Introduction

In recent years, cases of age estimation in the living individual have become more frequent. For the living, the aim is to solve judicial or civil problems concerning age of minors regarding questions of adoption, imputability and pedopornography. For adults, civil issues on pensionable age (50, 55, 60, 65 years, depending on the country) and other similar matters for individuals (mainly immigrants and refugees) lacking valid documents of identification.[1]

In children, age determination from teeth is a relatively simple, accurate procedure and is based on the stages of development and eruption of teeth. However, in adults it is a challenge to medico-legal science.[2] Until now, a multiplicity of methods have been applied to estimate age based on dental tissue and tooth morphology. These methods can be broadly classified as: Morphologic and radiologic methods.

Radiography being a non-destructive method plays a vital role in forensic dentistry to uncover the hidden facts, which cannot be seen by means of physical examination. Radiographic age estimation using teeth rely on developmental stages of teeth especially in children whereas in adults; the continuous deposition of secondary
dentin throughout life depicted by reduction in pulp area can be employed.[3]

The present study was undertaken to assess the chronological age of adults of Karnataka aged between 18 and 72 years based on the relationship between age and measurement of the pulp/tooth area ratio of maxillary canine, using panoramic radiographs and a computer-aided drafting program. The purpose of the present research was to derive a population-specific formula based on Cameriere’s method.

Materials and Methods

Subjects and Materials: Panoramic radiographs of 200 patients of Karnataka were analyzed [Table 1] based on the following criteria:
- Patients aged between 18 and 72 years
- The selected tooth on the panoramic radiograph was the right or left maxillary canine, which had fully erupted into the oral cavity
- The root of the canine was fully formed.

Canines with any pathology, such as, caries or periodontitis or periapical lesions, malaligned canines or rotated canines, canines with any prosthetic fittings and orthodontic appliances, fractured canines, severely attrited canine secondary to parafunctional habits and canines with any developmental anomalies were excluded from the study. Ethical clearance and informed consent were obtained.

Panoramic radiographs were digitized using scanner, and images were recorded in a computer file. Radiographic images of canines (RIC) were processed using a computer-aided drafting program -ImageJ. Twenty points from each tooth outline and ten points for each pulp outline were identified and used to evaluate tooth and pulp areas. Measurements of the RIC, yielded pulp and tooth areas [Figure 1]; tooth, pulp and root lengths [Figure 2]; and pulp and root widths [Figure 3], at three different levels.

![Figure 1](image1.png) (a) Measurements of the RIC yielded tooth area; (b) Measurements of the RIC yielded pulp area and pulp and root widths

![Figure 2](image2.png) Measurements of the RIC yielded tooth, pulp and root lengths

![Figure 3](image3.png) Measurements of the RIC yielded pulp and root widths

| Table 1: Age and gender distribution of the study groups |
| --- |
| **Group** | **Age (years)** | **Gender** | **Total** |
|   |   | **Male** | **Female** | **Total** |
| I   | <20 | 3 | 4 | 7 |
| II  | 20-29 | 27 | 15 | 42 |
| III | 30-39 | 21 | 22 | 43 |
| IV  | 40-49 | 22 | 28 | 50 |
| V   | 50-59 | 9 | 11 | 20 |
| VI  | 60-69 | 30 | 2 | 32 |
| VII | >=70 | 5 | 1 | 6 |
| Total | | 117 | 83 | 200 |
|       | | 58.5% | 41.5% | 100.0% |
All measurements were made without prior information about personal data of the subjects. To test intra and inter observer reproducibility, a random sample of forty RIC were re-examined after an interval of two weeks.

**Statistical analyzes**

The following morphological variables were recorded: 

\[ P = \text{pulp/root length}; \ r = \text{pulp/tooth length}; \ a = \text{pulp/root width at enamel-cementum junction (ECJ) level}; \ c = \text{pulp/root width at mid-root level}; \ b = \text{pulp/root width at midpoint level between ECJ level and mid-root level}; \ AR = \text{pulp/tooth area ratio}. \]

These measurements were normalized to allow for angulation distortion.

Data was entered in a Microsoft EXCEL spreadsheet. Correlation coefficients were evaluated between age and predictive variables. To obtain an estimate age as a function of the morphological variables and gender of the subjects, a multiple linear regression model was developed with first order interactions by selecting those variables that contributed significantly to age estimations using the stepwise selection method.

Mean comparison of morphological variables between male and female was carried out by using student’s t-test. Intra and inter observer reproducibility of measurements was studied using the concordance correlation coefficient. Statistical analysis was performed with Statistical Package for the Social Sciences software (SPSS, version 10.5) package. The significance threshold was set at 5%.

**Results**

There were no significant difference between the morphological variables among the males and females, indicating that gender did not influence the regression model used to estimate chronological age for both female and male groups. There were excellent intra and inter observer agreement between the paired sets of measurements carried out on the re-examined panoramic radiographs with correlation coefficient ‘r’ values 0.946 and 0.882, respectively.

