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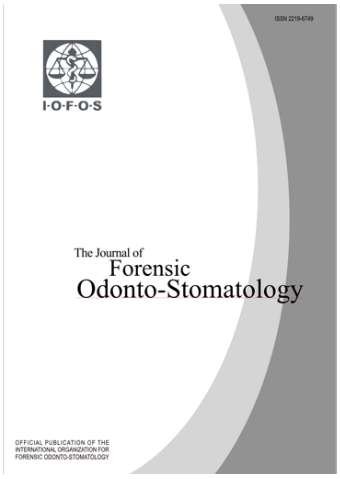

THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

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CONTENTS

Reliability of determining the age of majority: a comparison between measurement of open apices of third molars and Demirjian stages.

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KEYWORDS

Age estimation;
Third molar maturity index;
Demirjian's method;
Age of majority;
India

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ABSTRACT

Aim: This study examines the open apices of third molars in discriminating between individuals who are or are not 18 years of age or older and to assign a cut-off for estimation of the age of 18 years. Furthermore, this method was compared to those based on Demirjian's stages 8 and 9.

Methodology: Orthopantomographs (OPGs) of 1062 individuals (14 and 23 years) were assessed, to verify Cameriere's third molar maturity index (I3M). The apical ends of the roots of the left mandibular third molar were analysed. Mineralization of the third molar was also evaluated.

Results: A cut-off value of I3M =0.08 was taken. The sensitivity of this test was 70.76% and specificity was 82%. The results of the test showed a better specificity for Stage 9 and better sensitivity for stage 8 for adult age. Accuracy was 74.58% for third molar maturity index as compared to 72.41% for stage 9.

INTRODUCTION

Forensic age estimation of living individuals is one of the most intimidating challenges for the forensic community. However, it has been extremely advantageous in helping authorities in searching for unknown victims, determining eligibility for social benefits and assisting immigration services in the processing of undocumented immigrants. Human dentition has been proved to be one of the most reliable estimators of chronological age.[1,2] Various morphological methods can be used during human skeletal growth and development. The last tooth to initiate and complete development is the third molar and thus, is the last available dental morphological predictor of age.[3] After reaching the age of legal majority, the treatment of an individual changes dramatically within the criminal and civil legal courts in India. In these scenarios, forensic odontologists are often consulted by government agencies in estimating the ages of adolescents or juveniles who may or may not have reached that legally crucial age. Eighteen years of age is the threshold at which an individual is legally considered to have attained adulthood and, consequently, the legal system considers the person's activities, differently.

The Indian Juvenile Justice (Care and Protection of Children) Act, 2000, states that an individual who has not attained the age of 18 years is considered as a juvenile. While, as per the Juvenile Justice (Care and Protection of Children) Amendment Bill, 2006, a "juvenile in conflict with law", i.e. a juvenile affirmed to have committed an offence, cannot be sentenced to death or life imprisonment or committed to prison. On the contrary, such young offenders are counselled by Juvenile Justice Boards and remanded to a special home, usually for 3 years or until the time he/she attains majority status.[4] In India, according to Section 87 of the Indian Penal Code or IPC, eighteen years is also the legally acceptable age for giving/obtaining consent; while the Child Marriage Restraint Act, 1978 accepts it as the legally permissible age for marriage of females. Furthermore, it is the minimum age to enter government service in India.[5] Therefore, estimating whether an individual has (or has not) reached the age of majority can be very detrimental in India in a number of legal cases when the age is not confirmed or under dispute.

In particular, the third molar, whose development commences much later than other teeth, is usually the only tooth that is still undergoing calcification at this stage. Hence, although its development may be erratic and the tooth itself has a relatively high incidence of agenesis, the third molar has been the subject of immense interest as a predictor of 18 years of age and status of majority.[6,7,8]

In 1973, Demrjian et al[9] published a new classification of stages of tooth mineralization. In 1993, commissioned by the American Board Forensic Odontology, Mincer et al[3] studied 823 American children, prevalently Caucasians, aged between 14 and 24 years, to evaluate the radiographic reliability of the third molar as an age indicator and used Demirjian's tables to determine the mineralization stages. Using the revised grading system,[10] where alphabetical grading (A to H) was replaced with a numerical scale (Stages 0-9) on the mandibular (left) third molar, Acharya et al[11] determined majority/minority status applying three statistical approaches, i.e. traditional regression analysis, logistic regression analysis and Bayesian prediction.

The high number of subjects over 18 years of age with the third molar still not mature remains an important problem. The third molar has been used to evaluate age in late adolescence by various researchers from different countries like USA, South Africa, Japan, Austria, Turkey and Spain.[12-17] All these studies emphasized the dilemma of using the third molar as a determinator of age particularly for the age of majority i.e. 18 years. Actually, average age at the end of mineralization, i.e. Demirjian's stage 9, is usually reported to be more than 20 years and therefore classifying an individual as being over 18 only if the third molars which are in phase according to Demirjian yield a large number of errors (false non-adult).

Thus, this study was planned to examine the open apices of third molars in discriminating between individuals who are or are not 18 years of age or older and to fix a cut off for evaluation of the age of 18 for forensic purposes. Secondly, we aimed to compare sensitivity and specificity of this method with stages 8 and 9 of Demirjian.

MATERIALS AND METHODS:

Subjects and materials

Digitalized orthopantomographs (OPGs) of 1062 individuals aged between 14 and 23 years were retrieved from the Department of Oral Medicine and Radiology (Table 1). We evaluated the third molar maturity index by analysing the apical ends of the roots of the left mandibular third molar from the OPGs, to distinguish between individuals above and below 18 years of age. The third molar maturity index has been defined as: when the apical ends of the roots are completely closed, the inference is that the third molar has completed its root development i.e. $I_3M = 0$. While, if the apical ends of the roots are open, then I_3M is evaluated as the sum of the distances between the inner sides of the two open apices divided by the tooth length (Fig 1). Therefore, the maturity index, I_3M, is calculated in a similar way to the ratios Ai to Li, I = 6,7 as is reported for the other two molars with two roots as in the studies carried out by Cameriere et al.[18,19]

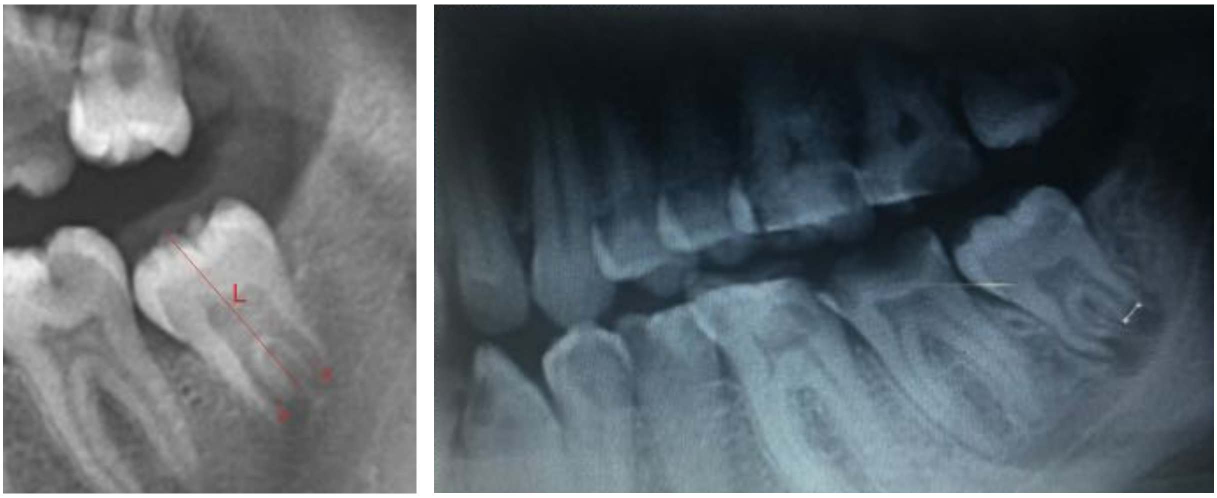
Statistical analysis

To evaluate inter-observer reliability, all measurements were carried out by two observers. The two observers made repeated observations of 30 OPGs at an interval of 2 weeks. The inter-observer reproducibility of the third molar maturity index, I_3M, was studied with the concordance correlation coefficient and k (kappa) statistics were used to measure the inter-observer reproducibility of the Demirjian stages. Analysis of variance (ANOVA) was performed to compare age distributions among Demirjian stages and gender. With individual age as a dichotomous response, variable (E=1 if an individual is at least 18 years of age, E=0 otherwise) and gender and the third molar maturity index, I_3M, as predictor variables, a generalized linear model was formulated to predict whether an individual is more than (E=1) or less than (E=0) than 18 years

Table 1: Sample of Orthopantomographs according to sex and age categories.

Age (years)	Male	Female	Total
14	18	13	31
15	44	18	62
16	77	54	131
17	75	62	137
18	78	24	102
19	35	55	90
20	84	83	167
21	80	82	162
22	57	54	111
23	49	20	69
Total	**597**	**465**	**1062**

Figure 1: Measurement of the length and width of the root apices in mandibular third molar.



of age by using a logistical model such as link function. The predictive accuracy of the model was assessed by the determination of the characteristic receiver operating curve (ROC) [Fig 2]. Receiver operating characteristic curve was used as a linear scale to determine the different levels of predicted probability that an individual is of age 18 years or older.

All significant variables were used to examine the medico-legal question as to whether an individual is older or younger than 18 years of age. The test was carried out to ascertain a threshold (cut-off) that could be used to assign an individual to the population of those younger (T=0) or older (T=1) than 18. The sensitivity p_1 of the test (i.e. the proportion of children for or older than 18 years of age, which verifies event T=1) was determined and also its specificity, p_2 ((i.e. the proportion of children younger than 18 years of age, who verify the event T=0).

Open apices in teeth can prove to be extremely crucial to distinguish between individuals who are

or are not aged 18 years or more, by the post-test probability of being 18 years of age or more (i.e. the proportion of individuals aged 18 or over in whom event E= 1 is verified).
The observations were entered in Microsoft excel file and the statistical analysis as well as the related graphs were completed with the SPSS 17.0 version statistical programme and the Microsoft Excel® programme. The significance level was set at 5%.

RESULTS

Representation of Demirjian stages was done by observing any disagreement between two measurements made by different observers, k=1.
The inter-observer representation of Demirjian stages was very good with Cohen's kappa statistic (±standard deviation) at k= 0.830±0.09, indicating substantial homogeneity of evaluation between operators. In the case of the reproducibility of the third molar maturity index, I3M, the estimated concordance correlation coefficient (±standard deviation) for inter-observer variability was pc = 0.999±0.001, when the measures of both observers were compared. Our results showed very good reliability between the two observers.
We distinguished the individual age in the sample using the Demirjian stages of third molars and gender [Table 2].
We applied ANOVA to ascertain the differences in age distributions among Demirjian stages and gender; it exhibited that gender had no influence on the mean value of the age distributions (p= 0.620) [Table 3].

Figure 2: Receiver operating characteristic curve for assessment of the sensitivity and specificity of age of majority. Diagonal segment are produced by ties

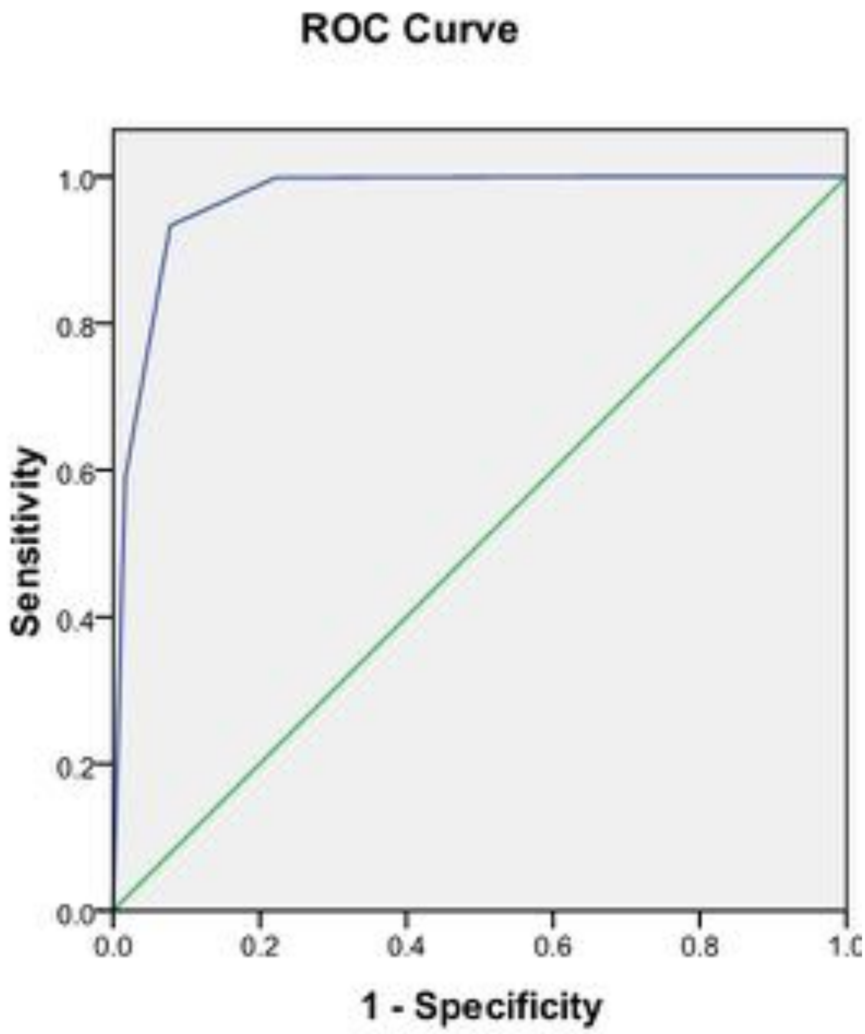


Table2: Mean and standard deviations for mineralization stages in relation to age and gender in study population

	Male Mean (SD)	Female Mean (SD)
Stage 4	14.13 (0.35)	14.00 (0.00)
Stage 5	15.32 (0.70)	15.33 (0.77)
Stage 6	16.39 (0.76)	16.49 (0.69)
Stage 7	17.39 (0.76)	17.54 (1.26)
Stage 8	19.38 (1.51)	19.65 (1.29)
Stage 9	21.02 (1.54)	20.93 (1.36)

Table 3: Percentage of individuals at least 18 years old or older by stage and gender

	Male N (%)	Female N (%)
Stage 4	0	0
Stage 5	0	0
Stage 6	1 (1.2)	0
Stage 7	31 (50.8)	15 (40.5)
Stage 8	117 (91.4)	123 (91.8)
Stage 9	234 (98.3)	180 (98.9)

Table 4 affirmed the frequency distribution by gender and stages of individuals older than or at least 18 years old. From this table, the inference drawn is that only Stage 9 can be used dependably to test adult age. We observed 98.3% of males 99% of females in Stage 9 to be adults.

When we used the third molar maturity index, I3M, we found a cut-off value of I3M for adult age which maximized the post-test probability and, at the same time, minimized the frequency of false negatives (i.e. the proportion of individuals of 18 years of age or older who were wrongly classified to the sub-adult population).

Setting p = P (E=1) as the probability that an individual was at least 18 years old, the probability on I3M and gender was formulated with a linear logistic model:

$\text{Logit}(p) = b_1 + b_0\, I_{3M}$

In sum, the probability that an individual is 18 years or older depends on the degree of maturity of the third molar I3M, but it does not significantly depend on gender.

The maximum likelihood estimates of parameters of the logistical model used to estimate the probability that an individual was 18 years of age or older, p, given the values of the factor I3M, are listed in Table 5.

