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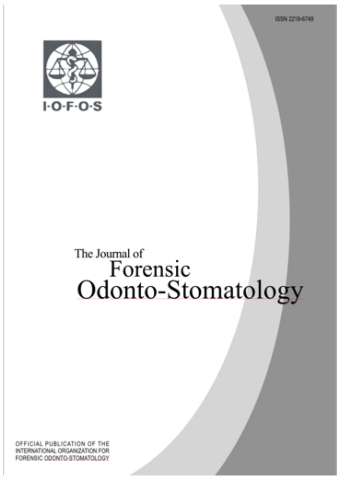

THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

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CONTENTS

Proposal of a formula mouth opening reduction assessment, for forensic purposes

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KEYWORDS

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Mouth;
Disability Evaluation;
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ABSTRACT

Objectives: To propose a formula for determining reduced mouth opening due to oral and maxillofacial trauma, based on the normal standard of the Brazilian population. Materials and Methods: First, the maximum mouth opening was established, in millimeters, using a digital pachymeter, in patients between 22 and 60 years of age. The opening was measured from the upper to the lower incisor, at maximum amplitude, without pain and overbite. Second, the facial profile type and height were determined. A formula was developed to calculate the percentage of reduced mouth opening based on the normal average. Results: The average mouth opening was found to be 51.71 mm in men and 47.94 mm in women, thus establishing a statistically significant difference in mouth opening between sexes. However, there was no statistically significant difference between age and profile type with mouth opening. The following formulas were developed to calculate the reduced mouth opening, based on the averages found, by using RA= [100-(A.1.93)].0.3 for males and RA= [100-(A.2,08)] .0.3 for female patients. Conclusion: Considering that mouth opening tends to be larger in men than in women, valid formulas can be used to determine the correct percentage of reduced mouth opening.

INTRODUCTION

The maximum mouth opening is formally considered as the interincisal distance without pain, plus an overbite [1]. Its amplitude can be modified by several factors, such as gender, height, age, and facial profile. [2-5]

Mandibular movements are complex and depend on the harmonious functioning of the structures that make up the stomatognathic system, namely the mandible, maxilla, temporomandibular joint, skull bones, hyoid bone, and musculature[6]. The face is the most commonly affected part of the body in cases of trauma because it is not protected. Lesions in this region can be highly devastating because of their physical and psychological consequences.[7,8]

The main causes of trauma in the oral and maxillofacial regions are traffic accidents, falls, and aggression. The most prevalent consequence is fractures of the mandibular region[9]. As a result, major detriments may include pain and clicks in the temporomandibular joint, facial asymmetry, change in occlusion, and limitation of mouth opening.[10]

The Brazilian civil code states that any injury caused by an unlawful act should be repaired, whether the damage is to material or non-material property.[11] Damage to the bodily integrity must be reimbursed proportional to the injury caused to the victim, whether psychological or physical.[12]

