

Physiological Changes in Teeth as a Tool to Estimate Age.

Dr. Amandeep Singh*, Dr. V.P. Gupta, and Dr. Sanjoy Das

Department of Forensic Medicine and Toxicology, Himalayan Institute of Medical Sciences, HIHT University, Swami Ram Nagar, Dehradun-248140, Uttarakhand, India .

*E-mail: dramandeep@gmail.com

ABSTRACT

With increasing age there are many changes in teeth such as attrition, periodontal disease, deposition of secondary dentine, root translucency, cementum apposition, root resorption, color changes, and increase in root roughness. By taking into consideration these secondary changes in teeth with advancing age, various studies were done to estimate the age of an individual. Such research has resulted in multifactorial methods that help in age estimation.

A score was calculated noting the various physiological changes in the teeth and a graph was plotted with actual age on one axis and the score calculated on the other. Regression formulae were derived from the obtained graph. Previous regression formulae were used to calculate the age using total score recorded from the physiological factors of teeth. Formula A (newly derived formula) derived from the regression curve obtained by plotting total score against actual age was $y = 4.7x + 9.66$ where y is calculated age and x is total score. Correlation of the morphological changes occurring in teeth with other factors like age and sex were also studied.

(Keywords: age estimation, secondary changes in teeth, regression analysis)

INTRODUCTION

Age is one of the essential factors in establishing the identity of a person. Estimation of human age is a procedure adopted by anthropologists, archaeologists, and forensic scientists. Different factors have been used for age estimation but none has withstood the test of time for adults above 25 years [1].

Age estimation can prove to be a critical part in victim identification process. In cases of

unidentified dead bodies, age estimation becomes necessary if there is no antemortem information available and a personal profile has to be reconstructed. In addition, age estimation can be done in precious archaeological skeletal material dating back hundreds of years [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].

Teeth, in many ways, form a unique part of the human body (e.g. they are a most durable and resilient part of the skeleton). It has now clearly been established that dental evidence can be invaluable in personal identification and criminalistics. In the eyes of most law enforcement agencies and courts, it is valid and reliable method, ranking favourably with other methods of comparison such as fingerprints and blood grouping procedures [4].

Age can be estimated from the teeth by various methods like eruption of teeth, which is known to be a good indicator of the age of the person. Teeth erupt in two sets (i.e. temporary and permanent teeth). Temporary teeth will guide from six months to thirty-three months while the permanent teeth will help from six years to twenty-five years in age determination. Teeth age can also be estimated from microscopic examination of a section of central part of teeth by counting the cross striations which appear daily as devised by Boyde and Gustafson's method. This is used for the age estimation from teeth specially for the

age above 25 years [5]. The teeth also help in identification if one has detailed records of teeth and presence of any peculiarities like decay, malposition, overlapping, malrotation, fillings with different materials, gaps or dentures.

At no time during the life of an individual is a tooth unit static. It is constantly undergoing changes of one sort or another (e.g. eruption, tilting, or even lateral movement through jaw). The epithelium of the mouth on crown of teeth also moves rootwards. Other changes which are appreciable with increasing age are attrition, periodontal disease, deposition of secondary dentine, root translucency, cementum apposition, root resorption, color changes, and increase in root roughness. By taking into consideration these secondary changes in teeth with advancing age various studies were done to estimate the age of an individual. Such research has resulted in multifactorial methods that help in age estimation.

Translucency of teeth and periodontal regression can be useful for dental age estimation. Periodontitis or gingival regression is caused by "the degeneration of the soft tissue surrounding the tooth as it progresses from the neck to the apex of the root and appears as a smooth and yellowish area below the enamel and darker than it but clearer than the rest of the root". This measurement was taken from the labial surface and recorded the "maximum distance between the cemento-enamel junction and the line of soft tissue attachment".

Transparency of teeth is a physiological feature that does not appear before age 20. Transparency is a result of the deposition of hydroxyapatite crystals in the dentine tubuli and is emphasized when the tooth is placed on light box [6].

Gustafson in 1950 suggested the use of six retrogressive changes and ranked them on an arbitrary scale, allotting 0-3 points according to degree of the change. None of the changes singly proved more accurate than when these were studied together help in age estimation.

The aim of this study was to note the various physiological changes occurring in teeth with age and to find whether factors like age and sex affects the morphological changes occurring in teeth.