Table 6 revealed the discrimination performance of the test i.e. to ascertain the crucial question authorized by law whether an individual is older or younger than 18. This helped in assigning an individual to the population of those younger than 18 if the test resulted negative (T = 0) and to the older age group if the test resulted positive (T=1).

In forensic investigations, where age estimation is the main point of consideration, it becomes highly significant and detrimental in the Court of law that the test reveals a low proportion of individuals younger than 18 whose test is positive (T=1) and so it was more appropriate to pay more attention to the chance of a false positive than to that of a false negative.
Based on these assumptions, we affirmed that an individual is considered to be 18 years of age or older (the test is positive, T=1) if I3M is lower than the cut-off value of 0.08; otherwise, an individual is considered to be under 18 (the test is negative, T=0). In our study, 496 individuals were classified as 18 or greater than 18 years.
The sensitivity of this test (the proportion of individuals being 18 years of age or older whose test is positive) was 70.76% and its specificity (the proportion of individuals younger than 18 whose test is negative) was 82%. The proportion of correctly classified individuals was 88.41%. The accuracy was 74.58%. Hence, the probability that a subject positive on the test (T=1) was 18 years of age or older was 74.58% (Table 7).

Table 4: Summary table of ANOVA

	df	SSQ	MSSQ	F	Pr(F)
Gender	1	0.210	0.210	0.134	0.714
Stage	5	4633.43	926.68	592.188	<0.001**
Gender x Stage	5	5.51	1.103	0.705	0.620
Residuals	1050	1643.10	1.565		

**p<0.001; Highly significant

Table 5: Para estimates for logistical model

Parameter	Value	Std. Error	p value
b0	2.400	0.160	<0.001
b1	-2.767	0.225	<0.001

Table 6: Classification table describing discrimination performance of the test

	Age		Total
	<18	**≥18**	
T=0	296	205	501
T=1	65	496	561
Total	361	701	1062

Table 7: Percentage of sensitivity, specificity, correct classification, and post-test probability (95% confidence interval) of test of adult age when stages 8 and 9 and I_{3M} index <0.08 are used to discriminate between individuals who are or are not aged 18 years or more

	Phase 8	Phase 9	I_{3M}<0.08
Sensitivity	93.30	59.06	70.76
Specificity	92.24	98.34	81.99
PPV	95.89	98.57	88.41
NPV	87.63	55.30	59.08
Accuracy	92.94	72.41	74.58

PPV: Positive predictive value; NPV: Negative predictive value.

DISCUSSION

In view of global rise of incidence of crime rates by juveniles in India, it has become increasingly important to determine the age of majority with precision. Various research studies have focused on wisdom tooth eruption and later on its mineralization to assess the age of 18 years.[12-17,20] Mincer et al earlier[3] suggested the method of degree of third molar development to estimate ages in the living; however, it was subsequently concluded to be a less precise method in identifying the adult individuals. However, they also emphasized that in dire situations, third molar formation is the only usable datum for age estimation. The timing of mandibular third formation was documented for two groups of children in England and South Africa and it was found that children from London and Cape Town were significantly delayed in the mean age of initiation and almost all subsequent formation stage of the permanent mandibular third molar compared to black South African children.[21]

Our results revealed that, if the root apices of the third molar are closed (i.e. the third molar is at terminal grade 9), then there is a high probability that the subject is at least 18 years of age. However, in terminal stage 9, only 59% of individuals were there, and the homogeneity between "at or over 18 years or under 18 years" and belonging to Stage 9 or not was 98%. Stage 8 shows a greater sensitivity as compared to stage 9, however, it decreases the specificity and the positive predictive value.

Hence, if stage 8 is selected as a predictor of adult age, it improves test sensitivity with respect to Stage 9, but, it markedly increases the false positive individuals, which is considered an ethically unacceptable error in the judiciary system. If Cameriere et al's[22] I3M method with a cut-off of 0.08 is used to estimate the legal adult age of 18 years, it significantly increases test sensitivity with respect to Stage 9. Furthermore, it minimises the number of false positive individuals. From a forensic point of view, it is significant that the percentage of false positives is small, since it is a graver error to consider a subject younger than 18 as a criminal in the Court of law than the judgement which does not consider a subject older than 18 as chargeable.

In forensic science, the judges are most inclined to ascertain whether the individuals in question have reached the threshold of the age of majority; it becomes a cumbersome decision for them to make in borderline cases. There are two unacceptable errors in the Courts of judgement, technically unacceptable and ethically unacceptable errors. In the first category falls the errors of judgement due to forensic age estimation indicating that a subject actually over 18 is in fact a juvenile or minor. However, if a minor who is under the age of majority is declared as an adult in the Court, it is considered an ethically unacceptable error, as it implies the direct violation of minors' rights. If they are judged to be under 18, they will be treated as juveniles, with the advantages of child care in reform homes; a child wrongly judged to be over 18 may be at risk of exploitation if placed with adults.

Thus, we must minimise false positives and in our study third molar maturity index achieved the lesser number of false positives similar to Cameriere's results in Italian population.[22] Study on Albanian sample[23] showed substantial success of suggested value for I3M, with the 87.4% and 92.5% correctly classified females and males, respectively. Similarly, high accuracy was obtained using third molar maturity index as a determinant of the age of majority in the Croatia.[24]

CONCLUSION

Our research compared the efficacy of third molar maturity index and Demirjian stages in estimating 18 years of age in Indian population. The accuracy of forensic age estimation in living subjects can be increased by evaluating population-specific results. The present study conducted on Indian adolescent sample proved that the I3M method is fairly accurate and reasonably reliable, therefore, it can also be recommended to be used for assessment of age of majority in a forensic context.

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Accuracy of age estimation in 6-21 year old South Indian population – A comparative analysis of clinical and radiographic methods

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KEYWORDS

Dental age estimation; Demirjian method; Foti's mathematical Model 2; Panoramic radiograph

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ABSTRACT

Unavailability of chronological age brings to the forefront the importance of age estimation for human identification. Dental age is routinely assessed based on the calcification stages and/or the eruption of teeth, which exhibit wide variations amongst different ethnic groups. The current study aimed at estimating the dental ages in 384 South Indian subjects aged 6-21 years, using clinical and radiographic methods and comparing the predictive accuracy of these two dental age estimation methods. For the estimation of age by clinical method, Foti and co-workers' mathematical Model 2 was employed and for the radiographic method, Chaillet and Demirjian's method with Acharya's Indian formula was used. The clinical method yielded a mean error in the range of -3.16 to 4.07 years and -1.83 to 4.32 years among male and female subjects respectively whereas the radiographic method yielded an error of -9.52 to 1.96 years among males and an error of -10.72 to 2.66 years in females. The mean absolute error for the entire sample obtained by clinical method was 0.80 years and by radiographic method was 0.89 years. We found that the clinical method had a better accuracy in estimating dental age of children and adolescents when compared to the radiographic method in South Indian (Karnataka) population. However, the difference between the two is negligible implying that either of the methods can be employed in clinical practice

INTRODUCTION

Human identification is one of the most important, challenging and indispensable aspects of forensic science.[1] Its clinical applications are enormous both in situations requiring identification of a living or a deceased individual.[2] Various parameters are used to draw the identity of an individual, of which estimation of age plays a pivotal role. Requirement for age estimation has a wide role in situations of child adoption, child marriage, penal code, infanticide, rape, judicial punishment, commercial or sexual exploitation, domestic employment, requests for political asylum, issues of inheritance and pension claims of the elderly.[3,4] It is also important in the practice of medicine and dentistry for the evaluation of developmental progress, occasionally to achieve accurate diagnosis and in treatment planning.[4,5] Age of the individual mandates estimation when the chronological age is unknown[4,5] or unavailable.

Age estimation methods most frequently use the skeletal maturation and/or the teeth development, as these parameters have shown to correlate positively with the chronological age. Among the two, age estimation using teeth has demonstrated higher correlation[6,7] as teeth are minimally affected by environmental diversities like nutritional and endocrine disturbances and withstand post-mortem destruction.[7] Dental age estimation methods utilize numerous factors starting from the appearance of tooth germs to the post-eruption alterations of the teeth.[7] Age estimation of young and adolescent individuals on the basis of stages of tooth development and eruption pattern are the most reliable.[8]

Clinical observation of tooth eruption was the method chosen for dental age estimation before the advent of radiographic techniques and still remains a practical method in situations where other dental age estimations methods cannot be carried out. This method is non-invasive, technique insensitive and economical.[8,9] Foti and co-workers proposed Model Number 2 for age estimation in living individuals by clinical examination of the erupted teeth, specifically under conditions when radiographic evaluation is either not possible or not permissible.[10,11]

Tooth eruption is likely to be influenced by a multitude of factors such as premature loss of primary teeth and crowding.[12] However, development of teeth is not affected as such. As a result they show less variation during estimation of age.[12] Screening radiographs like panoramic radiographs allow for detailed evaluation of the developmental stages of all the teeth in a single view.[7,13] The most well-known radiographic method for ascertaining dental age is the Demirjian method. Although this method was considered reliable, several studies showed high dependence on the characteristics such as race, ethnicity of the specific population in question.[12] Acharya thus derived an Indian specific regression formula using Demirjian's 8-teeth method.[14]

With this, the present research aimed at evaluating the accuracy of Foti's clinical method and Chaillet & Demirjian's radiographic method (using Acharya's Indian formula) for age estimation in people of Karnataka, a state in southern India.

MATERIALS AND METHODS:

The study sample consisted of 384 South Indian subjects aged 6 to 21 years. The subjects were divided into 16 groups starting from 6 years to 21 years with a class interval of 1 year (e.g. Group I:- 6 – 6.9 years, Group II:- 7 – 7 .9 years........ Group XVI: - 21 – 21.9 years). Each age group consisted of 24 subjects with equal distribution of 12 subjects in both the genders. The rationale for this equal distribution was that the maturity scores for each tooth in radiographic method is gender specific. The review board of institutional ethical committee has given approval for this comparative study.

Subjects residing in South India for at least the past two generations who underwent clinical and radiographic examination for reasons other than that of the present study were included in the study after obtaining informed written consent. Further, only those subjects who were devoid of congenital anomalies or syndromes, metabolic disorders, dental disorders, malignancies and/or treatment for the same and those who provided proof for their date of birth were included in the study.

To eliminate observer bias, an identification number between 1 and 384 was randomly allocated to each subject by an individual who was not a part of the study. The date of birth of the subject was then documented against their allocated identification number.

Chronological age estimation

The chronological age of the subject was calculated by subtracting his or her date of birth from the date of examination. For the convenience of statistical analysis, the resultant age was converted into a decimal value (e.g. 6 years 3 months 25 days was recorded as 6.3 years and was included in the 6 - 6.9 year age group).

Dental age estimation by clinical method

A detailed intraoral examination was completed for each subject by using diagnostic instruments under adequate illumination. The eruption of maxillary incisors and molars of the deciduous dentition; maxillary canines and molars and mandibular premolars, 2nd molars and 3rd molars of the permanent dentition were recorded in a proforma sheet. A tooth was considered to be erupted if at least a portion of the tooth pierced the alveolar ridge mucosa and was visible in the oral cavity.

Dental age estimation by clinical method was calculated using the formula derived by Foti and co-workers' Mathematical Model 2.[10] The equation is as follows: Estimated age = 13.652 - (0.514 x number of erupted deciduous upper incisors) - (0.236 x number of erupted deciduous upper molars) + (0.314 x number of erupted permanent upper canines) - (1.748 x number of erupted permanent upper 1st molars) + (1.012 x number of erupted permanent upper 2nd molars) + (0.944 x number of erupted upper 3rd molars) + (0.252 x number of erupted lower premolars) + (0.285 x number of erupted permanent lower 2nd molars) + (1.537 x number of erupted lower 3rd molars).

Dental age estimation by radiographic method

Digital panoramic radiographs were obtained following the clinical examination as was indicated for the diagnosis. Patient's data pertaining to his/her identification number, name, age and gender was registered in the Sidexis XG software. Images obtained following the exposure were stored in the computer with an identification number unique to the subject to facilitate blinding.

In the chosen panoramic images, the calcification stage of all eight permanent teeth on the left (3rd) quadrant were assessed and graded from 0 to 9 based on the Chaillet & Demirjian's method[8,15] by the observer. In the absence of any tooth on the 3rd quadrant, the corresponding tooth on the 4th quadrant was considered for assessment.

Following assessment the grades of the developmental stages of calcification of all 8 teeth were recorded in the proforma. A gender specific maturity score was given to each grade using Demirjian's individual maturity score table.[5,15]

The resultant maturity scores of all 8 teeth were then summed to obtain the total maturity score (S). This value was substituted in Acharya's Indian formula[14] as given below

Age (Males) = $27.4351 - (0.0097 \times S^2) + (0.000089 \times S^3)$

Age (Females) = $23.7288 - (0.0088 \times S^2) + (0.000085 \times S^3)$

STATISTICS

The data obtained was subjected to statistical analysis using the SPSS (Statistical Package for Social Sciences) version 15.0 software. Student's unpaired t-test was used to compare the chronological age with the dental age as estimated by Foti's clinical method as well as Chaillet & Demirjian's radiographic method (using Acharya's formula). Comparison was done separately for males and females. Level of significance was set at p = 0.05 and 95% confidence intervals (CI). The values were represented as Mean ± SD and standard errors. Multiple linear logistic regressions were used to evaluate the relationship between the chronological age and estimated dental age by both the methods.

The mean of estimated dental age by both the methods were compared with the mean chronological age of the corresponding age group.

RESULTS

Results of statistical comparison between Foti's clinical age estimation method and chronological age

The mean age estimated by the clinical method for the entire male sample was 13.04 years and for the entire female sample was 12.99 years (Table 1).

observed in the group XV (20-20.9 years) and for females in the group VII (12-12.9 years). The minimum difference for males was seen in the group IX (14-14.9 years) and for females in the group X (15-15.9 years).

In the age groups of VI (11-11.9 years) and XIV (19-19.9 years), there was a statistically significant ($p < 0.05$) difference observed in the mean estimated age between males and females implying that in these age groups there is a necessity to apply formulae for both males and females separately (Table 2).

