To estimate the age by evaluating the area and length:

Dental age estimation, digital vernier caliper, the area of dentin translucency showed good

Ground sections of single rooted permanent teeth were made and stained with 1% methylene blue. The area and length of dentin translucency were measured using digital Vernier caliper and with the help of stereomicroscope.

Results: Linear regression analysis showed that estimation of age by assessing the area of dentin translucency with Vernier caliper was statistically significant and showed a high regression coefficient ($R = 0.7738$) when compared to evaluation of age by assessment of length. Multilinear regression analysis done to calculate age by both area and length also showed a high co-efficient of regression ($R = 0.7797$).

Conclusion: The area of dentin translucency showed good correlation with age when compared to the length.

Key Words: Dental age estimation, digital vernier caliper, multilinear analysis, regressive analysis, stereo microscope

Introduction

Identification of living individual or deceased is very important in forensic sciences. Nowadays age estimation is also very important in forensic science, and forensic odontology has played a key role in this. Dental age estimation and forensic odontology have been successfully employed in ethical and humanitarian grounds as well as in legal aspects and criminal investigations. There are different methods for estimation of dental age such as by morphological, radiographic, histological and biochemical means. Among these, histological methods are considered as important for dental age estimation, the reason being that the teeth are one of the major sources of evidence as it can withstand various environmental changes and insults for long time.

Dentin forms the major bulk of the tooth. Dentin develops uniformly from the infancy to adolescence. After adolescence, the dentin undergoes physiological changes such as sclerosis. This process of sclerosis in dentin is known as dentin translucency that gradually increases as age advances. Thus, these changes can help possibly us to estimate age.

Lamendin et al., in 1992 estimated age with the help of the following two criteria; extension of the root dentin translucency and periodontal height on the labial surface of single rooted teeth. Gustafson included six parameters for dental age estimation such as attrition, cemental angulations, attachment of cemento enamel junction, dentin translucency, secondary dentin deposition and root resorption. All these parameters he considered were in intact teeth. Among these parameters, dentin translucency is the sole significant parameter for dental age estimation as dentin translucency is considered to be less inclined to deviate in pathologic processes and resists environmental changes and other age-related changes.

This study was undertaken to assess the area and length of dentin translucency in single-rooted ground sections of extracted teeth and to correlate this with age. The length of dentin translucency was measured using digital Vernier caliper, and the area was calculated using a graph sheet and a stereomicroscope.

Materials and Methods

Fifty freshly extracted permanent anterior teeth (incisors and canines) from 50 individuals were collected from Department of Oral and Maxillofacial Surgery. Inclusion criteria for selection of patients: Those who complain of mobile teeth because of periodontitis, and for prosthodontic purpose. Exclusion criteria were mobility because of trauma, any pathological condition, external root resorption and abrasion. Among these 50 individuals 27 were males, and 23 were females with age range from 51 to 65 years. After obtaining the consent from the patient and Institutional Review Board, the extracted teeth were kept it in 10% neutral formalin to prevent dehydration. All the teeth were sectioned...
buccolingually using corborandum disc. After sectioning, 250 μm thick ground sections were made by using Arkansas stone (Figure 1). The thickness of sections was confirmed using digital vernier caliper. The ground sections were then dipped in 1% methyl blue for better appreciation of dentin translucency. The purpose of dipping in methyl blue was that it stains the entire teeth blue except the dentin translucency area because sclerotic dentin will never take up any stain (Figure 2). A horizontal line was drawn at cemento enamel junction area with lead marker pencil and from this line till the root apex, the total length and area of the root were measured. The length of the translucency in the root was measured using digital vernier caliper (Figure 3) before taking ground sections to observe under stereomicroscope. Before subjecting the sections to examination by the stereomicroscope, a graph paper was superimposed on a transparent overhead projector sheet (Figure 4). Over this superimposed graph paper ground sections were kept and the total number of squares were counted in the root area (i.e., from cemento enamel junction to apex of the tooth) in both labial as well lingual surfaces independently to obtain the values for total root area. The area of dentin translucency was also calculated. The counting and calculations were made as follows: If one square was completely filled or more than half of it was filled it was counted to be 1 mm², and if less than half the square was filled, it was not counted. The number of squares in the total length of the root gave the area of the root, whereas the total number of squares in the translucent zone gave the area of dentin translucency. The measurement of both length and area were done separately on labial and lingual separately, and average of both the sites was considered.

Figure 1: Armamentarium for preparation of the ground section of a tooth.

Figure 2: Ground section showing dentin translucency at the apex of root.

Figure 3: Dentin translucency determination of the translucent zone using digital vernier caliper.

Figure 4: Ground section under a stereomicroscope with a graph paper superimposed on overhead projector sheet.
Dentin translucency and age estimation ... Kattappagari KK et al

Statistical analysis
Statistical Package for Social Sciences version 20.0 (IBM, USA) was used for statistical purposes. The mean and standard deviation for area and length was calculated separately. The correlation between age and length & area was calculated using Pearson’s correlation coefficient. Regression analysis was done to find the estimated age from length and area separately and also combined. The significance level was set at 0.01.