MATERIALS AND METHODS

The medico-legal cases received for autopsy by the Department of Forensic Medicine, Government Medical College, Patiala, were taken for this study. A total of 100 cases were studied in adults (age group of 25 to 70 years). This range was further divided into six groups:

1. Group A - 25 - 30 years
2. Group B - 30 - 35 years
3. Group C - 35 - 45 years
4. Group D - 45 - 50 years
5. Group E - 55- 60 years
6. Group F - above 60 years

The following 6 dental parameter were studied in each case:

1. Attrition
2. Periodontal disease
3. Cementum apposition
4. Secondary dentine deposition
5. Root translucency
6. Root resorption.

Apparatus: The tools and materials used for the study included tooth extraction forceps, periosteal elevator, carborundum stone (rough and smooth), alcohol, xylene, formalin, light microscope, DPX, and slides, etc.

Method: After collecting the details regarding age and sex, the teeth to be studied were selected and this selection was made based on the study of Solheim (1980) with priority given to first premolars, second premolars and canines, and lastly incisors. In our study we took first premolars as they gave the strongest coefficient (Kvaal and Solheim 1994). The degree of attrition and extent of periodontal disease was recorded before the extraction of the tooth and scoring was done depending upon the scale given below. Then the tooth was extracted with extraction forceps and preserved in formalin until the ground section was prepared [7].

Preparation of the Ground Section: For examination of a tooth, hand grinding was done manually first with rough carborundum stone fitted on a motor until a section of 2 to 3 mm was obtained and then on static carborundum stone by hand until the thickness was 1 mm. At this thickness, the root translucency was noted. Grinding was further done using a fine stone until

a section of 0.25-mm thickness was left. Finally, the cleaned and dried section is mounted on a slide using DPX and viewed under a microscope for secondary dentine deposition, cementum apposition, and root resorption [8]. The factors seen in the tooth before and after sectioning were recorded in the Performa using a 4 point allotment system (Krishan Vij) as follows [4]:

(1) Attrition (A):

- A0- No Attrition
- A1- Attrition limited to enamel level
- A2- Attrition limited to dentine level
- A3- Attrition up to pulp cavity.

(2) Peridontal disease (P):

- P0- No obvious peridontal disease
- P1- Beginning of peridontal ds. but no bone loss
- P2- Peridontal disease more than 1/3rd of the root
- P3- Peridontal disease more than 2/3rd of the root.

(3) Secondary dentine (S):

- S0- No secondary dentine formation
- S1- Secondary dentine up to upper part of pulp cavity
- S2- Secondary dentin up to 2/3rd of the pulp cavity

S3- Diffuse calcification of entire pulp cavity.

(4) Root translucency (T):

- T0- No translucency
- T1- Beginning of translucency
- T2- Translucency more than 1/3rd of the apical root
- T3- Translucency more than 2/3rd of the apical root.

(5) Cementum apposition (C):

- C0- Normal cementum
- C1- Thickness of cementum more than normal
- C2- Abnormal thickness of cementum near the apex of the root
- C3- Generalized abnormal thickness of cementum throughout the apex of the root.

(6) Root resorption (R):

- R0- No resorption
- R1- Spotted resorption
- R2- Resorption limited to cementum
- R3- Extensive resorption of both cementum and dentin



No Attrition
(Score – 0)



Attrition involving enamel only
(Score – 1)



Attrition involving up to dentine
(Score – 2)

Figure 1: Showing Various Stages of Attrition and its Scoring.