Table 1: Mean age values of Clinical method and Radiographic method

	Gender	N	Mean (In Years)
Chronological age	Male Female	192 192	13.99 13.91
Clinical method	Male Female	192 192	13.04 12.99
Radiographic method	Male Female	192 192	14.81 15.07

Table 2: Comparison of estimated mean dental age by Clinical method and Radiographic method to chronological age for both genders

		Clinical method				Radiographic method			
Age groups	**Gende**	**Mean**	**SD**	**S.E**	**P-Value**	**Mea**	**SD**	**S.E**	**P-Value**
I (6-6.9 years)	M	9.58	1.62	0.47	0.08	15.94	2.28	0.66	0.17
	F	8.39	1.60	0.46		17.28	2.37	0.68	
II (7-7.9 years)	M	9.38	1.27	0.37	0.14	12.37	1.86	0.54	<0.001*
	F	8.42	1.76	0.51		16.27	2.00	0.58	
III (8-8.9 years)	M	8.97	0.70	0.20	0.24	10.69	0.75	0.22	0.004*
	F	8.54	1.00	0.29		12.61	1.93	0.56	
IV (9-9.9 years)	M	9.33	0.43	0.12	0.63	10.30	0.53	0.15	0.04*
	F	9.40	0.36	0.10		10.67	0.28	0.08	
V (10-10.9years)	M	10.59	1.72	0.50	0.42	10.39	0.95	0.27	0.19
	F	10.08	1.22	0.35		10.91	0.93	0.27	
VI (11-11.9 years)	M	12.19	1.89	0.55	0.01*	11.19	1.52	0.44	0.62
	F	10.39	1.37	0.40		10.94	0.80	0.23	
VII (12-12.9 years)	M	13.03	1.78	0.51	0.86	11.31	1.82	0.53	0.43
	F	12.89	2.15	0.62		11.97	2.17	0.63	
VIII (13-13.9 years)	M	13.57	1.64	0.47	0.42	14.14	1.68	0.48	0.72
	F	14.01	0.85	0.25		13.84	2.31	0.67	
IX (14-14.9 years)	M	14.34	0.10	0.03	0.48	15.45	1.32	0.38	0.14
	F	14.22	0.58	0.17		14.42	1.92	0.55	
X (15-15.9 years)	M	14.65	1.50	0.43	0.55	16.23	2.05	0.59	0.07
	F	14.39	0.00	0.00		14.51	2.43	0.70	
XI (16-16.9 years)	M	15.32	1.96	0.56	0.67	16.60	1.74	0.50	0.54
	F	15.01	1.54	0.45		16.10	2.07	0.60	
XII (17-17.9 years)	M	14.52	0.56	0.16	0.25	17.10	1.63	0.47	0.70
	F	14.96	1.17	0.34		17.34	1.43	0.41	
XIII (18-18.9 years)	M	15.51	1.67	0.48	0.12	18.12	1.19	0.34	0.86
	F	14.67	0.67	0.19		18.04	0.97	0.28	
XIV (19-19.9 years)	M	16.69	2.16	0.62	0.03*	19.07	1.34	0.39	0.45
	F	18.62	1.76	0.51		18.62	1.49	0.43	
XV (20-20.9 years)	M	16.50	2.28	0.66	0.98	18.61	2.45	0.71	0.91
	F	16.48	2.02	0.58		18.70	0.99	0.29	
XVI (21-21.9 years)	M	17.49	2.12	0.61	0.84	19.52	0.96	0.28	0.11
	F	17.31	2.13	0.61		18.97	0.66	0.19	

* Statistically significant

Table 3: Mean error and M.A.E (Mean Absolute Error) in years for Clinical method and Radiographic methods of age estimation in all age groups

		Clinical method				Radiographic method			
Age	**Gender**	**Mean**	**SD**	**M.A.E**	**P-Value**	**Mean**	**SD**	**M.A.E**	**P-Value**
I (6-6.9 years)	M	-3.16	1.83	0.53	0.09	-9.52	2.24	0.65	0.24
	F	-1.83	1.79	0.52		-10.72	2.58	0.75	
II (7-7.9 years)	M	-1.85	1.27	0.37	0.13	-4.83	1.98	0.57	<0.001*
	F	-0.86	1.73	0.50		-8.71	2.02	0.58	
III (8-8.9 years)	M	-0.46	0.61	0.18	0.30	-2.19	0.92	0.27	0.004*
	F	-0.07	1.12	0.32		-4.14	1.88	0.54	
IV (9-9.9 years)	M	0.26	0.51	0.15	0.85	-0.72	0.52	0.15	0.10
	F	0.22	0.37	0.11		-1.05	0.40	0.12	
V (10-10.9 years)	M	0.04	1.75	0.51	0.49	0.24	1.01	0.29	0.15
	F	0.47	1.18	0.34		-0.35	0.92	0.27	
VI (11-11.9 years)	M	-0.61	1.81	0.52	0.02*	0.39	1.51	0.44	0.68
	F	1.15	1.44	0.42		0.60	0.90	0.26	
VII (12-12.9 years)	M	-0.50	1.76	0.51	0.85	1.23	1.78	0.51	0.41
	F	-0.36	1.87	0.54		0.57	2.04	0.59	
VIII (13-13.9 years)	M	-0.08	1.66	0.48	0.58	-0.64	1.71	0.49	0.61
	F	-0.39	0.92	0.27		-0.21	2.30	0.66	
IX (14-14.9 years)	M	0.09	0.27	0.08	0.24	-1.02	1.36	0.39	0.11
	F	0.30	0.56	0.16		0.10	1.93	0.56	
X (15-15.9 years)	M	0.85	1.52	0.44	0.50	-0.73	1.95	0.56	0.07
	F	1.16	0.28	0.08		1.04	2.50	0.72	
XI (16-16.9 years)	M	1.21	1.98	0.57	0.89	-0.07	1.71	0.49	0.72
	F	1.31	1.49	0.43		0.21	2.06	0.59	
XII (17-17.9 years)	M	2.97	0.54	0.16	0.47	0.39	1.80	0.52	0.90
	F	2.68	1.24	0.36		0.30	1.50	0.43	
XIII (18-18.9 years)	M	3.04	1.53	0.44	0.26	0.43	1.07	0.31	0.66
	F	3.61	0.74	0.21		0.24	0.96	0.28	
XIV (19-19.9 years)	M	2.90	2.26	0.65	0.04*	0.52	1.42	0.41	0.39
	F	1.06	1.82	0.53		1.05	1.54	0.44	
XV (20-20.9 years)	M	4.07	2.35	0.68	0.89	1.96	2.56	0.74	0.77
	F	3.93	2.07	0.60		1.72	0.98	0.28	
XVI (21-21.9 years)	M	3.99	2.10	0.61	0.71	1.96	0.85	0.24	0.03*
	F	4.32	2.18	0.63		2.66	0.57	0.17	
		CLINICAL METHOD ---- MAE for whole sample = 0.80 years				RADIOGRAPHIC METHOD ---- MAE of Entire sample = 0.89 years			
		CLINICAL METHOD ---- MAE for male sample = 0.76 years				RADIOGRAPHIC METHOD ---- MAE for male sample = 0.88 years			
		CLINICAL METHOD ---- MAE for female sample = 0.84 years				RADIOGRAPHIC METHOD ---- MAE for female sample = 0.90 years			

* Statistically significant ; SD = Standard Deviation ; MAE = Mean Absolute Error

Accuracy of the Clinically Estimated Dental Age

The clinical method yielded a mean error in the range of -3.16 to 4.07 years among male subjects and -1.83 to 4.32 years in females subjects. The positive result indicates an over-estimation and negative result indicates an under-estimation. The estimated age was considered accurate if it was <±1 year from the chronological age "CA" and was considered inaccurate if it was >±2 years (Table 3).

Accuracy refers to how close the estimated dental age (EDA) is to the chronological age. Theoretically, the difference between the EDA and the chronological age must be zero or close to zero. In recent studies, the statistical tool that has been used to quantify accuracy is Mean Absolute Error (MAE). MAE is the average of all absolute errors. The main objective of MAE is to consider all the observations in the group and make the values unaffected by the extremes. It tells us how large an error we can expect from the predicted values. For example, MAE of 0.80 means that during the clinical application of age estimation the value obtained is likely to have an error up to +/- 0.80 years. At present, MAE is considered to be the best parameter to express the accuracy of any age estimation method.

The mean absolute error for the entire sample by the clinical method obtained was 0.80 years, for male sample was 0.76 years and for female sample was 0.84 years (Table 3).

Results of statistical comparison between Demirjian's radiographic method and chronological age

The mean age estimated by the clinical method for the entire male sample was 14.81 years and for the entire female sample was 15.07 years (Table 1). The maximum mean age difference for males was observed in group XV (20-20.9 years) and for females in group I (6-6.9 years). The minimum difference for both males and females was seen in the group IV (9-9.9 years). In the age groups II - IV (7-9.9 years), a statistically significant ($p < 0.05$) difference were observed in the mean estimated age between males and females (Table 2).

Accuracy of the Radiographically Estimated Dental Age

The radiographic method yielded a mean error in the range of -9.52 to 1.96 years among the male subjects and -10.72 to 2.66 years in female subjects.

The mean absolute error for the entire sample obtained was 0.89 years, for male sample was 0.88 years and for female sample was 0.90 years (Table 3).

Comparison of clinical and radiographic methods

An error of <± 1year was observed in 39.6% of the subjects for whom EDA was predicted by clinical method and in 32.8% of the subjects for whom EDA was predicted by radiographic method indicating that the clinical method a better predictor of age than radiographic method. Even though the clinical method is performing better, in 46.6% of the subjects the EDA is falling within ± 3 years when estimated by radiographic method suggesting radiographic method is efficient in estimating the DA within 3 years (Table 4).

The ages estimated by both the methods were correlated with the chronological age for the entire study population using Pearson's correlation. The p value was set at 0.001.

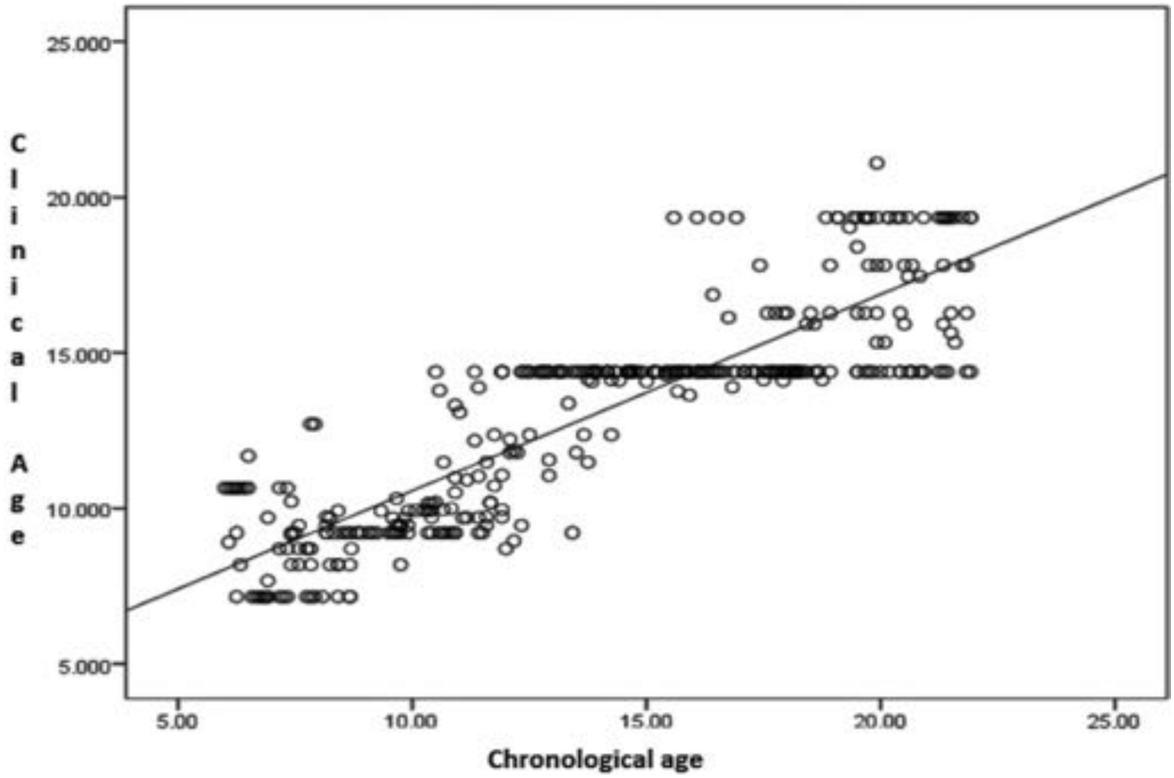
Clinical method correlated positively with chronological age ($r < +1$) for the entire study group and was highly significant ($p < 0.001$). The correlation coefficient was 0.87. The values being greater than 0.80 indicated a strong correlation (Graphs 1).

Similarly, the radiographic method also positively correlated with chronological age ($r < +1$) for entire subjects and was highly significant ($p < 0.001$). However the correlation coefficient was 0.64 for the entire study group. Values were ranging from 0.50 to 0.80 indicating a moderate correlation (Graphs 2).

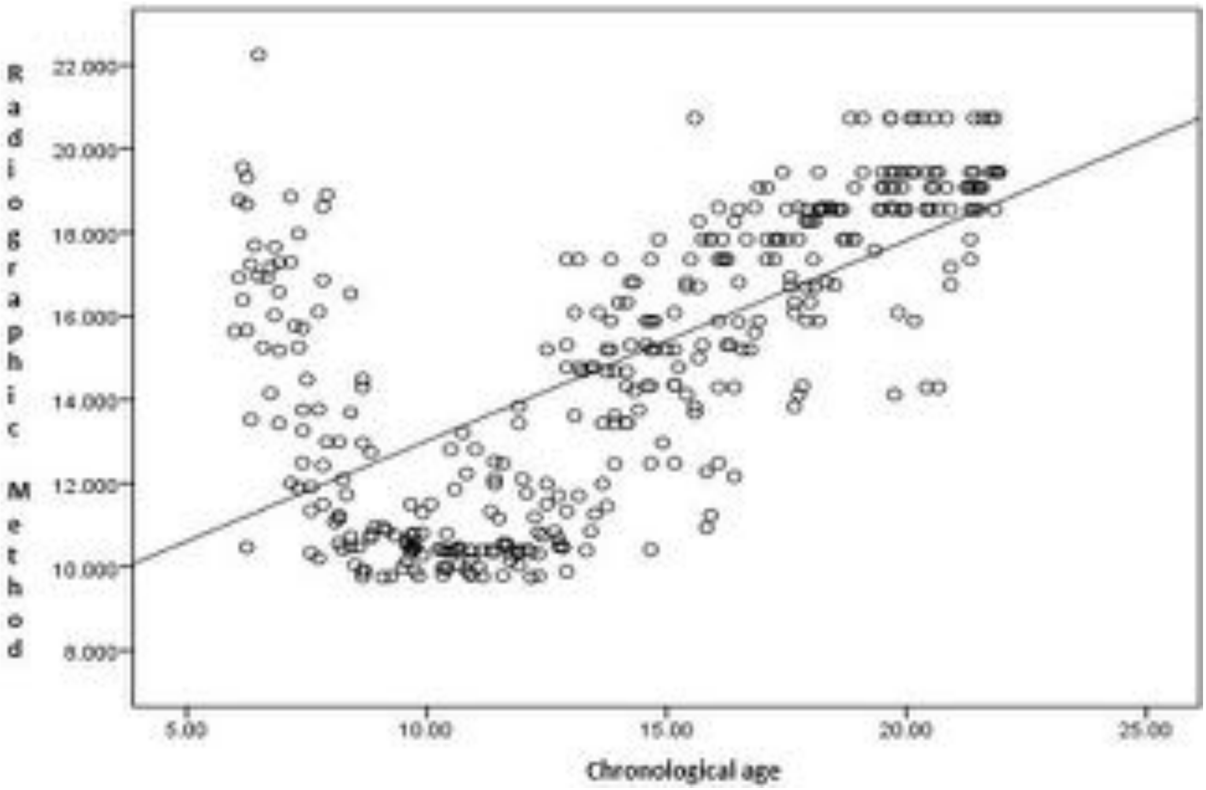
Table 4: Frequency distribution of total sample as per error range for Clinical and Radiographic methods

Error distribution	**≤ ± 1year**	**± 1.01 to ± 3 years**	**≥ ± 3.01 years**
Clinical Method	152	143	89
	39.6%	37.2%	23.2%
Radiographic Method	126	179	79
	32.8%	46.6%	20.4%

Graph 1: Scatterplot depicting the relationship btw CA and EDA by clinical method for entire study group



Graph 2: Scatterplot depicting the relationship btw CA and EDA by radiographic method for entire study group



DISCUSSION

Dental age estimation plays a crucial role in the identification of living and dead subjects.[3] Various physical, chemical and histological methods have evolved over the years to estimate age using the teeth.[16] However, the majority of them result in the loss of physical evidence.

Estimation of age by assessing the stages of tooth development has often been the preferred method as it closely coincides with the chronological age and it can be evaluated using radiographs.

