto osteological collection of Sassari (Sardinia) and are housed in the Museum of Anthropology, Department of Experimental and Evolutionistic Biology, University of Bologna. For each skeleton, dental maturity was evaluated by measuring the pulp/tooth area ratio on upper (x) and lower (x) canines. Very good agreement was found between intraobserver measurements. Statistical analysis was performed in order to obtain multiple regression formulae for dental age calculation, with chronological age as dependent variable, and gender, and upper and lower canines as independent variables. Stepwise regression analysis showed that gender did not contribute significantly to the fit (p>0.881) whereas variables x1 and x2 and the first-order interaction between them did. These two variables explained 92.5% of variations in estimated chronological age and the residual standard error was 4.06 years. Lastly, two simple linear regression equations were obtained for age estimation using canines from the maxilla and mandible separately. Both models explained 86% of variations in estimated chronological age and allowed an age-at-death estimate with a residual standard error of about 5.4 years.

The new advent such as digital radiography dramatically increases the quality and timeliness associated with the use of dental x-rays in forensic dental victim identifications, particularly when combined with computer-based dental chart matching software.
In fact, a very strong concordance correlation coefficient was found. Linear regression analysis showed a coefficient of determination ($r^2$) of 0.31 which suggests that there is a rather weak correlation between the volume ratio of pulp versus tooth volume and biological age. Although rather time consuming, this technique shows promising results for dental age estimation in a non-destructive manner using X-ray microfocus computed tomography.

**DENTAL AGE ESTIMATION THROUGH VOLUME MATCHING OF TEETH IMAGED BY CONE-BEAM CT**


The technique has shown promising results for dental age estimation in a non-invasive manner using cone-beam CT images in living individuals.

**Conclusion**

As with dental identification, one must bear in mind that the report of age estimation is addressed to law enforcement agencies. Therefore, it is very important that the wordings in the report reveal the underlying concepts of age estimation, the materials that were obtained for age estimation (such as radiographs or skeletal samples), as well as the method(s) used. In addition, it is important to address the applicability of the method(s) to the population on which it was used. Ultimately one need to remember that dental age cannot be expressed precisely, but at best, within an age-range.

**REFERENCE**