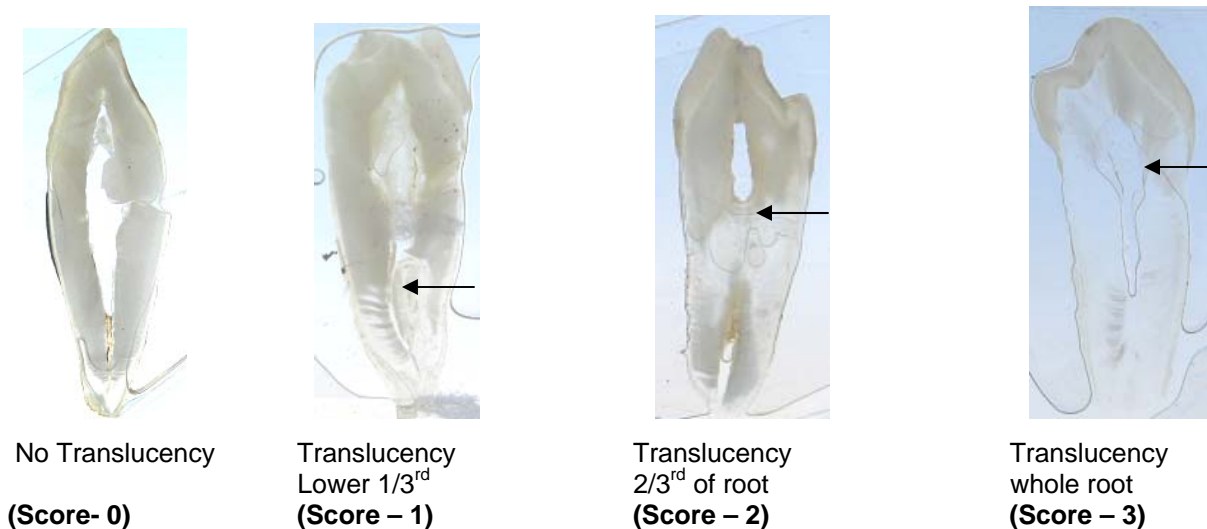


Figure 2: Showing Various Stages of Root Translucency and its Scoring.

After collecting the data and calculating the total score, a graph was plotted with actual age on one axis and the calculated score on the other. This graph can be used to derive a regression formula for the determination of age of the unknown cadaver.

The difference in the calculated age and actual age was found. From the above obtained difference maximum and minimum deviation of the calculated age from the actual age were also derived.

The total score was also used in various previous known formula and age calculated and the difference is calculated from the actual age. Following formulas were used:

1. Formula A: Newly Derived
2. Formula B: $y = 5.34 x - 4.08$
3. Formula C: $y = 4.56 x + 11.43$

4. Formula D: $y = 4.26 x + 13.45$
5. Formula E: $y = 6.26 x - 6.0$
6. Formula F: $y = 8.5 A - 26.073$
7. Formula G: $y = 6.37 T + 4.63 P + 2.7 S + 2.4 C + 3.08 A + 1.34 R + 8.57$

where y = calculated age,
 x = total score,
 A = Attrition score,
 T = Root Translucency score,
 P = Peridental Disease score,
 S = Secondary Dentine deposition score,
 C = Cementum Apposition Score
 and R = Root Resorption score

OBSERVATIONS

A total of 100 cases were taken in the study and divided in 8 different groups with maximum no. of cases between the age group of 25 – 35 years.

Table 1: Various Age Groups and Number of Cases in Each Group.

| Group | Age Group | No. of Cases |
|-------|--------------|--------------|
| I | 25 – 30 | 24 |
| II | 31 – 35 | 18 |
| III | 36 – 40 | 14 |
| IV | 41 – 45 | 8 |
| V | 46 – 50 | 12 |
| VI | 51 – 55 | 9 |
| VII | 56 – 60 | 11 |
| VIII | Above 60 | 4 |
| | TOTAL | 100 |

Table 2: Sex Distribution of Cases in this Study.

| Sex | No. of cases |
|--------|--------------|
| Male | 63 |
| Female | 37 |

Table 3: Distribution of Cases According to Diet.

| Type of Diet | No. of cases |
|----------------|--------------|
| Vegetarian | 76 |
| Non-Vegetarian | 24 |

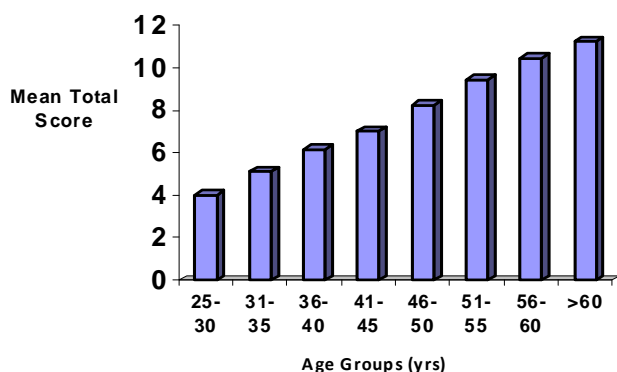


Figure 1: Mean Total Score with Increasing Age.