Age estimation by analysing the dental development was done using radiographs. Demirjian's method[8] is one of the oldest and most widely used radiographic method for ascertaining dental age due to its simplicity, pre-set criteria for evaluating tooth maturity, schematic illustrations and gender specific maturity scores. Nevertheless, it has demonstrated significant differences between the predicted and the true age in non-Canadian population. Several authors[5,17] therefore have developed an Indian-specific formula for accurate age prediction in an Indian population. As our study was on an Indian population, we used Demirjian's radiographic method in combination with Acharya's formula for the radiographic method of age estimation.

Next best and non-invasive means of dental age estimation is perhaps the clinical method as proposed by Foti and co-workers wherein the age is estimated by assessing the pattern of teeth eruption. We applied their regression model 2.

Accuracy of Foti's Clinical Method

In our study, Foti's clinical method yielded age estimates for the ages 7 – 15 years with a difference within ± 1 year between estimated and true age (Table 4), which was in concordance with the study by Dinakar et al in a Goan population.[5] But, a negligibly higher error of 1.16 years was observed among female subjects of 11-12 years and 15 -16 years. This over-estimation can be attributed to the early pubertal changes observed in females compared to males.

The error of more than 1 year observed in our study in the subjects younger than 7 years old is comparable with the results observed by Foti et al.[10] When Foti and co-workers[10] validated all of their 4 models on French population aged 6 – 21 years, their DAE using model 2 in the subjects below 10.5 years showed a significant error of >1 year; this could be due to inherent limitation in Foti's Model 2 regression formula, as it does not consider deciduous canines, mandibular incisors, molars and permanent 1st molars, teeth commonly present in a 6 year old child.

Similarly, Foti's clinical method failed to make precise estimates for the age group of 16 – 22 years. An error of 1.06 – 4.32 years in EDA was bound to occur in these age groups (Table 3) as 3rd molars show a diverse eruption pattern in the population of the present generation, most often, remaining occult.

The mean error of 0.92 $\pm$ 1.39 (S.D) years obtained for the entire study sample is comparable with Foti and co-workers' [10] achieved mean error of - 0.47 $\pm$ 1.85 years in their study group.

We found a statistically significant difference ($p<0.05$) in the mean estimated ages between males and females in the age group of 11-11.9 years and 19-19.9 years (Table 2). This difference may be due to the absence of gender specific formulae thus imploring a need to derive gender specific regression formulae.

Accordingly, in our study Foti's method estimated a mean absolute error of 0.80 years for the entire study sample (Table 3). This implies that age estimated by clinical method using Foti's Model 2 regression formula is likely to estimate the chronological age within 1 year difference. Dinakar et al[5] observed a MAE of 2.33 years in a Goan population.[5] The MAE of as low as 0.80 years achieved in our study suggests that Foti's method can predict the age of South Indian (Karnataka) population with greater accuracy and hence can be effectively used during forensic age estimation.

Accuracy of modified Demirjian's method

For the radiographic method of DAE, we chose to use Chaillet and Demirjian's 8-teeth[15] method as our study subjects aged between 6-21 years. It is based on the assessment of calcification stages of mandibular left permanent teeth including 3rd molars using panoramic radiographs. It provides a gender specific dental maturity scoring system and tables for conversion of them to dental ages.[8,15]

As Demirjian's 7-teeth method over-estimated the dental age by 1.2 – 3 years in South Indian population,[18,19] Acharya[14] used Chaillet and Demirjian's 8-teeth method on 547 panoramic radiographs of South Indian population and derived new regression formulae for them. We applied Acharya's regression formulae in our study as the subjects are from Karnataka, South India.

In the present study, radiographic method accurately estimated dental age of 9-19.9 year old subjects with an error less than ± 1 year (Table 3). Similar results were achieved by Mohammed RB et al[20] and Sonali et al.[13]

Although the radiographic method estimated DA accurately in 9-19.9 years old subjects, large variations were observed in estimated dental ages of 6-8.9 year old subjects (Table 3) which is probably due to two reasons: a) The Indian formula that we used was derived from individuals aged 7-25 years and thus the formula is not accurate/applicable for the younger age groups b) The second and most likely reason is perhaps dependent on the assessment of tooth development stage (TDS) during age assessment. Under-estimation of DA is directly related to the lower scoring of the TDS. As per the criteria laid down by Demirjian for TDS, a lower score has to be assigned to the calcification stage when in doubt.[8] In individuals of 6-9 years, the mandibular anterior teeth will be in different stages of calcification. Further, the use of panoramic radiographs will have variable degree of cervical spine shadow superimposition over the mandibular anterior region. These inherent limitations may result in under-scoring thus influencing TDS and calculated age.

In the age group of 20-21 years, we observed an over-estimation of DA in both genders with an error of 1.72 – 2.66 years (Table 3). This may be because of genetically influenced early maturation of 3rd molars leading to score 9 or the interplay of environmental effects such as nutrition and diet. Acharya in his study also observed over-estimation in this age group.[14]

In the age groups of 7-8.9 years and 21-21.9 years, we observed a significant difference ($p<0.05$) in the estimated ages of males and females (Table 3). Though the formulae that we used for radiographic age assessment are gender specific, they have been derived by applying the French weighted maturity scores. This necessitates the calculation of maturity scores specifically for an Indian population.

Our study estimated the MAE to be 0.89 years for the entire sample (Table 3) which implies that in any clinical situation during forensic investigation, the age estimated for a South Indian population by Chaillet and Demirjian method using Acharya's formula is likely to estimate the chronological age within 1 year difference. Our results are in concordance with the results of Acharya[14] and Sonali[13] et al on a South Indian population and significantly lower when compared to the MAE in a Goan population. Their higher MAE of 2.33 could be due to the variation in the sample population and the age groups of the subjects included in their study.

Comparison of the Foti's clinical method and Modified Demirjian's radiographic method

The clinical method was able to accurately assess dental age in 39.6% of the population when compared to 32.85% by radiographic method. By considering the error rate of ± 1 year as accurate, this comparison suggests that the clinical method is a better predictor of dental age than the radiographic method (Table 4).

To the best of our knowledge, ours is the first study to compare the accuracy of clinical and radiographic methods in age assessment of a South Indian population.

The clinical method was accurate in DA assessment of subjects in the age range of 7-15 years. The radiographic method accurately assessed the DA of 10-20 years old subjects (Tables 3).

In a study on a Goan population,[5] DA estimated by using Foti's regression model 2 as well as Acharya's formula was accurate in 44% of their population. Although in our study, the clinical method was a better predictor of age than the radiographic method, the accuracy achieved among our population is less when compared to Goan subjects. This variation reflects the racial differences in the tooth maturation and emphasises the necessity to derive population specific regression formulae. This difference could also be the result of using radiographs to estimate DA by Foti's regression model[5] 2 since it seems inappropriate to predict the duration required for the eruption process by radiographs. When Pearson's correlation statistical test was applied to our study population, the DA estimated by clinical method showed strongly positive association with true age and this correlation was highly significant ($p<0.001$) with correlation coefficient of 0.87 years (Graph 1). These results are at parity with the correlation coefficient of 0.86 achieved by Dinakar et al.[5]

Our values are also comparable with the correlation coefficient of 0.78 observed by Foti & co-workers' [10] in their study on a French population. Assessment of association between the radiographic method of DAE with the CA also indicated a highly significant ($p<0.001$) positive correlation (Graph 2). However, with a coefficient of 0.64 the values expressed a moderate correlation between the two.

The results of our study imply that clinical method of age estimation (Foti's method and formula) provides an age estimate that is on average more accurate (MAE = 0.76 years for males and 0.84 for females) than the radiographic method (Demirjian grades and scores assessed using Acharya's Indian Formula) (MAE = 0.88 years for males and 0.9 years for females) in the population of Karnataka, India.

CONCLUSION

Clinical method is a better predictor of age than the radiographic method. But the difference between the two may be considered as practically small implying that either of the methods can be employed in clinical practice. However, one must be judicious while extrapolating the observations made from the current study to the entire South Indian population as there exists an enormous amount of genetic admixture and cultural diversities which necessitates population and sub-population specific studies.

In future, there is a need to derive gender-specific regression model 2 for an effective application of Foti's clinical method in age assessments. Further, the maturity scores specific for an Indian population need to be studied and regression formulae derived in order to improve the predictive accuracy of the radiographic method.

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Colour stability of dental restorative materials submitted to conditions of burial and drowning, for forensic purposes

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Human identification, Forensic Odontology, Dental Materials, Colour stability

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ABSTRACT

The aim of this study was to evaluate the effect of earth and water on the colour stability of tooth-coloured dental restorative materials: composite resin (CR) and glass ionomer cement (GIC). Aiming to distinguish between one and another tooth-coloured material and to estimate the period in which they could be submitted to the factors earth and water, the proposed method may contribute to the proceedings of human identification of victims of burial and submersion in water. Forty bovine incisors were prepared (6 x 6 x 2mm) and restored with CR Filtek™ Z250 XT (3M ESPE™) and GIC Ketac™ Fil Plus (3M ESPE™). After initial colour read-outs (VITA™ Easyshade spectrophotometer), the samples were separated into two groups (n=10), according to the conditions to which they were submitted: simulations of burying and submersion in water, for periods of 1, 3, 6 and 12 months, when new read-outs were taken. The values of colour change (ΔE, ΔL^*, Δa^*, and Δb^*) were subjected to 3-way ANOVA statistical analysis, repeated measures, Bonferroni ($p<.05$), and it was verified that both factors produced colour changes in the restorative materials, which were higher for glass ionomer cement ($p<.05$) after 12 months of burial, and 6 months of submersion in water. The authors concluded that the analysis of colour change in the material contributed to the forensic odontology casework depending on the time during which the victim was submitted to the condition of burial or submersion in water.

INTRODUCTION

Floods are often cited as being the most lethal of all natural disasters; during the twentieth century, floods killed at least eight million people.[1] On December 26, 2004, a major earthquake and the resulting tsunami killed more than 230,000 people in 12 countries on the shores of the Indian Ocean.[2-4] The devastating consequences of that event have led to the most significant international effort undertaken so far to identify victims of natural disasters.[4] On January 2011, a devastating tropical storm hit the mountain area of Rio de Janeiro State in Brazil, resulting in flooding and mud slides and there were 845 immediate deaths. One year after the disaster, the number of dead and missing persons was estimated to be 1,300.[5]

In such cases, the search and rescue of victims may require extended time due to the local climate and geographical conditions.[6-8]

Also, bodies may be entirely or partially decomposed or fragmented, making the proceedings of human identification a challenge for the forensic expert teams.[9-12] Given the poorly preserved remaining tissues or lack thereof, the feasibility of fingerprint analysis can be forgone[7, 8, 13, 14].

The durability of teeth is a feature that makes forensic dentists regular participants in forensic investigation[3, 15]. The teeth can stand up to most post mortem events that can disrupt or alter other body tissues.[10, 11, 13] Teeth are often the only findings that can be analyzed and, in these cases, Forensic Odontology becomes the most viable, practical, fast, if not the only possible identification method available.[9, 12] If there are any dental restorations, the restorative dental materials are exposed to the same agents as the teeth, for example, earth and water. Thus, these agents cannot destroy the teeth but could modify the dental restorative materials.

Restorative dental materials are subject to intrinsic changes over time; moreover, their physical and mechanical properties characterize and distinguish them from the others.[16] Studies have been carried out with the aim of identifying changes occurring in dental materials when subjected to extraordinarily high[7, 17] or low[8] temperatures; but to date, there have been no studies on the changes caused by earth and water in these materials. Among the possible changes in the physical and mechanical properties of restorative dental materials, the colour stability has been studied, to establish which agents are responsible for the changes, as well as to estimate the period during which the materials were exposed to such conditions.[7, 8]

In this context, the authors considered it essential to carry out an experimental study to learn more and improve the knowledge base about the changes that restorative materials undergo when exposed to the effect of the earth and water. Thus, this study may contribute to the human identification proceedings of forensic odontology teams involved in Disaster Victim Identification (DVI) cases. Thus, the aim of this study was, by the simulations of burial and submersion in water, to evaluate the effect of earth and water on the colour stability of the dental restorative materials most commonly used in the daily dental practice.

MATERIALS AND METHODS:

Forty sound bovine incisors were restored in the central region of the buccal surface and randomly separated into two groups according to the restorative material used (Table 1). After restoring the teeth, the first colour read-outs were taken, using a portable spectrophotometer (Easyshade™, VITA, Bad Säckingen, Germany). Three colour read-outs were taken, according to the CIE $L^*a^*b^*$[18] scale, and the mean of these values was considered the initial value. The CIE $L^*a^*b^*$ scale (Commission Internationale de L'Eclairage) consists of three Cartesian coordinates in which L^* indicates lightness, a^* indicates the green-to-red shade, and b^* indicates the blue-to-yellow shade.[18]

The teeth restored with each material were randomly separated into groups (n=10), according to the environment to which they were submitted: burial or submersion in water. To simulate the burying environment, the teeth were inserted into an excavated 4 feet deep hole, and covered by earth. For the simulation of submersion in water, the teeth were inserted into a nylon bag with open and reticular wefts and chained from a fixed point on the shore of a lake and then submerged into that lake within a bird cage.

After periods of 1, 3, 6 and 12 months, the samples were withdrawn from the environmental conditions and new colour readings were performed by the same operator who carried out the initial readings. The colour stability of the restorative materials was calculated by the formula:[18]

$$\Delta E = \sqrt{(\Delta L^*)^2 + \Delta a^*)^2 + (\Delta b^*)^2}$$

Where $\Delta L^* = L^*_f - L^*_i$; $\Delta a^* = a^*_f - a^*_i$; $\Delta b^* = b^*_f - b^*_i$, being L^*_i, a^*_i e b^*_i referred to as the initial read-outs, and L^*_f, a^*_f e b^*_f as final read-outs for colour coordinates. The colour values (ΔE) and the changes in the coordinates (ΔL^*, Δa^* and Δb^*) were analyzed according to 3-way ANOVA, repeated measures, Bonferroni, $p<.05$, to compare the materials, the environments and the periods tested.

Table 1: Materials used, commercial brands, manufacturers, colour and method of restoration.

Category	Brand Name	Manufacturer	Colour	Method of restoration (Clinical steps)
Composite resin	Filtek™ Z250 XT	3M ESPE™, Sumare, SP, Brazil	A3	1. Acid etch (37% phosphoric acid, Alpha Etch DFL™, Rio de Janeiro, RJ, Brazil) for 15 seconds, washing, and drying; 2. Bonding system application (Adper Single Bond 2, 3M ESPE™, Sumare, SP, Brazil) and light curing (Ultralux EL, Dabi Atlante™, Ribeirao Preto, SP, Brazil) for 10 seconds; 3. Material insertion in increments and light curing for 20 seconds; 4. Finishing and polishing (flexible discs Sof-Lex™ Pop-On, 3M ESPE™).
Glass ionomer cement	Ketac™ Fil Plus	3M ESPE™, Sumare, SP, Brazil	A3	1. Powder/liquid (1:1) agglutination up to 1 minute; 2. Material application in increments until the cavity filling.

RESULTS

The comparisons of the ΔE mean values are shown in Table 2. There was no statistically significant difference ($p>.05$) in colour change for composite resin (CR), irrespective of the period that the samples were submitted to the agents. The most significant ($p<.05$) change for the glass ionomer cement (GIC) occurred after 12 months, in both environments. Water produced a higher change ($p<.05$) than earth, after 6 months for CR and GIC, and 12 months for GIC (Fig. 1).