Pearson’s correlation coefficients between age and morphological variables showed that the variables ‘AR’, ‘a’, ‘b’ and ‘c’ correlated significantly with age, AR correlating the best among them (correlation coefficient ‘r’ value -0.974) [Graph 1]. The ratios between length measurements correlated worst with age. In particular, the variable ‘r’ was not significantly correlated with age (P value 0.140) and was therefore excluded from further statistical analysis. All the morphological variables had an inverse relation with each other.

Subjects’ age was modeled as a function of the morphological variables (predictors), and to optimize the model, a stepwise linear regression analysis was performed to find these statistically significant predictors of chronological age.

The results [Table 2] show that variables ‘AR’ (pulp/tooth area ratio) and ‘b’ (pulp/root width at midpoint level between ECJ level and mid-root level) contributed significantly to the fit, yielding the following linear regression formula:

\[
\text{Age} = 87.305 - 480.455(\text{AR}) + 48.108(\text{b})
\]

The regression equation with selected variables explained 96% of total variance (R² = 0.960) with the standard error of estimate of 3.018 years and median of the residuals (observed age minus predicted age) of 0.161 years.

The scatter plot [Graph 2] shows that the values are equally distributed; hence, the regression model fits the trend of the data reasonably well. The residual plot [Graph 3] shows no

**Table 2: Stepwise regression analysis**

| Model     | Unstandardized B | Std. Error | Standardized Beta | t     | ’P’ value | 95.0% confidence interval for B | Lower bound | Upper bound |
|-----------|------------------|------------|-------------------|-------|-----------|---------------------------------|-------------|-------------|
| Constant  | 91.134           | 0.855      |                   |       |           |                                 |             |             |
| AR        | -435.702         | 7.262      | -0.974            | 106.651 | < 0.001   |                                 | 89.449      | 92.820      |
| Constant  | 87.305           | 0.907      |                   |       |           |                                 |             |             |
| AR        | -480.455         | 8.709      | -1.074            | 96.235 | < 0.001   |                                 | -450.022    | -421.381    |
| b         | 48.108           | 6.344      | 0.148             | 7.584 | < 0.001   |                                 | 35.598      | 60.618      |

*Dependent variable: Age
obvious pattern and no outliers, hence supports our chosen regression model to estimate age.

There was no significant difference between chronological and estimated age for any of the age groups (P value > 0.05) thus signifying that the derived formula is appropriate for all the selected age groups [Table 3].

Discussion

The apposition of secondary dentin can be indirectly measured by the reduction in pulp size in the radiographs. In 1925, Bodecker[4] established that the apposition of secondary dentin correlated with age. In 1995, Kvaal et al.,[3] presented a method for age estimation, which was based on investigation of periapical radiographs, whereas Paewinsky et al.,[5] verified the applicability of this method on orthopantomograms.

In 2004, Cameriere et al.,[6] for the first time conducted a preliminary study to evaluate the variations in pulp/tooth area ratio (AR) as an indicator of age and their method of age estimation seems promising. The method originally examined the maxillary canine but subsequently included the second molar and mandibular canine.[7,8] While the authors obtained high levels of accuracy in age prediction (mean error - 3 to 4.5 years), they advised that future research should investigate “the effect of race and culture in model parameters”. Indeed, others have also advocated the verification of age estimation methods on independent samples and some have concluded that best results are derived when population-specific formulas are used.[3,9] Babshet et al., found that Cameriere’s formula, based on the Italian population, is not as applicable to the Indian population.[10] The present study was, therefore, undertaken to assess the chronological age of adults of Karnataka origin.

Maxillary canines were chosen as they are the single-rooted teeth with the largest pulp area and thus the easiest to analyze. The smaller size of the other single-rooted teeth leads to less clear measurement of the pulp/root ratio. In multi-rooted teeth, pulp changes are clear in the canal but less evident in the root. In addition, in adult subjects, molars and premolars are often missing or damaged as a result of wear. Maxillary canines are normally the oldest teeth and undergo less wear as a result of diet than posterior teeth.[8] Since Kvaal et al.,[3] did not find significant differences between teeth from the left and the right side of the jaw; teeth from either the left or from the right side were processed depending on whichever were best suited for measurement.