Table 4: Mean Total Score Calculated for all Six Factors for Various Groups.

| Group | Age Group | Total Score of six factors |
|-------|-----------|----------------------------|
| I | 25 – 30 | 4.0 ± 0.41 |
| II | 31 – 35 | 5.11 ± 0.32 |
| III | 36 – 40 | 6.14 ± 0.36 |
| IV | 41 – 45 | 7.0 ± 0.53 |
| V | 46 – 50 | 8.25 ± 0.45 |
| VI | 51 – 55 | 9.44 ± 0.52 |
| VII | 56 – 60 | 10.45 ± 0.68 |
| VIII | Above 60 | 11.25 ± 0.50 |

In Table 4 various age groups and the mean of the total score calculated from six physiological changes occurring with age are shown. It was observed that score was significantly increasing with the increasing age.

Table 5: Comparison of Total Score According to the Sex of the Person.

| Sex | No. of cases | Range of total score | Mean ± S.D. | t | p | S |
|--------|--------------|----------------------|-------------|------|-------|----|
| Male | 63 | 4 – 12 | 6.92 ± 2.41 | 0.97 | >0.05 | NS |
| Female | 37 | 3 - 11 | 6.43 ± 2.43 | | | |

In the study the number of females was half that of males and the mean of total score of various physiological factors in males were slightly higher as compared to that of females but statistically the relation of the total score to that of sex of person was non-significant.

Table 6: Comparison of Total Score According to the Diet of the Person.

| Diet | No. of cases | Range of total score | Mean ± S.D. | t | p | S |
|----------------|--------------|----------------------|-------------|------|-------|----|
| Vegetarian | 76 | 3 – 12 | 6.60 ± 2.17 | 0.99 | >0.05 | NS |
| Non-vegetarian | 24 | 4 - 11 | 7.16 ± 2.21 | | | |

There were more vegetarians in the study group but the total score calculated was higher in the non-vegetarian group as compared to vegetarian.

Table 7: Comparison of Age Calculated by New Derived Formula and Actual Age.

| Age | Range | Mean ± S.D. | t | p | S |
|------------|---------------|---------------|------|--------|----|
| Actual | 25 – 65 | 41.23 ± 11.65 | 0.06 | > 0.05 | NS |
| Calculated | 23.76 – 66.06 | 41.33 ± 11.38 | | | |

In the study, the new regression formula derived was used to calculate age of the person using total score and was compared with the actual age of the person. It was found that difference between the actual age and calculated age using score of physiological factors of teeth was statistically non-significant.

Table 8: Maximum and Minimum Difference in Actual Age and Calculated Age by Various Formulae.

| Formula | | Maximum | Minimum |
|---------------------------|--|---------|---------|
| A (New Formula) | $y = 4.7x + 9.66$ | - 4.24 | + 3.46 |
| B (Pillai and Bhaskar) | $y = 5.34x - 4.08$ | - 16.06 | - 3.34 |
| C (Gustafson) | $y = 4.56x + 11.43$ | - 3.53 | + 4.67 |
| D (Maple and Rice) | $y = 4.26x + 13.45$ | - 4.69 | + 5.49 |
| E (Singh and Mukerjee) | $y = 6.26x - 6$ | - 15.22 | + 4.86 |
| F (Tomaru et al.) | $y = 8.50A + 26.073$ | - 21.92 | + 18.07 |
| G (Kwak and Kim's) | $y = 6.37T + 4.63P + 2.70S + 2.40C + 3.08A + 1.34R + 8.57$ | - 17.42 | + 1.28 |

Table showing maximum and minimum difference of the age calculated using various previous regression formulae and it was seen that regression formula derived by Gustafson (formula C) and Maple & Rice (formula D) gave nearest value to the newly derived formula [1,9-13].

Table 9: Mean Calculated Age and Standard Deviation Using Various Physiological Factors.

| No | Factors | Range (yrs) | Mean (yrs) | Standard Deviation |
|----|------------------------------|---------------|------------|--------------------|
| 1 | Attrition | 30.58 – 64.36 | 41.22 | ± 8.04 |
| 2 | Peridontal disease | 32.32 – 45.70 | 41.21 | ± 3.30 |
| 3 | Secondary Dentine Deposition | 22.43 – 55.99 | 41.22 | ± 9.01 |
| 4 | Cementum Apposition | 31.63 – 47.89 | 41.22 | ± 8.03 |
| 5 | Root Translucency | 34.30 – 62.52 | 41.21 | ± 9.92 |
| 6 | Root Resorption | 34.17 – 59.79 | 41.21 | ± 8.21 |

Table showing the mean age calculated using regression formula derived from each individual factor taken alone and their standard deviation. It was seen that all factor had almost same mean age although maximum and minimum deviation from the actual age was very large.