The comparisons of the ΔL* mean values are shown in Table 3. Both materials lost luminosity when submitted to submersion in water for 1 month, a different result ($p<.05$) in comparison with the same period of burial. For GIC, the most significant change ($p<.05$) was verified after 12 months of submersion in water. The most significant difference ($p<.05$) between CR and GIC occurred after 1 month of burial, and 3 months of submersion in water (Fig. 2).

The comparisons of the Δa* mean values are shown in Table 4. There was significant difference ($p<.05$) for the CR submerged in water for 12 months, and for CR after 6 months, and GIC after 12 months when submitted to both environments. The most significant difference ($p<.05$) between CR and GIC occurred after 1 month of burial, and 1 and 12 months of submersion in water (Fig. 3).

The comparisons of the Δb* mean values are shown in Table 5. There was a higher change in both materials after 6 months of burial and submersion in water, with statistically different results ($p<.05$) for CR. The most significant difference ($p<.05$) between CR and GIC occurred after 1 month of burial, and 1 and 6 months of submersion in water (Fig. 4).

Representative photographs of the colour changes, before and after the submission to the proposed tests, are shown in Figs. 5 and 6. It was verified a change in the colour of CR after 1 month of burial, remaining stable after 3 months. After 6 and 12 months, new colour changes were observed. Regarding GIC, despite the apparent loss of brightness after the first month, the most significant colour changes were seen after 3 months and were maintained until 12 months of burial. A significant change in the CR colour was observed after 1 month of submersion in water, remaining stable after 3 months. The highest change was verified after 6 months, looking much clearer in relation to the other periods. Concerning GIC, there was a permanent darkening after 1 and 3 months, but the most significant change in the colour was observed after 6 months, the restoration was very whitish.

Table 2: Comparison of means of ΔE (standard deviation) of the agents, in different periods, for the same material.

Material	Periods (months)	Earth	Water
CR	1	3.08 ±0.69 aA	3.64 ±1.55 aA
	3	1.59 ±0.52 aA	2.07 ±0.73 aA
	6	4.01 ±1.13 bA	5.28 ±1.52 aA
	12	2.31 ±0.84 aA	2.17 ±0.93 aA
GIC	1	2.94 ±1.41 aB	7.23 ± 3.82 aC
	3	4.60 ±3.99 aB	4.96 ± 2.50 aC
	6	5.24 ±3.68 bB	12.30 ±7.50 aB
	12	12.17 ±6.61 bA	24.07 ±5.42 aA

Different letters, lower case letters on the line and capital letters in the column indicate statistically significant results (p<.05).

Figure 1: Graphic representation of the comparison of ΔE between CR and GIC. Horizontal lines above the bars indicate statistically significant results ($p<0.05$).

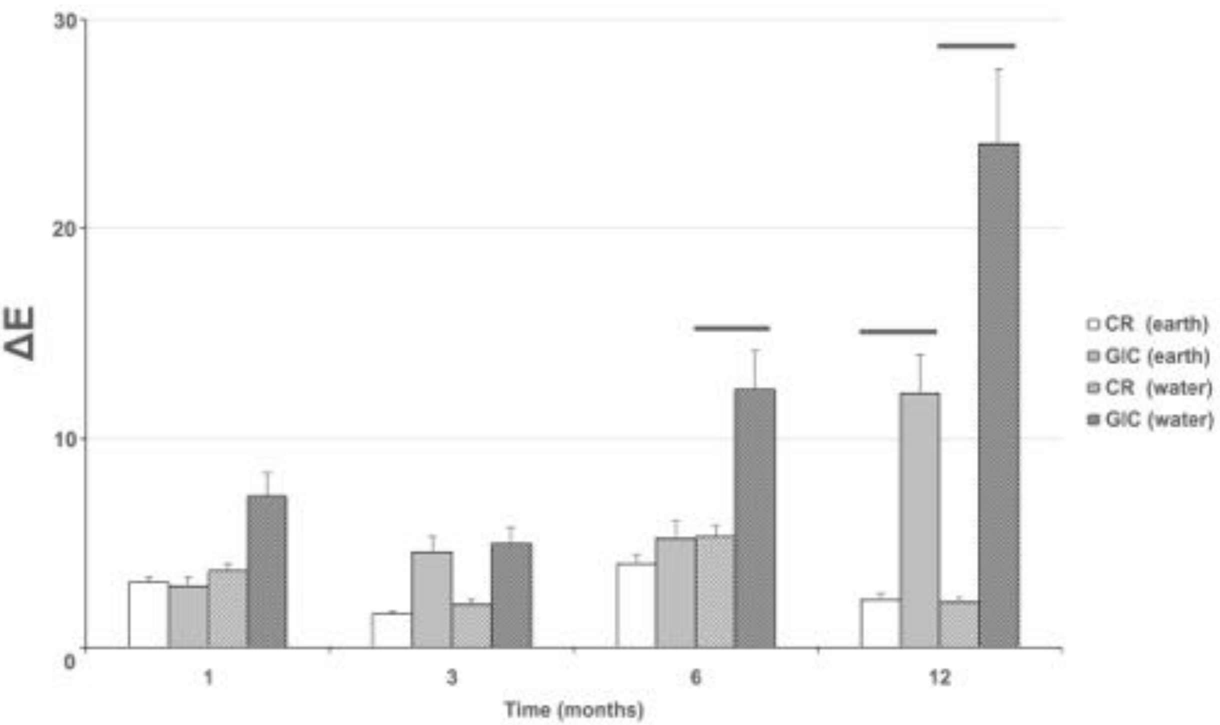

Table 3: Comparison of means of ΔL* (standard deviation) of the agents, in different periods, for the same material.

Material	Periods (months)	Earth	Water
CR	1	1.19 ±0.93 bA	-1.88 ±1.53 aA
	3	0.27 ±0.70 aA	0.48 ±1.17 aA
	6	1.58 ±1.02 aA	1.53 ±0.95 aA
	12	0.35 ±0.82 aA	-0.10 ±0.89 aA
GIC	1	0.00 ± 2.71bc	-6.87 ± 4.00 aC
	3	-3.76 ±4.41 aB	-3.86 ± 3.24 aC
	6	-2.96 ± 4.42 bBC	-11.37 ±7.88 aB
	12	-11.62 ±6.54 bA	-23.44 ±5.84 aA

Different letters, lower case letters on the line and capital letters in the column indicate statistically significant results (p<.05).

Figure 2: Graphic representation of the comparison of ΔL* between CR and GIC. Horizontal lines above the bars indicate statistically significant results (p<0.05).

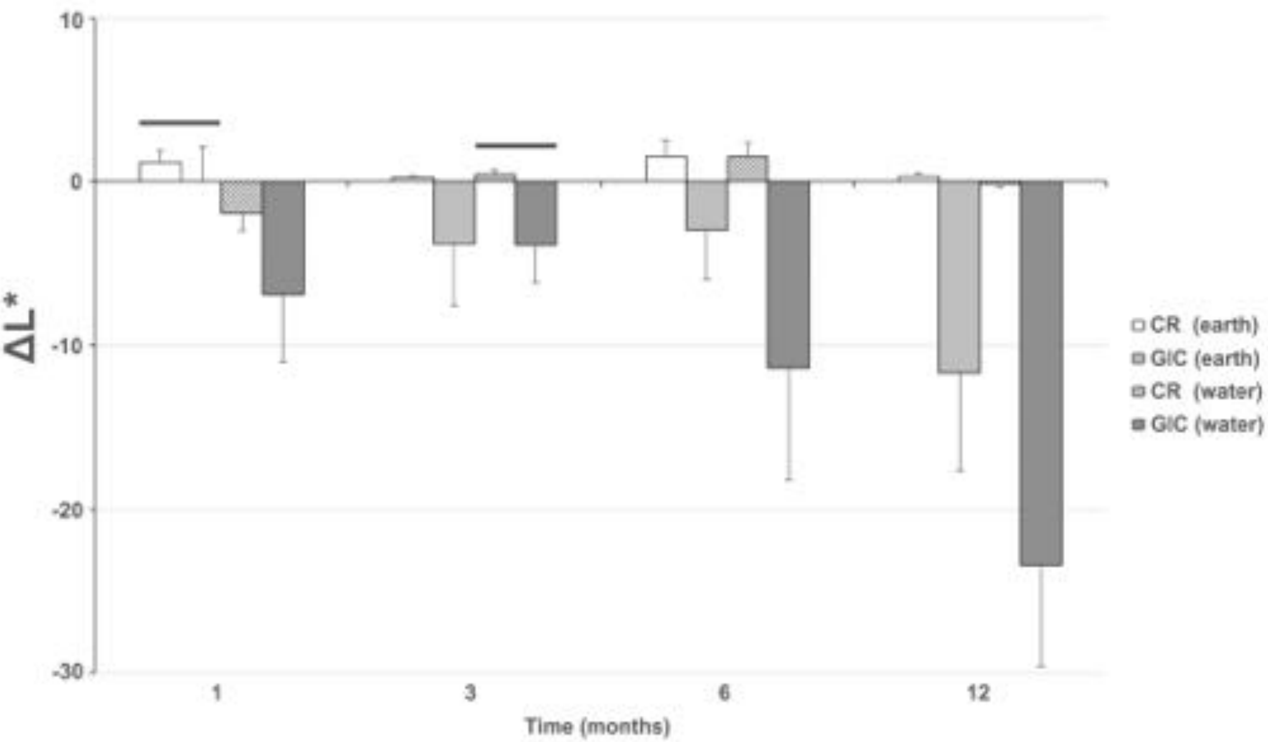

Table 4: Comparison of means of Δa* (standard deviation) of the agents, in different periods, for the same material.

Material	Periods (months)	Earth	Water
CR	1	1.98 ±0.26 aA	1.51 ±0.47 aB
	3	1.18 ±0.41 aB	1.23 ±0.33 aB
	6	2.34 ±0.48 aA	2.36 ±0.57 aA
	12	1.76 ±0.51 aAB	1.17 ±0.58 bB
GIC	1	0.01 ± 0.68 aC	0.40 ± 0.61 aC
	3	0.31 ± 0.87 aC	0.67 ± 0.84 aC
	6	1.34 ±1.77 aB	1.77 ±0.99 aB
	12	2.60 ±1.74 aA	3.27 ±0.96 aA

Different letters, lower case letters on the line and capital letters in the column indicate statistically significant difference (p<.05).

Figure 3: Graphic representation of the comparison of Δa* between CR and GIC. Horizontal lines above the bars indicate statistically significant results (p<0.05).

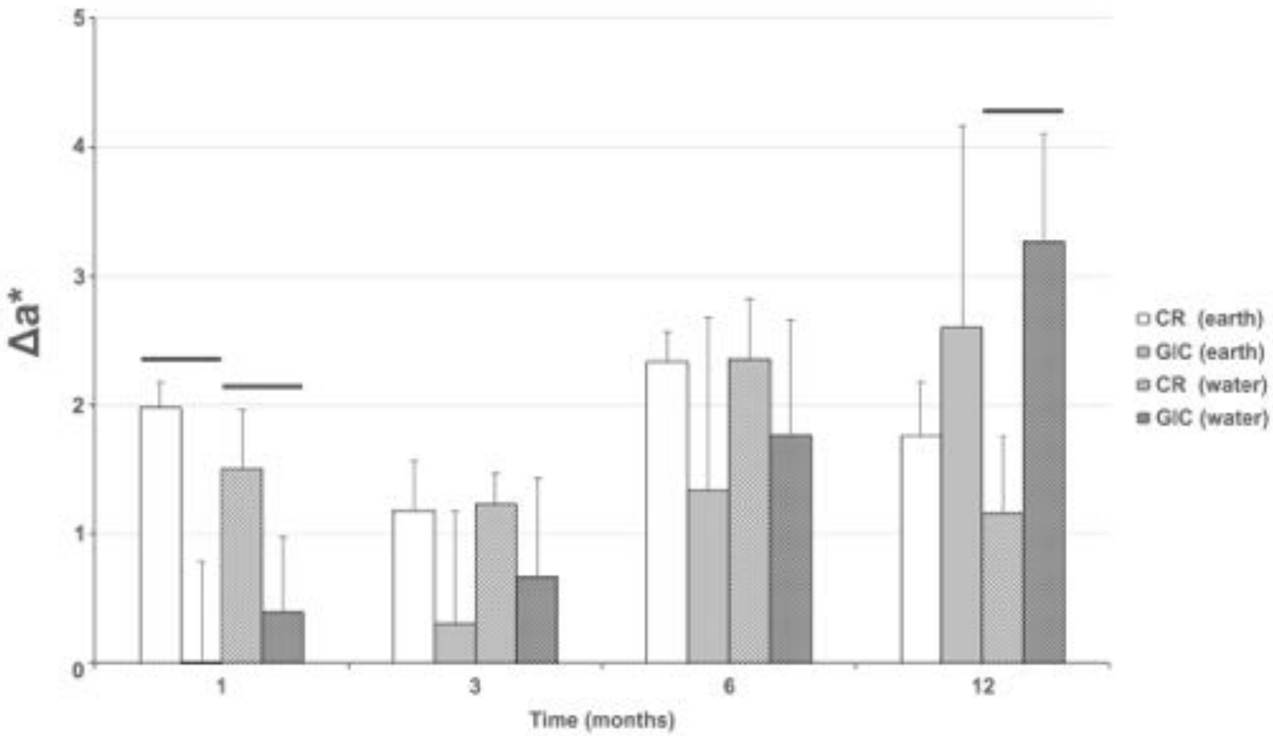

Table 5: Comparison of means of Δb* (standard deviation) of the agents, in different periods, for the same material.

Material	Periods (months)	Earth	Water
CR	1	1.85 ±0.62 aAB	2.38 ±1.35 aB
	3	0.36 ± 0.81 aC	1.15 ±0.64 aB
	6	2.70 ±0.96 bA	4.42 ±1.28 aA
	12	1.10 ±0.87 aBC	1.31 ±1.23 aB
GIC	1	-1.16 ± 1.50 aC	-1.64 ±0.85 aB
	3	-0.97 ± 1.53 aC	0.89 ±2.12 aA
	6	1.43 ±2.72 aA	1.66 ±3.34 aA
	12	0.34 ±2.17 aB	0.17 ±3.99 aAB

Figure 4: Graphic representation of the comparison of Δb* between CR and GIC. Horizontal lines above the bars indicate statistically significant results ($p<0.05$).