Results
The mean age of the sample was 51.28 ± 12.1 years. The mean age of males was 52.11 ± 13.0, whereas the mean age of females was 50.30 ± 11.27 (Table 1).

Regression analysis of age by area with a stereomicroscope showed that the Y intercept (minimum age at which length of translucency is 0) is 10.03. The coefficient of slope was 7.50 (when age advances by a year, there is a 0.075 mm² increase in the translucency area, and the correlation coefficient (R) was 0.7738 which denotes the degree of correlation between age and area of translucency and was statistically significant (P < 0.01). Thus the prediction equation of age obtained was: Age = 10.03 + 7.50 (area) (Table 2 and Graph 1).

Regression analysis of age by length with a Vernier caliper showed that the Y intercept is 32.08. The coefficient of the slope was 0.83, and the correlation coefficient (R) was 0.1417. The correlation between age and length was not statistically significant (P > 0.01) and the prediction equation of age was: Age = 32.08 + 0.83 (area) (Table 3 and Graph 2).

Multilinear regression analysis done to estimate age by both parameters i.e., area and length in vernier caliper technique also showed a high correlation coefficient (R = 0.7797). The prediction equation of age obtained was: Age = 3.0544 + 7.4446 (area) + 0.5591 (Length) (Table 4).

Discussion
In the 20th-century application of dentin translucency in relation to forensic odontology and age estimation has leapt several strides. Although there is lot of speculation with regard to how dentin translucency forms, it is a well-accepted fact that it increases as age advances which have been proven by numerous studies. Gustafson considered dentin translucency to be one of the six parameters to estimate age of an individual. Solheim in 1989 reported that translucency can be used for dental age estimation. The application of 1% methylene blue dye for ground sections for better appreciation of dentin translucency was first introduced by Thomas in 1994 who

Table 1: Mean age of the sample distribution among males and females.

| Gender  | Mean | N   | SD  |
|---------|------|-----|-----|
| Males   | 52.11| 27  | 13.00|
| Females | 50.30| 23  | 11.27|
| Total   | 51.28| 50  | 12.10|

SD: Standard deviation

Table 2: Regression analysis of age by area.

| Variable | Regression coefficients | SE  | t value | P value |
|----------|-------------------------|-----|---------|---------|
| Intercept| 10.03                   | 5.65| 1.7762  | 0.00001*|
| Area     | 7.50                    | 1.16| 6.4654  |         |

R²=0.7738, SE of estimate=9.4813, SE: Standard error, *: P<0.01

Table 3: Regression analysis of age by length.

| Variable | Regression coefficients | SE  | t value | P value |
|----------|-------------------------|-----|---------|---------|
| Intercept| 34.08                   | 14.40| 2.3674  | 0.4549  |
| Length   | 0.83                    | 1.09 | 0.7579  |         |

R²=0.1417, SE of estimate=14.8187, SE: Standard error

Table 4: Multilinear regression analysis of age by area and length.

| Variable | Regression coefficients | SE  | t value | P value |
|----------|-------------------------|-----|---------|---------|
| Intercept| 3.0544                  | 10.4770| 0.2915  | 0.00001*|
| Area     | 7.4446                  | 1.1699| 6.3633  |         |
| Length   | 0.5591                  | 0.7049| 0.7932  |         |

R²=0.7797, SE of estimate=9.5447, SE: Standard error, *: P<0.01
obtained satisfactory results. The same staining technique has been used by us in this study. The rationale behind usage of this stain is very simple. 1% methylene blue is taken up by all the structures of the root such as dentin (blue), cementum (dark blue) except the areas of the dentin translucency. Thus, it helps us to appreciate translucency better. The reason why sclerotic dentin does not take up this stain is because it is completely mineralized, and the dentinal tubules do not allow the dye to penetrate. This technique was also successfully adapted by Whittaker and Bakri. This translucency is initiated in the apical portion of the root. The reason being the lesser diameter of dentinal tubules in the apical dentin as compared to the coronal part, and thus they get obliterated fast. Another reason that can be attributed for this is that there are lesser number of tubules per unit area in the apical portion of the root. Azaz et al., in 1977 found that an increase in the dentin translucency with age can be considered as a physiological change even in impacted canines.

The present study shows that there is a strong correlation between dentin translucency and age advancement, and thus these results are strongly concomitant with previous studies. The area of translucency linearly increases as age advances. The correlation coefficient obtained in our study by regression analysis was 0.77 and coefficient slope was 0.075, which is in correlation with the study by Singhal et al., and Vasiliadis et al. Multilinear regressive analysis of age by area and length also showed a high correlation coefficient.

Summary and Conclusion
The general conclusion that can be made out of this study is that translucency noted in the apical root portion can be used for age estimation. The two variables that have compared here are length and area of dentin translucency. Statistical analysis showed that the translucency area is more reliable and accurate when compared to the length. This technique can be used to estimate age using the formula by length, by area as well combined (area and length). However estimation of age of individuals who are above 70 years is difficult as translucency in dentin seems to become static beyond this age, which may be due to the fact that there is complete blockage of all tubules. Furthermore, longitudinal studies are required with large sample sizes in different populations to conclude anything positively.

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