Table 10: Maximum and Minimum Difference in Age Calculated from Actual Age.

| No. | Factors | Minimum difference (yrs) | Maximum difference (yrs) |
|-----|------------------------------|--------------------------|--------------------------|
| I | All factors | - 4.24 | + 3.26 |
| II | Attrition | - 17.53 | + 22.47 |
| III | Peridontal Disease | - 20.99 | + 19.7 |
| IV | Secondary Dentine Deposition | - 15.79 | + 14.21 |
| V | Cementum Apposition | - 17.11 | + 17.89 |
| VI | Root Translucency | - 14.70 | + 15.52 |
| VII | Root Resorption | - 25.83 | + 17.92 |

Table showing maximum difference and minimum difference of actual age of the person and calculated age from all factors taken together and each individual physiological factor taken alone. It was seen that although range of age difference between actual age and calculated age from each factors taken alone was very large still secondary dentine deposition and root translucency were the factor giving least range of deviation amongst all the factors.

DISCUSSION

A total of 100 cases were examined in this study and six physiological factors were recorded. The teeth selected in this study were first premolars. Incisors although having maximum affect of attrition were not taken as these were had less thickness and tended to get broken while making their ground section which is necessary to score four physiological factors (Figure 5).

Premolars were selected for the study as these are known to give best coefficient as compared to other teeth as per study of Kvaal and Solheim (1994) [7].

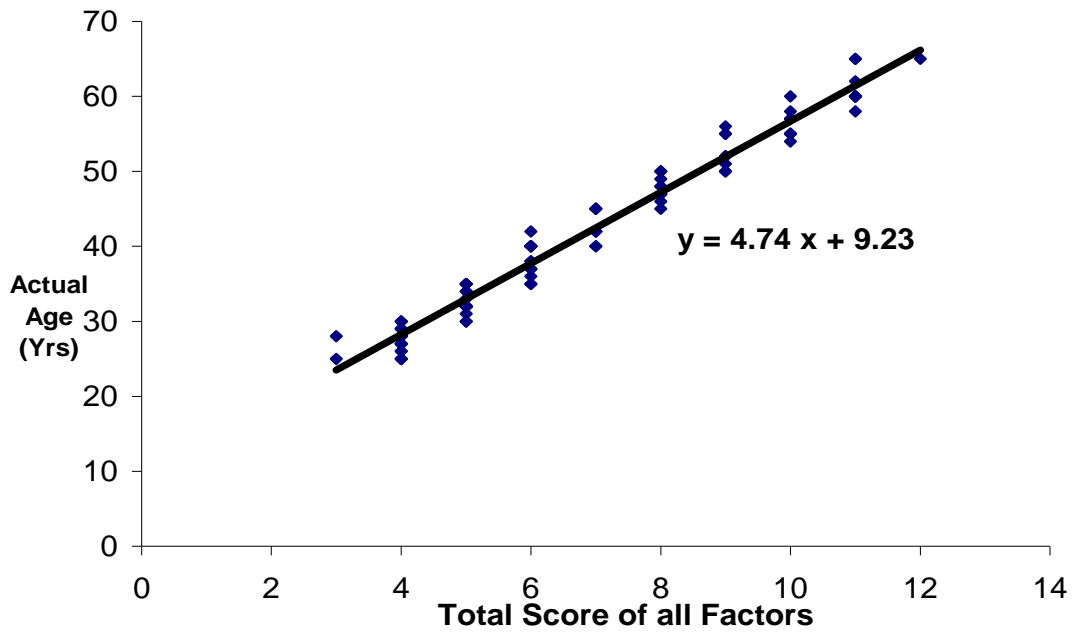


Figure 4: Regression Curve Depicting Relation of Calculated Total Score to Actual Age and its Regression Formula.



Figure 5: Ground Sections Prepared of Premolar 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 (Santini, Land and Raab 1990) [14] and in our study we were to include only the normal teeth. Third molars too were very variable in eruption and usually not present in all individual. Premolars were thus best in this respect (i.e., having optimum strength and are less brittle as compared to incisors).