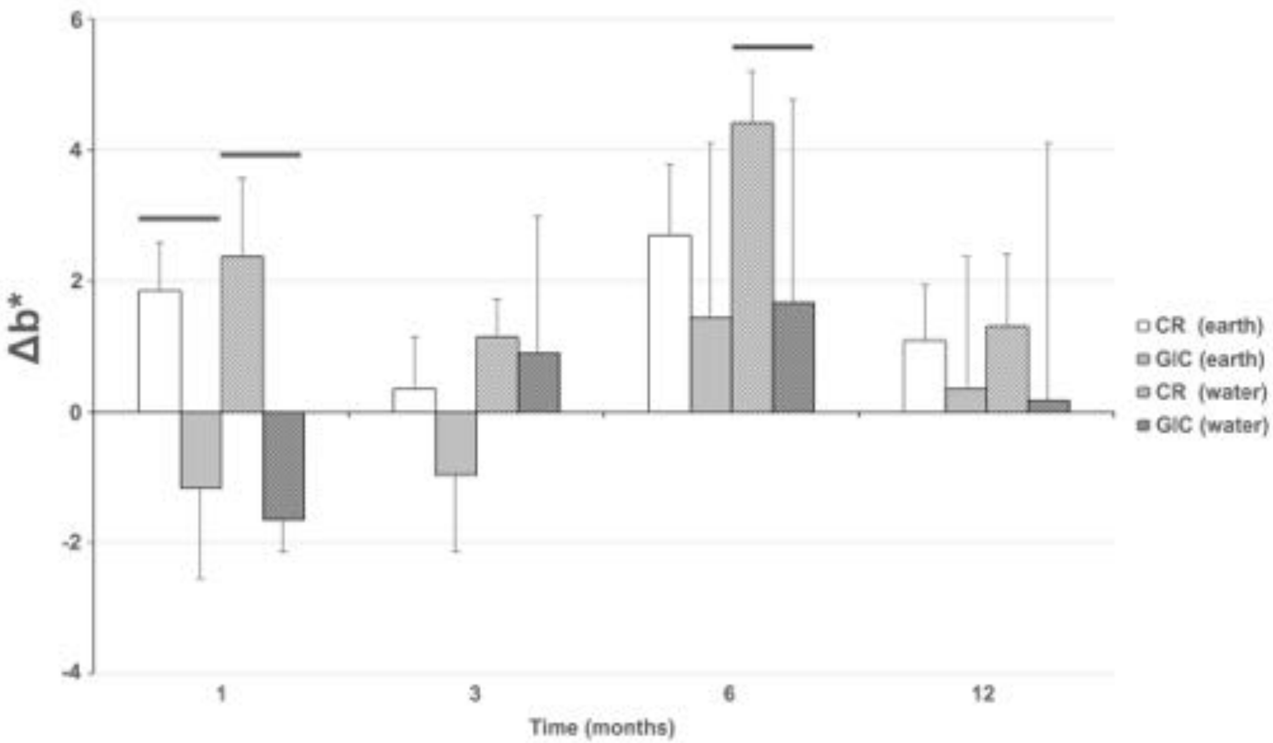

Figure 5: A photographic comparison of the restorations of CR (above) and GIC (below) submitted to different periods of burial.

Figure 6: A photographic comparison of the restorations of CR (above) and GIC (below) submitted to different periods of submersion in water.

DISCUSSION

In cases of a delayed discovery of a body, it is not possible to estimate the time of death by analysis such as cooling the body, cadaveric rigidity or hypostasis, for example.[19] However, the study of the physical and mechanical properties of the materials is relevant to distinguish between them and hence to assist in the identification procedure of victims.[7, 8]

The analysis of the colour change of the dental restorative materials, at different periods, may contribute to the forensic expert works, when the victims are found buried or submerged in water, providing information that allows distinguishing the materials found in the oral cavity. Such discrimination may help in the comparison between the ante-mortem information (in the dental records of the subject, if any) with the post-mortem information, collected and reported by the forensic odontologists.

Colour matching is a routine procedure in daily dental practice, and it is critical to the success of the restoration.[20] Therefore, this information must be recorded on the patient's chart. Moreover, dentists have a medico-legal obligation and a social responsibility to exercise great care in the documentation of the treatment procedures[21]. The quality of the ante-mortem dental records, which serve as evidence, is very important in forensic work and deficient ante-mortem charting could hamper forensic odontology casework.[2, 12, 21] Records with accurate and complete data are obviously much more likely to be matched.[3]

Thus, this study aimed to evaluate the effect of earth and water on the colour stability of the dental restorative materials most commonly used in the daily dental practice.[22] The authors started with the null hypothesis that the agents would not produce changes in the colour stability of each material, irrespective the period; and that the differences between the materials could distinguish between them. The results indicated that the null hypothesis could not be accepted because there were significant ($p<.05$) changes in the colour of the materials. However, the second hypothesis can be accepted because that difference allows distinguishing between the materials, according to the method proposed.

The colour changes were analyzed using a portable spectrophotometer, that is the most accurate and reliable method for this type of analysis because it does not allow for subjective evaluations and avoids errors in the colour interpretation. The instruments are more accurate than the eyes and the inconsistent perception of colour by the observers.[23]

Bovine teeth were used as the substrate for the restorations, because of the bioethical issues.[24, 25] They show similarities with the human teeth, as the direction of the enamel prisms, an equivalent percentage by weight of calcium, and protein matrix formed by the same amino acids. [24] The literature shows that bovine dentine is feasible for adhesion, providing adequate bond strength when compared to human dentine.[25] Moreover, this study evaluated changes occurred in the restorative materials, not dental structures.

The periods established for the analysis of this study (1, 3, 6, and 12 months) were determined after other studies showed that the majority of missing corpses were found up to 1 year after their disappearance.[26, 27] Furthermore, the Council of Europe[28] recommends that when the circumstances of a person's disappearance are reasonable to conclude that death is likely, searches must be closed within a maximum of one year after the disappearance, as it was done in the tragedy that occurred in Rio de Janeiro, Brazil, in 2011.[5]

The results showed that composite resin (CR) had significantly different colour changes ($p<.05$) than GIC after 1 year of burial and 6 months of submersion in water. Characteristics of the chemical structure of the composites, as well as the composition of the resin matrix, may interfere with their colour stability.[16] The resin matrix is susceptible to water penetration, which is associated with the discolouration of the composite, so that the greater the percentage of resin matrix in its composition, the greater the possibility of colour change.[29]

The composition of the resin matrix can also have an effect on water absorption because the monomer TEGDMA absorbs more water than UDMA, which in turn absorbs less water than Bis-GMA.[29] The higher predisposition to water absorption of the monomer TEGDMA increases the solubility of the formed polymer, giving these composites less colour stability when combined the monomers Bis-GMA and TEGDMA, due to the increase in the free volume of the formed polymer and, hence, the larger room for the water molecules to diffuse into the polymeric structure. [29] But the hydrolytic degradation reaches its limit with the saturation of the polymer network, and the structure of the composite achieves stability. Thus, the changes in mechanical properties cease, and no longer change over time.[16, 29]

The glass ionomer cement (GIC) is synthesized by an acid-base setting reaction between the polyacrylic acid and the calcium aluminum fluorosilicate glass particles. The resulting product is glass polyalkenoate. It presents a slow setting reaction and initial sensitivity to water loss (syneresis) and gain (soaking).[30] Thus, because it is susceptible to imbibition caused by submersion in water, GIC may present a more significant colour change, as the results showed. There was also significant colour change when the material was buried, especially when compared to CR.

The comparison of the coordinates (L^*, a^*, and b^*) allows analyzing the contribution of each of them to the total colour change of the samples (ΔE). The L^* value, lightness indicator, increased after burying the CR, showing that the restorations became clearer at any period. For the GIC, this value decreased, suggesting a loss of luminosity and consequent darkening of the restorations. When subjected to the action of water, both materials lost luminosity, mainly the GIC, because of its susceptibility to imbibition.[30]

The a^* coordinate values increased, suggesting saturation of the red hue, verified by the changes in CR, up to 6 months, and in GIC, after 12 months. Possibly, this result was caused by the earth colour of the burial place. In turn, the change in the b^* coordinate was positive for both materials, what explains the yellowing of the restorations.

Distinguishing between the tooth-coloured restorative materials would be one of the possibilities in which this study could contribute to forensic works of human identification. Thus, the results allow us to state that the statistically significant colour change ($p<.05$) is a determining factor for this discrimination.

Another significant contribution would be to provide information about the periods in which the materials were submitted to the action of earth and water, and thus help with the estimation of the time lapse between death and the necroscopic examination of the victim. Thus, this study could correlate the existence of dental remnants with a chronology of the thanatological changes, becoming a practical and accessible tool to help in establishing the medical and legal causa mortis. It could also provide clues as to whether the corpse suffered ante-mortem or post-mortem wounds or displacements, thus contributing to the investigations and even positively identifying or excluding suspects. The results show that the colour change (ΔE) of the materials is time-dependent, that is, the longer the agent acts, the greater the discrimination potential of the materials, from 6 months. This is justified by the inherent properties of each material, described above.

CONCLUSION

The authors concluded that burial and submersion in water produce changes in the colour of the tooth-coloured restorative dental materials, contributing to Forensic Odontology in procedures of human identification, depending on the period that the victim was submitted to these conditions.

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Radiographic evaluation of dental and cervical vertebral development for age estimation in a young Brazilian population

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ABSTRACT

Age estimation is guided by the evaluation of events that happen during the processes of bone and dental development. The purpose of this study was to validate the method of age estimation proposed by Lajolo et al. (2013) through oro-cervical radiographic indices in Brazilians. The study aimed to verify the effectiveness of age estimation equations through dental and cervical vertebrae examinations, in addition to including dental and cervical vertebrae data in new age estimation equations. The sample consisted of panoramic radiographs and teleradiographs from 510 subjects (8-24.9 years). Age estimation methods were applied by assessing the development of seven mandibular teeth, cervical vertebrae and third molars. Techniques used previously have been combinations of radiographic indices: Oro-Cervical Radiographic Simplified Score (OCRSS) and Oro-Cervical Radiographic Simplified Score without Wisdom Teeth (OCRSSWWT). In the second phase of the study, dental maturation, vertebral measurements, and real age were estimated by regression equations. OCRSS and OCRSSWWT had success rates of 67.4% (R^2=0.64) and 70.8% (R^2=0.62), respectively. When age estimation equations for tooth evaluations were applied, the average error was 1.3 years, and for cervical vertebrae measurements, the error was 1.9 years. When dental variables and the measurements of cervical vertebrae were included, the average error of equations was 1.0 year. Radiographic indices were easy to perform, and after adequate training, are reliable and can be used in forensic practice. The use of the new equations presented in this study is recommended because including cervical vertebrae and dental data provides greater accuracy for age estimation.

INTRODUCTION

In forensic sciences, estimating the age of living individuals and corpses represents a challenge with significant anthropologic, social, penal, and civil implications. Age estimation supports human identification, provides information of ancestors of a population and assists in situations of birth certificate missing, questionable documentation, resolution of cases involving adoption, criminal responsibility and asylum application. Over the past few years, age estimation cases have become increasingly frequent and relevant worldwide, as economic globalization, associated with increasing migrations related to socio-political issues, has led to a major demand for age estimation in living individuals with no official documentation, particularly children and adolescents.[1,2] To this end, it is necessary to develop highly accurate and reliable non-invasive methods.

Given the current global context, there is an increase in the number of requests for medical and dental experts to establish an individual's chronological age by using age estimation techniques.[3,4] Methods employed are based on determining biological age through the evaluation of teeth and bone structure development.

Tooth eruption and mineralization are widely studied parameters in radiographic images and are successfully applied to estimate chronological age. When tooth development is finishing, dental age estimation becomes much more difficult. The third molar mineralization is the last option of age estimation by teeth, however variability of third molars morphology is usual and it is often an absent tooth. In addition to teeth, modification in the size and shape of cervical vertebrae from birth to bone maturity is an important indicator of bone development, which can be evaluated using lateral teleradiography.

Despite the various studies and age estimation methods already published, there is still great variability between estimated and chronological ages.[5] In this context, association between age estimation methods – dental and bone – leads to better results, the verification of possible discrepancies, and the evaluation of developmental differences between sexes.[6]

To improve results obtained by age estimation studies, research that assesses the developmental stages of different human body structures, and not only teeth, can potentially contribute to a greater reliability of age estimation through x-ray analysis, which is commonly requested in clinical practice. In the pilot study carried out by Lajolo et al. (2013) in Italian caucasians,[5] two new oro-cervical radiographic indices – oro-cervical radiographic simplified score and oro-cervical radiographic simplified score without wisdom teeth – were developed to facilitate, expedite, and increase age estimation reliability.

Given the relevance of assessing more than one anatomical structure and considering the possibility of technical improvements to achieve better results, as well as the importance of validity and reliability tests for age estimation methods in different population groups, the first phase objectives of this study consisted of validating an age estimation method using oro-cervical radiographic indices in a Brazilian mixed population without definition of a specific race, as well as assessing the relationship between chronological and estimated age by using the indices. In the second phase, this study assessed the effectiveness of regression equations for age estimation through the dental examinations described by Chaillet & Demirjian[7] and the equations to estimate age by examining cervical vertebrae developed by Caldas et al.,[8] in addition to devising new equations by including dental records and cervical measurements from previous methods.

MATERIALS AND METHODS

This research was submitted and approved by the Ethics and Research Committee of Faculty of Dentistry of University of São Paulo, and was approved by opinion No. 754.502.

The study sample consisted of a total of 1020 radiographic images (510 panoramic radiographs and 510 lateral teleradiographs) from 510 Brazilian individuals aged between 8 and 24.9 years, stratified by sex (female and male) and age group, a total of seventeen groups, each one with the same number of female and male. All radiographic images were obtained previously at Rizzo Clinic – Digital Dental Radiology – located in the city of Feira de Santana (Bahia) – for clinical diagnosis and orthodontic treatment reasons. Panoramic radiographs and teleradiographs were selected from each participant, simultaneously gathered from the collection of images and assessed in this study exclusively for the purpose of age estimation.

Use of oro-cervical radiographic indices – Phase I

In order to apply the radiographic index methodology proposed by Lajolo et al.,[5] the following age estimation methods were initially applied: Demirjian's Method (DM)[9] to estimate the age of the seven lower teeth from the left side; the Cervical Vertebral Maturation Method (CVM);[10] and the Demirjian method modified by Mincer et al.[11] to estimate the age of third molar development (TMD). Following the evaluation of the radiographs and the application of the previously described methods, the two oro-cervical radiographic scores proposed by Lajolo et al.[5] were applied. The Oro-Cervical Radiographic Simplified Score (OCRSS) is based on the sum of the scores adopted for the three previously applied techniques. The second index – Simplified Oro-Cervical Radiographic Score Without Third Molar (OCRSSWWT) – is derived from the combination of the first two methods mentioned above.

Therefore, the sum of the values for the simplified scores obtained for each method corresponds to the radiographic index scores. Based on the simplified values of the oro-cervical radiographic scores, the study sample was divided into three (3) groups (A, B, and C), which represented the increase in chronological age. Group A includes individuals aged 8-13.9 years, group B includes adolescents aged 14-17.9 years, and group C includes individuals aged 18-24.9 years (Figure 1). In order to compare the classification generated by the scores, chronological ages of the studied population were also distributed into groups A, B, and C to allow for the assessment of the accuracy rate of the scores provided by the radiographic indices.

Use of regression equations for age estimation – Phase II

For age estimation using equations, the model established by Chaillet and Demirjian[7] was used. Eight lower teeth from the left side (central incisor to the third molar) were assessed and a score was given for the maturation of each tooth according to tables recommended by the technique. Next, the scores of each of the 8 teeth were summed to obtain a Total Maturity Score (S). The S value was used in regression formulae and chronological ages were estimated for all study participants.

To estimate age through cervical vertebrae using the equations by Caldas et al.,[8] digital lateral teleradiographs were analyzed using the ruler tool in Adobe Photoshop CS6 (Adobe Systems Inc., USA) and, according to the technique, the following measurements were carried out: anterior height of the vertebral body (AH), vertebral body height (H), posterior vertebral body height (PH), and antero-posterior vertebral body length (AP), in the third and fourth cervical vertebrae. After all measurements were taken, regression equations were applied, and chronological ages were estimated for all study patients.

Reliability assessment and statistical analysis

Prior to the analysis of the radiographic images, examiners underwent a period of study and improvement of techniques. The inter-examiner concordance test used a blind methodology and two independent and experienced examiners to apply the age estimation methods.

The data for statistical calculations were entered in Microsoft Excel (Microsoft Co., Redmond, USA) and analyzed using MedCalc v16.4.3 (MedCalc Software, Ostend, Belgium). To assess the reliability of radiographic indices, the Kappa coefficient was calculated for intra and inter-examiner data. A descriptive statistical analysis of the sample was carried out using mean, standard deviation, minimum, and maximum values. Determination coefficients (R^2) for the age estimation methods, accuracy rates of radiographic scores, mean error, and regression equations were also determined. The level of significance for the tests was set at 5%.