| Table 3: Comparison of chronological and estimated age in subjects grouped according to age |
| Age (years) | N | Mean age | SD | Min. | Max. | 't' value | 'P' value |
|-------------|---|----------|----|------|------|-----------|-----------|
| <20         |   |          |    |      |      |           |           |
| Chronological | 7 | 18.6     | 0.34 | 18.3 | 19.1 | 0.505     | 0.491     |
| Predicted   | 7 | 18.0     | 2.28 | 15.9 | 22.6 |           |           |
| 20-29       |   |          |    |      |      |           |           |
| Chronological | 42 | 24.3    | 2.66 | 19.9 | 29.4 | 0.002     | 0.966     |
| Predicted   | 42 | 24.3    | 3.91 | 18.2 | 37.7 |           |           |
| 30-39       |   |          |    |      |      |           |           |
| Chronological | 43 | 34.2    | 2.50 | 29.8 | 39.0 | 2.212     | 0.141     |
| Predicted   | 43 | 35.3    | 4.10 | 28.7 | 45.0 |           |           |
| 40-49       |   |          |    |      |      |           |           |
| Chronological | 50 | 44.4    | 2.88 | 39.8 | 49.3 | 0.448     | 0.505     |
| Predicted   | 50 | 44.8    | 3.84 | 37.2 | 52.1 |           |           |
| 50-59       |   |          |    |      |      |           |           |
| Chronological | 20 | 54.4    | 2.95 | 50.0 | 58.7 | 0.023     | 0.880     |
| Predicted   | 20 | 54.5    | 2.67 | 50.5 | 58.7 |           |           |
| 60-69       |   |          |    |      |      |           |           |
| Chronological | 32 | 63.8    | 2.76 | 59.6 | 68.8 | 3.791     | 0.056     |
| Predicted   | 32 | 62.1    | 4.02 | 52.0 | 67.8 |           |           |
| >=70        |   |          |    |      |      |           |           |
| Chronological | 6  | 70.9    | 0.93 | 69.6 | 72.0 | 2.036     | 0.184     |
| Predicted   | 6  | 68.7    | 3.59 | 62.4 | 73.3 |           |           |

SD: Standard deviation

Graph 2: Scatter plot of predicted age vs. chronological age

Graph 3: Scatter plot of predicted age vs. residual
In the present study, there was excellent intra and inter observer agreement indicating that Cameriere’s method is conducive to repeat measurements, both within and across examiners. The latter is of importance since Cameriere et al., had only evaluated intra-observer error and had advised that “repetitive measurements must be carried out by other independent observers in order to verify inter-observer reproducibility”.[6]

In the present study, Pearson’s correlation coefficients between age and morphological variables showed that the variables ‘AR’, ‘a’, ‘b’ and ‘c’ correlated significantly with age, AR correlating the best among them (correlation coefficient ‘r’ value =−0.974). This was consistent with the study of Cameriere et al.[6]

Subjects’ age was modeled as a function of the morphological variables (predictors), and to optimize the model, a stepwise linear regression analysis was performed to find these statistically significant predictors of chronological age. It was found that the variables ‘AR’ and ‘b’ contributed significantly to the fit, yielding the following linear regression formula: Age = 87.305 − 480.455(AR)+48.108(b). This was a new finding in our study contrary to the findings in previous studies by Cameriere et al.[6] and Saxena et al.[11] where the variables ‘AR’ and ‘c’ contributed significantly to the fit and yielded the formula for age estimation.

In our study, it was found that the regression equation with selected variables explain 96% of total variance (R² = 0.960) with the standard error of estimate of 3.0186 years and median of the residuals (observed age minus predicted age) of 0.1614 years. It is therefore comparable to other age estimation methods in adult.

According to the study conducted by Cameriere et al.[6] the regression equation, with the considered variables, explained 84.9% of total variance (R² = 0.849) with the standard error of estimate of 5.35 years and median of the residuals of 3.7 years. In the present study we got more accurate results. This could be attributed to the double sample size studied in our study.

Study conducted by Saxena et al.[11] showed 99.7% variance and 0.60 standard error of the estimate, when selected variables were used. The study was conducted on Indian population. India has a population of mixed ethnicity and people belonging to various origins reside here but ethnicity was not considered in the study. Our study was conducted on Indian population of Karnataka origin ensuring ethnic uniformity of the study sample.

In the present study, there was no significant difference between chronological and estimated age for any of the age groups (’P > 0.05‘) signifying that the derived formula is appropriate for all the selected age groups. This finding was consistent with the studies by Saxena[11] and Singaraju et al.[12]

The results of the study are promising; however, it cannot be generalized to other populations. The study was limited to the maxillary canines because it is the long lasting tooth and is easiest to analyze due to largest pulp area among all the single-rooted teeth. But, in conditions where these teeth are missing the method cannot be employed. Some questions concerning precision and accuracy of the measurements have been reported in literature when using digital measurements. So in future, image analysis programs which can recognize pulp outlines in radiographic images could be developed, which will be very useful in minimizing human manual measurements of morphological parameters, and will probably reduce both intra and inter observer variability.

Conclusion

Thus, within the limitations of the present study, it can be concluded that there is significant correlation between age and morphological variables ‘AR’ and ‘b’, and based on these variables chronological age can be determined with an accuracy of 96% in Karnataka population. The derived population specific regression equation can be potentially used for estimation of chronological age of individuals of Karnataka origin.

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How to cite this article: Juneja M, Devi YB, Rakesh N, Juneja S. Age estimation using pulp/tooth area ratio in maxillary canines-A digital image analysis. J Forensic Dent Sci 2014;6:160-65.

Source of Support: Nil, Conflict of Interest: None declared

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