Cases were divided into different age groups and the maximum number of cases in the study belonged to age group of 25 to 30 years, with the next being 35 to 40 years. This age group was largest as this is the most active age group and most of the postmortems done in the hospital are from this age group.

In each age group a score was given to every physiological factor and a total score was calculated. It was seen that the total score increased with the increasing age. It was 4.0 ± 0.41 in the age group of 25 to 30 years increasing to mean total score 11.25 ± 0.50 in the age group of above 60 years. This increase was statistically analyzed and was found to be significant. This finding was similar to the finding of Pillai and Bhaskar (1974) that also noted and scored these six physiological factors in their study [1].

In our study, 63 subjects were males and 37 were females. We found that the mean total score in males was slightly higher (6.92 ± 2.41) than that in females (6.43 ± 2.43), but statistically this difference was non-significant. This finding was consistent with the finding of Pillai and Bhaskar (1974) [1].

The diet of the persons was also noted. A person taking any kind of meat was recorded as non-vegetarian while persons eating only vegetables and eggs were kept in the group of vegetarians. It was seen that in the study group, vegetarians were the larger population as compared to non-vegetarians. The mean total score of the vegetarian group was compared to that with the mean total score of non-vegetarians. Mean total score in non-vegetarians was 7.16 ± 2.21 and slightly more as compared to mean total scores in vegetarians (6.60 ± 2.17). But statistically this difference was non significant and similar to the finding of Pillai and Bhaskar (1974) study. As the selected premolars are less frequently used in routine mastication, the attrition factors and

others factors usually are very less effected with type of diet and habits of the person [1].

In the present study, the regression line was obtained by plotting total score allotted against the actual age of the person. This regression line was used to derive the regression formula and it came out as Formula A: $y = 4.7x + 9.66$, where y is calculated age and x is total score allotted.

The values of total scores allotted by macroscopic and microscopic examination of teeth, was used in the regression formulae derived in previous studies:

- Pillai and Bhaskar (Formula B: $y = 5.34x - 4.08$)
- Gustafson (Formula C: $y = 4.56x + 11.43$)
- Maple and Rice (Formula D: $y = 4.26x + 13.45$)
- Singh and Mukerjee (Formula E: $y = 6.26x - 6$)
- Tomaru et al. (Formula F: $y = 8.50A + 26.073$)
- Kwak and Kim (Formula G: $y = 6.37T + 4.63P + 2.70S + 2.40C + 3.08A + 1.34R + 8.57$)

These formulae were also used and analyzed for age calculation differences as noted in Table 8.

Lastly, Table 7 shows the calculated age obtained by using values of total score allotted in the newly derived regression equation (Formula A: $y = 4.7x + 9.66$). [1,9-13]

The comparison of the mean actual age and mean calculated age, using the newly derived regression equation was done. It was found that mean calculated age (41.33 ± 11.38) was slightly higher as compared to the mean actual age (41.23 ± 11.65) but statistically this difference was non-significant.

Maximum and Minimum deviation was calculated from each of the six formulae and it was seen that newly derived regression formula (Formula A) gave least deviation of the calculated age from the actual age (- 4.24 to + 3.26 years) which was similar to that calculate using Gustafson's (- 3.53 to + 4.67 years) as well as Maple and Rice's formula (- 4.69 to + 5.49). The rest of the formulae gave large range of deviation. The reason could be different study group as well as due to use of multiple regression equation as in the method of Kwak and Kim.

All of the factors taken together and total score used for calculating age gave less deviation as compared when each factor was taken alone. Deviation of calculated age from the actual age

was ± 4 years (range - 4.24 to + 3.26 years) which was better than that found by Maple and Rice (i.e. ± 7 years) but more as compared to that found by Bang and Ramm in their study (± 3.6 years) [10, 15].

Thus in our study we found that physiological factor studied gave the best result when all the six factors were scored and used collectively together to find the age of the person.