RESULTS

Oro-cervical radiographic indexes results

Overall intra-examiner and inter-examiner reliability for the OCRSS was 0.91 ($p<0.001$; CI 95% = 1.0–0.71) and 0.85 ($p<0.001$; CI 95% = 1.0–0.66), respectively. For the OCRSSWWT, intra-examiner Kappa coefficient was 0.83 ($p<0.001$; CI 95% = 1.0–0.61) and inter-examiner Kappa coefficient was 0.73 ($p<0.001$; CI 95% = 0.92–0.53).

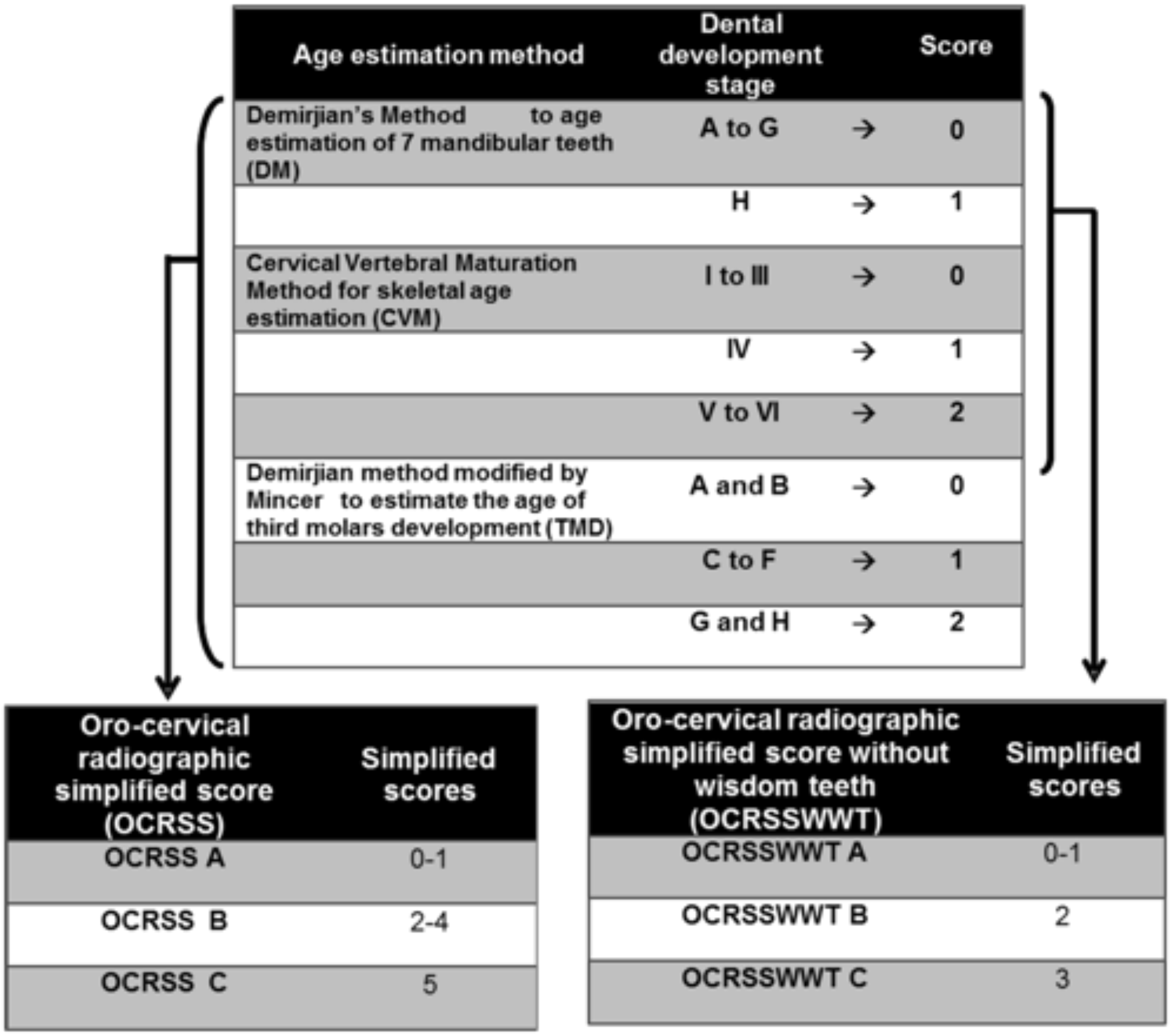
Table 1 . Characterization of the sample studied by chronological age group, OCRSS, and OCRSSWWT.

	N	Mean	SD	Minimum	Maximum
Real age group A	180	10.970	1.6979	8.000	13.920
Real age group B	120	15.991	1.1265	14.000	17.920
Real age group C	210	21.510	1.9905	18.000	24.920
OCRSS A	98	10.003	1.4052	8.000	14.000
OCRSS B	256	15.976	3.6285	9.170	24.830
OCRSS C	156	21.342	2.3187	15.830	24.920
OCRSSWWT A	167	11.092	2.1303	8.000	19.420
OCRSSWWT B	114	17.393	3.8411	9.580	24.830
OCRSSWWT C	229	19.933	3.0727	12.750	24.920

A = 8 – 13.9 years, B = 14 – 17.9 years, C = 18 – 24.9 years, SD = standard deviation, OCRSS = oro-cervical radiographic simplified score, OCRSSWWT = oro-cervical radiographic simplified score without wisdom teeth.

The characteristics for the studied population and its distribution based on chronological ages using OCRSS and OCRSSWWT, are reported in Table 1. Distribution of the number of individuals in both the OCRSSWWT and the real age groups A, B, and C was more similar when compared to the distribution of individuals in the OCRSS groups. However, the mean estimated ages using OCRSS were closer to the mean chronological age.

Figure 1. Three radiographic methods of age estimation with their respective stages and scoring for obtaining the simplified score proposed by Lajolo et al. (2013)[5].



For the assessment of the entire study sample, the accuracy using OCRSS was 54% for the group aged 8-13.9 years, 86.8% for individuals aged 14-17.9 years, and 67.8% for the group aged 18-24.9 years. The success rate was 67.4% (R^2 = 0.64) across all groups when applying OCRSS. Regarding OCRSSWWT, the accuracy of the method was 85% for group A, 38.5% for group B and 77.4% for group C. The overall accuracy for the OCRSSWWT method was 70.8% (R^2 = 0.62).

The analysis of results using the OCRSS method for females and males shows a higher accuracy for males (70.7%), except for group B in which the results for females had an accuracy of 90.3% compared to 83.3% for males. According to the OCRSSWWT results, age estimation for males was more accurate for all age groups (A, B, and C), with an average accuracy of 75.4% for males and 66.1% for females (Table 2).

Table 2. Distribution of the sample based on OCRSS and real age, and OCRSSWWT and real age.

OCRSS		REAL AGE		
	A	B	C	
A	97	1	0	98
B	83	106	67	256
C	0	15	141	156
Total	180	122	208	510
Hit rate (%)	54%	86.8%	67.8%	67.4%
OCRSSWWT		REAL AGE		
	A	B	C	
A	153	12	2	167
B	22	47	45	114
C	5	63	161	229
Total	180	122	208	510
Hit rate (%)	85%	38.5%	77.4%	70.8%
OCRSS (Females)		REAL AGE		
	A	B	C	Total
A	42	0	0	42
B	48	56	37	141
C	0	6	65	71
Total	90	62	102	254
Hit rate (%)	46.6%	90.3%	63.7%	64.2%
OCRSSWWT (Females)		REAL AGE		
	A	B	C	Total
A	67	2	0	69
B	18	23	24	65
C	5	37	78	120
Total	90	62	102	254
Hit rate (%)	74.4%	37.1%	76.5%	66.1%
OCRSS (Males)		REAL AGE		
	A	B	C	Total
A	55	1	0	56
B	35	50	30	115
C	0	9	76	85
Total	90	60	106	256
Hit rate (%)	61.1%	83.3%	71.7%	70.7%
OCRSSWWT (Males)		REAL AGE		
	A	B	C	Total
A	86	10	2	98
B	4	24	21	49
C	0	26	83	109
Total	90	60	106	256
Hit rate (%)	95.5%	40%	78.3%	75.4%

Group A = 8 – 13.9 years, Group B = 14 – 17.9 years, Group C = 18 – 24.9 years, OCRSS = oro-cervical radiographic simplified score, OCRSSWWT = oro-cervical radiographic simplified score without wisdom teeth.

To determine how much of the chronological age variability could be explained by the individual and combined age estimation methods, the adjusted determination coefficients were calculated. The R^2 value of the DM was 69%, higher than the value of 46% found for CVM.
The method that detected maturation stages of the third molar showed the highest determination coefficient (R^2 = 0.83). For the radiographic indices associated with the previously mentioned methods, the R^2 value for OCRSS was 0.64 and 0.62 for OCRSSWWT.

Results concerning the use of linear regression equations for age estimation
The use of age estimation equations described by Chaillet and Demirjian[7] for the whole study sample (510 participants), showed a maximum age prediction limit of 16 years using this technique. For the method of Caldas et al.,[8] the maximum value of estimated age was approximately 19 years. Although the equations were used for the whole sample (8-24.9 years), for analyzing the data, the group corresponding to the age group of 8 to 18 years (300 individuals) was selected.
A comparison between chronological age and age estimated by the two equations (scoring eight lower teeth on the left side and measuring cervical vertebrae) was carried out using the Mean Squared Error (MSE) for each method, calculated as follows:

$$MSE = \frac{1}{n}\sum\left(Chronological\,Age - Age\,predicted\,by\,the\,equation\right)^2$$

The MSE for the equations of Chaillet and Demirjian[7] was 1.65. A Mean Error (ME) of 1.29 was obtained by applying the square root (√), i.e., on average, this method showed an error of 1.3 years. For the equations developed by Caldas et al.,[8] the MSE was 3.57 and ME was equal to 1.9 years older or younger than the chronological age.
With the aim of developing new age estimation equations, a linear regression model was used, with chronological age as the dependent variable and using the same variables adopted by Caldas et al.[8] and Chaillet and Demirjian[7] as independent variables, in addition to their interactions with the variable 'sex'. The estimated parameters of the final model are reported in Table 3.
The new equations developed from the combination of variables obtained by dental measurements and cervical vertebrae are:

Chronological age (female) = - 285.6183 + 2.699277 x (AH3/AP3) + 0,451021 x (AH4/AP4) + 0,0007371 x (S value^3) - 0.1637731 x (S value^2) + 12.05215 x (S value).

Chronological age (male) = - 215,7111 + 1.755609 x (AH3/AP3) + 1.564029 x (H4/AP4) + 0,0005675 x (S value^3) - 0,1251991 x (S value^2) + 9.181172 x (S value).

For this proposed model, the MSE was 1.016; with a square root of 1.008 years. Thus, the new equations in this study showed an average error of 1.04 year (Table 4). Therefore, the equations produced estimated ages with high accuracy when compared to the equations which used individual age markers.

Table 3. Estimated parameters for the new proposed model for age estimation by including dental and cervical vertebrae data.

Estimated coefficients	Estimated	Standard error	t value	P value
Intercept: Female	-285.6183	38.9638	-7.33	0.000
(AH3 / AP3)	2.699277699	1.569079	0.28	0.087
(AH4 / AP4)	0.451021	1.623739	9,61	0.000
(S value³)	0.0007371	0.0000767	-8.79	0.000
(S value²)	-0.1637731	0.0186311	8.10	0.000
(S value)	12.05215	1.487366	-7.33	0.000
Intercept: Male	-215.7111	38.9147	-5.54	0.000
(AH3 / AP3)	1.755609	1.160963	1.51	0.132
(H4 / AP4)	1.564029	2.413496	0.65	0.518
(S value³)	0.0005675	0.0000809	7.02	0.000
(S value²)	-0.1251991	0.0192922	-6.49	0.000
(S value)	9.181172	1.511698	-6.07	0.000

AH3 = distance from the top of the front part to the tangent of the third vertebrae lower part, AP3 = Antero-posterior distance at middle of third cervical vertebral body; AH4 = distance from the top of the front part to the tangent of the fourth vertebrae lower part, AP4 = Antero-posterior distance at middle of fourth cervical vertebral body, S value = Total maturity score of the eight lower teeth.

Table 4. Results regarding the mean square error (MSE) and mean error (ME) of the age estimation equations applied in this study.

Age range	Equations of Chaillet and Demirjian (2004)		Equations of Caldas et al. (2007)		Equations with methods association	
	MSE	ME	MSE	ME	MSE	ME
8 – 18 years (n = 300)	1.65	1.29	3.57	1.89	1.01	1.04

MSE = mean square error, ME = mean error.

DISCUSSION

In a pilot study with sixty Italians,[5] the intra and inter-examiner reliability of radiographic indices were not evaluated, being one of limitations presented by the authors. In the present study, the reproducibility tests of the method were performed and it was found that the OCRSS and OCRSSWWT showed a satisfactory intra and inter-examiner agreement.

The accuracy of the OCRSSWWT method for group A (8-13.9 years) was 85%, higher than the 54% accuracy of the OCRSS method, confirming the hypothesis that in many cases, the assessment of the development stage of third molar results in an over-estimation of the chronological age due to its advanced maturation, which can be compatible with an age above the real age of a child or adolescent. Aynsley-Green et al.[12] emphasized the possibility of attributing a more advanced age to individuals who presented early maturation and, nevertheless, a lower chronological age regarding their development stage.

In general, the index which did not evaluate third molars (OCRSSWWT), produced more accurate results. However, it is important to note that accuracy was greater for OCRSS group B, which suggests the importance of assessing third molar mineralization for the age group between 14-17.9 years. For OCRSSWWT group C, accuracy was 77.4 %, with some cases of individuals in group B and a few in group A. In the pilot study,[5] ages of individuals between 18-24.9 years were also under-estimated. One possibility to improve results for individuals between 18 and 21 years of age consists of examining both the third molars and the clavicle, so that combination of these two methods produces better results.[13] Clavicular epiphysis can be easily examined in dry bone or through imaging with computed tomography.[14,15]

Martrille et al.[16] recommend that a large number of dental and skeletal indicators should be used to estimate chronological age. However, to increase the potential of each method, the final assessment should consider the method or methods with higher accuracy for a particular age group.

According to the guidelines of previous studies,[17,18] to improve the performance of research in age estimation, a study sample should consist of females and males distributed in age groups with equal numbers in order to compose a more homogeneous sample group, thus favoring more reliable statistical results. However, the radiographic indices proposed by Lajolo et al.[5] categorize individuals into groups A (8 to 13.9 years), B (14 to 17.9 years), and C (18 to 24.9 years), that is, combine several age groups into a single group. For many individuals over 21 years of age, dental and vertebral maturation have already been completed. Moreover, age estimation by these intervals has the complicating factor of the analysis of individuals who are close to the cut-off points between groups, since it is common for these individuals to have their ages over-estimated for the adjacent age group.[19]

Females ages were over-estimated more often and therefore had lower accuracy compared to male ages, which can possibly be explained by hormonal factors that result in an earlier maturation of females, leading to an over-estimated age.[5] These findings corroborate previously published results,[20,21] in which all variables for age estimation showed higher accuracy in males in comparison with females, since females reached maturation stages earlier than males.

The accuracy of each method in estimating chronological age through the stages of development of biological characteristics is limited, since it is necessary to consider biological variability and variation of maturation rates among individuals of the same age. Acceleration or delay of biological development of the different structures of the human body does not proceed in perfect agreement, the combination of more than one age estimation technique can provide results of estimated ages with more accuracy when compared to the practice of an isolated method, where error variance tends to be greater.[13,22] Association of methods can improve results to discriminate whether an individual is under or over 18 years old, this critical age represents the imputability age in Brazil and other countries in South America. In this sense, a method of age estimation with regression equation seems to be better to predict age than only scores classification, since difficulty is on the threshold of 18 years.