SUMMARY AND CONCLUSION

1. Six physiological factors were noted in this study that could help in the age estimation. These were attrition, periodontal disease, secondary dentine deposition, cementum apposition, root translucency/transparency and root resorption.
2. Physiological factors of teeth that could help in age estimation had no significant correlation with the sex of the person.
3. Diet, whether vegetarian or non-vegetarian, had no effect on physiological factors of premolar teeth which help in age estimation.
4. Regression formula was derived from the graph obtained by plotting total score against actual age.
5. Previous regression formulae were used to calculate the age using total score recorded from the physiological factors of teeth.
6. Previous regression formulae were evaluated and Gustafson's and Maple & Rice's formulae were found to give least range of deviation from actual age.
7. Formula A (newly derived formula) derived from the regression curve obtained by plotting total score against actual age was $y = 4.7x + 9.66$ where y is calculated age and x is total score.
8. Mean calculated age when total scores of all the physiological factors were taken together was 41.33 ± 11.38 while mean actual age was 41.23 ± 11.65 .
9. Mean calculated age with all physiological factors taken together had a deviation of about ± 4 years (- 4.24 to +3.26 years).

10. Age calculated using all the six factors had less deviation from the actual age as compared when individual factor was taken alone.
11. All the six physiological factors showed large deviation when they were taken separately. Least deviation of calculated age from actual age was seen with the secondary dentine deposition and root translucency factors.

REFERENCES

1. Pillai, P.S. and Bhaskar. G. 1974. "Age Estimation from the Teeth using Gustafson's Method – A Study in India". *Journal of Forensic Science*. 3: 135-141.
2. Soomer, H., Ranta, H., Lincoln, M.J., Pentilla, A., and Leibur, E. 2003. "Reliability and Validity of Eight Dental Age Estimation Methods for Adults". *Journal of Forensic Science*. 48(1): 149-152.
3. Pathak, S.K., Mathur, P.N., Jain, S., and Saini, O.P. 1999. "A Study of Eruption of 3rd Molar in Relation to Estimation of Age in People of Thirteen to Twenty-Five Years Age Group". *Journal of Forensic Medicine and Toxicology*. 16(1):17-9.
4. Vij, K. 2002. *Textbook of Forensic Medicine and Toxicology*. B.I. Churchill Livingstone: New York, NY. 4: 71-72.
5. Swami, D., Mishra, V.K., Bahal, L., and Rao, C.M. 1992. "Age Estimation from Eruption of Temporary Teeth in Himachal Pradesh". *Journal of Forensic Medicine and Toxicology*. 9: 3-7.
6. Prince, D.A. and Ubelaker, D.H. 2002. "Application of Lamendin's Adult Dental Aging Technique to a Diverse Skeletal Sample". *J forensic Sci*. 47(1): 107-116.
7. Kvaal, S and Solheim, T. 1994. "A Non-Destructive Dental Method for Age Estimation". *Journal of Forensic Odontostomatol*. 12(1): 6-11.
8. Metzger, Z., Buchner, A., and Gorsky, M. 1980. "Gustafson Method for Age Determination from Teeth – A Modification for the Use of Dentists in Identification Teams". *Journal of Forensic Science*. 25(4):742-9.
9. Gustafson, G. 1980. "Age Determination from Teeth". *Journal of American Dental Association*. 41: 45-54.
10. Maples, W.R. and Rice, P.M. 1979. "Some Difficulties in the Gustafson's Dental Age Estimations". *J Forensic Science*. 24(1):168-72.

11. Singh, A.M. and Mukerjee, J.B. 1985. "Age Determination from Teeth of Bengalee Subject by following Gustafson's Method". *Journal of Indian Academy of Forensic Science*. 24(2):1.
12. Tomaru, Y., Uchiyama, Y., Kobayashi, K., Kudo, Y., Mikumi, H., Endo, M., Tsukamoto, T., and Terazawa, K. 1993. "Age Estimation from Tooth Attritions of Lower Incisors: Discussion on Amano's Method". *Nippon-Hoigaku-Zasshi*. 47(1): 13-17.
13. Kwak, K.W. and Kim, C.Y. 1993. "Comparative Study of Age Estimation Accuracy in Gustafson's Method and Prediction Formula by Multiple Regressions". *Journal of Forensic Odontostomatol*. 10(1): 43-48.
14. Santini, A., Land, M., and Raab, G.M. 1990. "The Accuracy of Simple Ordinal Scoring of Tooth Attrition in Age Assessment". *Forensic Science International*. 48(2): 175-84.
15. Bang, G. and Ramm, E. 1970. "Determination of Age in Human from Root Dentine Transparency". *Acta Odontologica Scandinavica*. 28: 3-35.

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