In this work, equations that evaluated dental characteristics presented an average error lower than the equations that only analyzed measurements of the cervical vertebrae. When we tested a new model with the combination of the data from the vertebrae and the teeth, the error was attenuated and the model produced age estimates with an average error equal to 1 year. Therefore, the new equations presented in this work produced estimated ages with greater accuracy when compared to the equations that used individual age markers. This result is in accordance with previous studies,[23-25] which state that the use of regression equations using a combination of data from different anatomical structures has the ability to reduce errors in the estimated age group by reducing global variability. It is important to mention that the newly developed formulae including dental and skeletal variables were adjusted according to the sample in our study, which favours the results. Thus, it is recommended that future research apply these equations to a new, independent sample.

CONCLUSIONS

This study represents the first research on the applicability of the method proposed by Lajolo et al.[5] in Brazilians, using a large sample. The simplicity of the method, following adequate training, confirms the possibility that it can be used in forensic practice. However, it is suggested that the method can be used to support other techniques which produce an estimated age value, not only to classify an individual into a comprehensive age group. Thus, it is possible to apply the new equations proposed in this study, which include data from cervical vertebrae with dental development information, to obtain age estimation with greater accuracy.

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CASE REPORT

Taurodontism and its forensic value: a case report

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The authors declare that they have no conflict of interest.

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ABSTRACT
Taurodontism is an anomaly that affects posterior teeth, vertically increasing the size of the pulp chamber, mimicking the shape of bovine teeth, being only evidenced in diagnostic images. This report describes a case of taurodontism in a mandibular second molar, highlighting the forensic importance of this dental anomaly with relevant potential for human identification, not only for its morphological aspect but also for its relatively low frequency in mandibular second molars. In the case under study, the individual did not have any restored teeth. Thus, the set of diverse imaging modalities is fundamental to identify the anatomy of teeth and roots, the only information that could be used in a hypothetical identification situation, assigning to this anomaly an exceptional relevance as a potential characteristic for positive identification.

INTRODUCTION
Tooth abnormalities are disorders that affect permanent or deciduous teeth due to inherited, congenital or acquired factors. Failures in development of the Hertwig's epithelial root sheath cause root anomalies, which may only be observed on imaging examinations.[1,2]
The term taurodontism was proposed to describe the vertical increase in pulp chamber size, mimicking the shape of bovine teeth.[3] This anomaly may be classified as hypo, meso or hypertaurodontism, according to the level of apical displacement of the pulp chamber floor.[4]
This developmental disorder may be associated with syndromes.[5-7] A prevalence of 60% was observed in Brazilian individuals with Down's syndrome,[6] but it also occurs in non-syndrome individuals.[8-10] Moreover, it is seen in individuals presenting other tooth abnormalities, such as dental agenesis.[11-12] Its prevalence varies according to the study population, without gender predilection, affecting molars and premolars in both dentitions, involving one or more teeth in the same individual (Table 1).[13-23] Most cases are classified as hypotaurodontism (75%), while hypertaurodontism is much less frequent (6%).[16]
From the standpoint of forensic investigations, taurodontism is a valuable tooth characteristic for human identification. In Forensic Dentistry the combination of specific dental characteristics of an individual allows differentiation from others when identity determination is necessary, such as in mass disasters.[24] Therefore, this paper reports a case of taurodontism of the mandibular second molar, highlighting its forensic relevance.

Table 1: Prevalence of taurodontism in recent studies in different populations.

Study	Population	N	Type of image	Teeth analyzed	Prevalence (%)	2LM*
Porto et al. (2009)[21]	Brazilian	1300	PAN/FMS	All molars	5.54	22/108
Bürklein et al. (2011)[14]	German	800	FMS	All molars	2.25	8/30
Afify et al. (2012)[15]	Saudi Arabia	878	PAN	Posterior teeth	0.01	n/a
Patil et al. (2013)[16]	Indian	4143	PAN	Posterior teeth	0.41	6/32
Gupta, Saxena (2013)[20]	Indian	1360	FMS	All molars	2.50	32/118
Çolak et al. (2013)[19]	Turkish	6912	PAN/FMS	Posterior teeth	0.26	16/46
Shokri et al. (2014)[18]	Iranian	1649	PAN	All molars	3.34	42/228
Gonçalves Filho et al. (2014)[22]	Brazilian	503	PAN	Posterior teeth	27.19	n/a
Puttalingaiah et al. (2014)[13]	Indian	946	PAN	First mandibular molars	17.30	n/a
Bharti et al. (2015)[17]	Indian	1000	FMS	All molars	2.80	n/a
Pedreira et al. (2016)[23]	Brazilian	562	PAN	Posterior teeth	4.98	12/26

PAN: Panoramic radiography; FMS: Full mouth survey; 2LM: Second lower molar. * Number of 2LM taurodont/number of taurodont teeth; n/a: not available.

CASE REPORT

A 27 year-old man presenting with good general health was submitted to dental evaluation for job purposes (pre-hiring examination), also called administrative expert examination for entry in the public service staff. Besides clinical examination, previous images provided by the individual were also evaluated, including periapical and panoramic radiographs and cone beam computed tomography of the left posterior mandibular region.

Analysis of the images revealed hypertaurodontism of the mandibular left second molar, which presented crown with typical permanent mandibular second molar morphology and wide pulp chamber without nodules or obstructions (Fig. 1, Fig. 2). No other clinical or radiographic abnormalities were found that might preclude the job activity of the individual. Therefore, he was considered in good condition to take the job.

Consent was obtained from the individual, and the professional performing the expert examination prepared the material, assuring anonymity of the individual's data.

Figure 1: Mandibular left molars, highlighting the clinical aspect without anatomical abnormalities of the crown (A), and periapical radiographic image evidencing the particular aspect of hypertaurodontism on the mandibular left second molar (B).

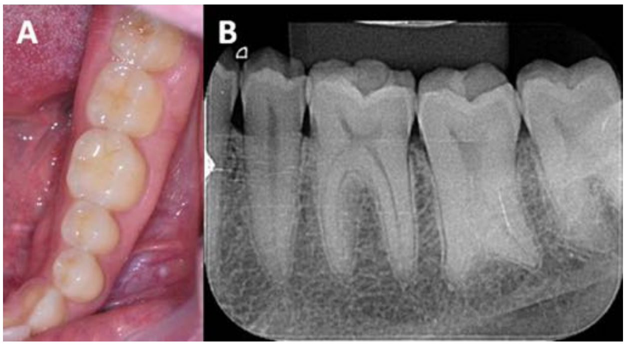
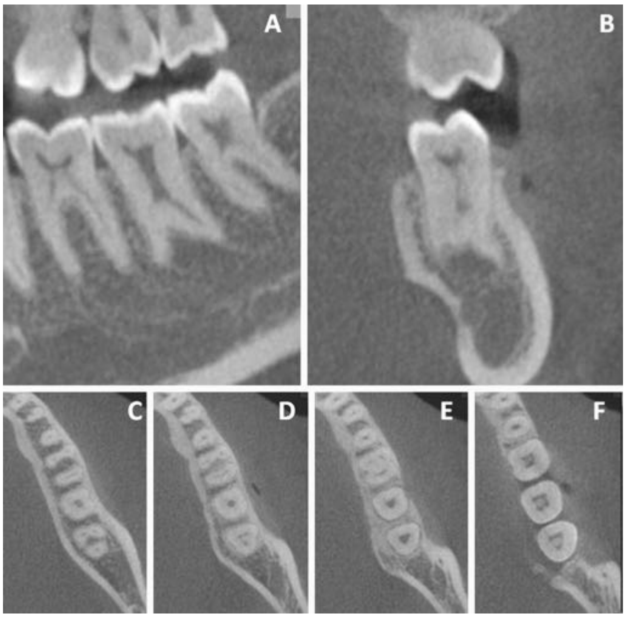


Figure 2: Cone beam computed tomography: panoramic (A), parasagittal (B) and axial reconstructions (C to F).



DISCUSSION

In Forensic Science, positive identification of an individual is achieved by the analysis of dental features in ante-mortem and post-mortem records, considering the combination of unique characteristics. This process is facilitated by the presence of variations that are not very frequent in the population, which serve as individualizing features. Within this context, taurodontism is a tooth abnormality with relevant potential for human identification, not only for its morphological aspect but also due to its relatively low frequency in some populations. It is even more relevant when associated with parameters such as presence/absence of syndrome, type of taurodontism (hypo, meso or hypertaurodontism) and affected tooth.

Even though mathematical calculations are not routinely used in human identification, and considering that only an image exhibiting the anomaly for comparison would allow identification of an individual, the present case would require knowledge on the frequency of hypertaurodontism only in second molars, which should be added to other dental features observed in the identification examination. According to the literature reviewed, only four studies allowed calculation of the prevalence of taurodontism in mandibular second molars in different populations,[18-21] which would range from 0.13% to 1.2%.

The pre-hiring examination is within the classification of administrative expert examinations conducted in the public service, requested by an institutional authority usually related to human resources administration (of either Federal, State or Municipal governments).[25,26]

According to Brazilian regulation, the official dental expert is the only professional allowed to advise individuals about the occasional need of treatment, and refer them to rehabilitation if necessary. This highlights the function of health promotion, avoiding absenteeism of the newly hired individual for the provision of dental treatment.[27] In the present case, the individual did not require any treatment, since the teeth were healthy; however, he was informed about the taurodontism observed on radiographic images.

The second function of administrative expert examination is to record specific features that may be helpful in the occasional need of forensic dental identification. In the present case, the individual did not have any restored teeth. Therefore, the combination of several imaging modalities is fundamental to identify the anatomy of teeth and roots, the only information that might be used in a hypothetical identification situation, assigning to this anomaly an exceptional relevance as a potential characteristic for positive identification.

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BOOK REVIEW

The comparative palaeopathology of males and females in English medieval skeletal samples in a social context

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This reproduction of a PhD thesis is aiming to compare male and female palaeopathology, based on analysis of skeletal samples from medieval England, focusing on sex differences in mortality, general health status, activity related pathology and dental disease. It has great informational and educational value for different professionals interested in the research on human remains, like forensic anthropologists, forensic archeologists, forensic odontologists, and students connected with this field.

The book comprises 10 chapters and a total of 240 pages. The 10 chapters go through a brief introduction, description of material used, subadult sex determination based on tooth measurements, database design, demography, general health indicators, trauma, joint diseases, dental pathology and research conclusions. After the completion of the thesis in 2000, the author has updated this project, by adding a postscript (2016), following the same chapter structure for fluency of reading, and highlighting research advances in this field. All chapters have clear sub-headings, and follow a similar structure to include introduction, methodology, results and discussions, limitations are extensively evaluated and recommendations for future research are made.

Potential differences in health and mortality are systematically assessed using a large volume of existing cemetery data from a clearly defined historical period, interpreted in a bio-cultural context referring to influence of both 'sex', as a biological feature, and 'gender', as the social idea of sex in a medieval system where women were generally subordinate to men. There is a significant input of historical and medical evidence in the process of evaluation, based on extensive and well-documented research. The cultural generalization of women's 'inferior' social status was integrated in the biological framework of physiological differences between males and females impacting on health status.

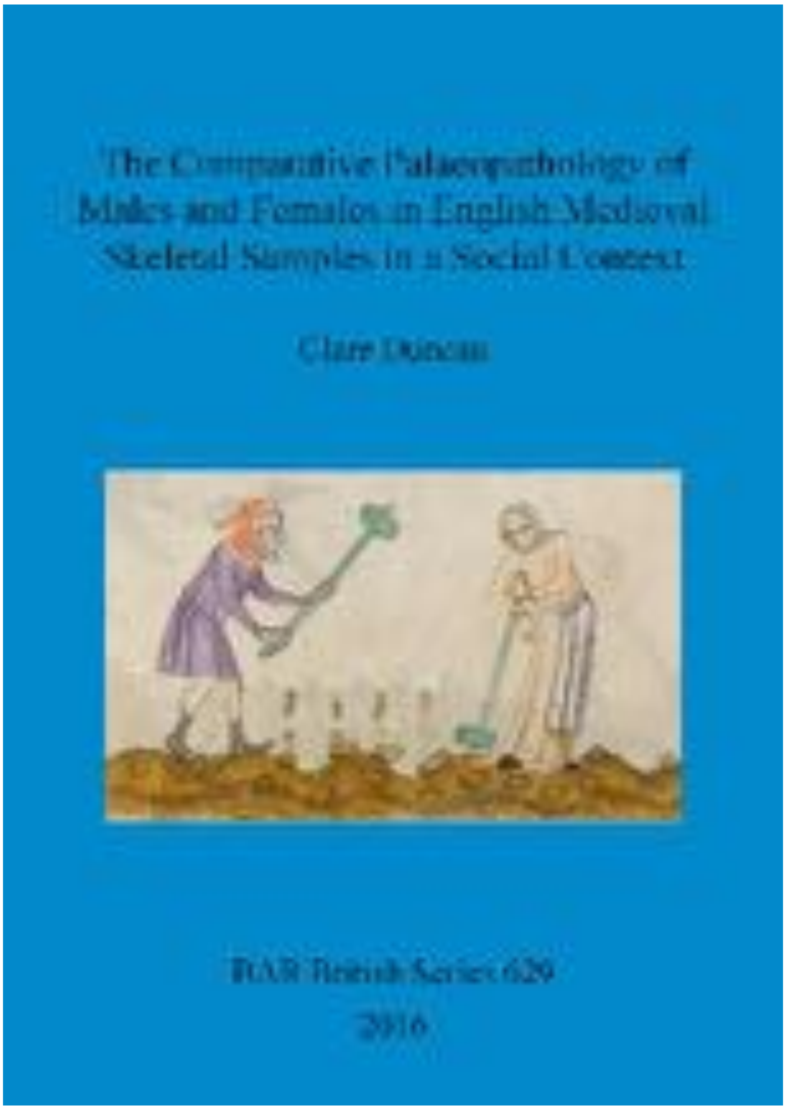
Over-representation of male skeletons, common characteristic to many archeological series, and earlier age at death in female, although with substantial site differences in the latter, were the main demographic features. A poorer general health status was identified in males, in contrast to women subordination expected to be in detriment of their health. On the other hand, dental health of adult females was inferior to that of males. Furthermore, considerable differences between cemeteries revealed the influence of living environment, urban/rural divide, socio-economic factors like migration and population aggregation on health and mortality.

Methods employed for skeleton's sex estimation were essentially the same for all cemetery samples and based on currently accepted anthropological techniques. Potential bias of sexing methodology towards over-representation of males was highlighted together with subsequent significant consequences on validity of pathology prevalence figures. One chapter aimed at validating a subadult sex estimation method based on tooth measurements, with the mandibular canine having the highest discriminative potential.

Age at death in adult and subadult was estimated using methods based on dental attrition and eruption respectively, together with osteological ageing methods. Unlike sex estimation, osteological methods used varied between different collections, all subject to recent data confirming increasing inaccuracy and bias with increasing age. Dental attrition on the other hand had been applied to all the samples, and due to relatively greater confidence, it was used as basis to categorise data for statistical analysis. Also subadult age estimation methods are considered to yield accurate and reliable estimates.

There is an undeniable research potential revealed by this book, in terms of using skeletal evidence as a tool to study sex and gender in past human populations. Although the study material comprised a large number of skeletons, an even larger collection could be used to overcome most of the study limitations, this being also highlighted by the author herself.

In summary this book should generate interest among both general public and specialists in the field like anthropologists, archeologists and forensic scientists. It adds evidence to the growing recognition of the value of skeletal human remains to complement historical evidence in the research on past human societies.



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