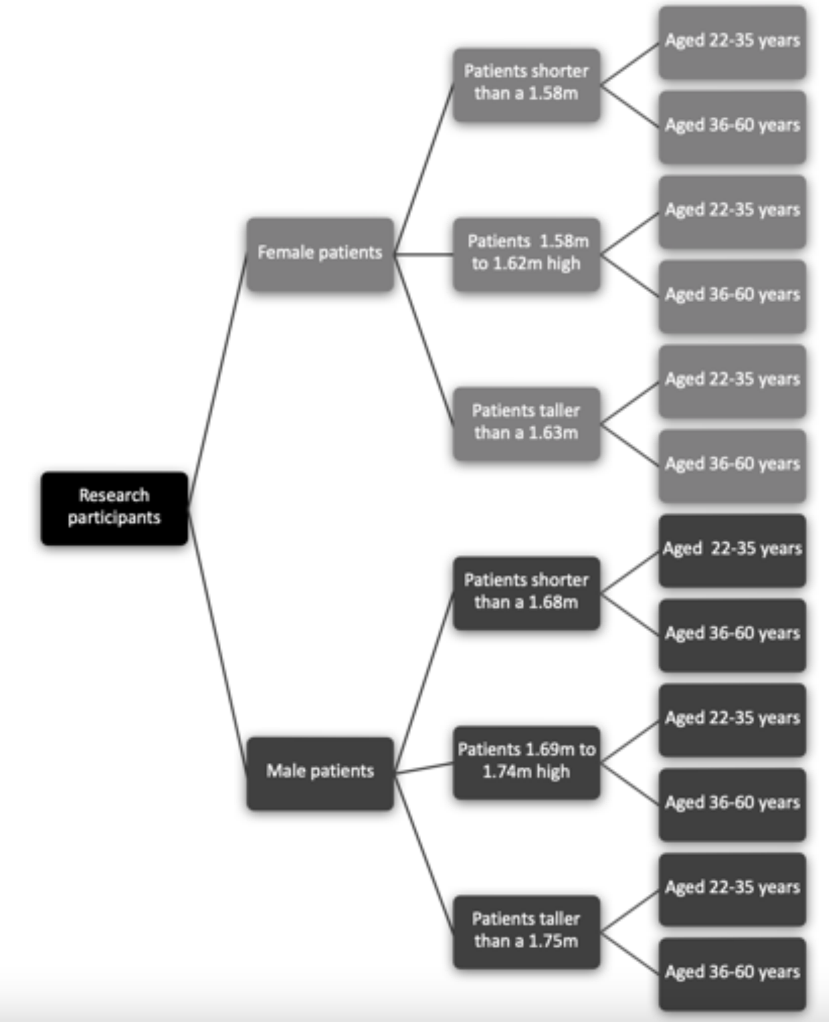
Since the expert examination is subjective, the auto insurance for personal injury caused by motorized land vehicles (DPVAT), Brazil's federal regulatory body on private insurance issues, and the Portuguese table of disabilities for quantifying bodily harm are important elements for unifying language and criteria. The upshot is that the same situation can be assessed and understood in the same way by more than one expert.[13] In legal terms, a table of disabilities serves as a tool, listing diseases or sequelae that can be correlated to a value, normally expressed as a percentage.[14] For this reason, it is of utmost importance to know the standard of normality of the mouth opening of a population. This is so that any permanent consequences caused to the victim can be established.[2-5]

This study aimed to propose a formula for determining reduced mouth opening due to oral and maxillofacial trauma, based on the normal standard of the Brazilian population.

MATERIAL AND METHODS

This project was approved by the ethics committee of the School of Dentistry of the University of São Paulo (FOUSP), Protocol no. 59004816.7.0000.0075. Informed consent was obtained from all participants included in the study. The participants selected included those in the range of 22 to 60 years of age, who had never suffered from trauma to the face, who were not in pain at the time of the research, and who had at least one upper and lower central tooth. All participants were selected from the waiting room of the emergency clinic run by FOUSP, whether patients or visitors, and were stratified according to sex, height, and age. The facial profile of all the participants was assessed but not paired (Fig. 1).

Figure 1. Sample distribution according to the variables analyzed



A questionnaire was administered for possible traumas involving the face and pain sensations at the time of the examination. The mouth opening was measured in millimeters using a digital pachymeter. The opening was measured from the upper to the lower incisor, at maximum amplitude without pain, plus overbite, as recommended by Machado et al.[15] The same instrument was used to measure the middle third and the lower third profile, to determine the facial profile at a later date, according to the study by Reis et al.[16] The patient's facial height was measured with a portable stadiometer, with him standing barefoot, with his head positioned so that the Frankfort plane was parallel to the ground, based on methodology by the Brazilian Institute of Geography and Statistics (IBGE). [17] A formula was developed based on the mean of normality to calculate the percentage of reduction in mouth opening.

This study was conducted by one examiner, and intra-examiner calibration was performed. The measurements were obtained at two different moments, and the kappa was calculated.

The results are tabulated in Excel®. An analysis was conducted to determine the statistical difference in mouth opening between sex, height, age, and facial profile. The student's *t*-test was used to perform the statistical analysis of sex and age. Whereas, the one-way ANOVA test was used to analyze the stature and facial profile. A significance level of 5% was considered.

RESULTS

The kappa agreement test result was adequate (p > 0.9).

A total of 486 participants enrolled in this study (286 women and 200 men). Table 1 shows the mean mouth opening, standard deviation, and confidence interval for the females and males studied, as well as the statistically significant difference in mouth opening between the sexes. The table shows that the range of mouth opening tended to be larger in men than in women.

Tables 2 and 3 show statistically significant differences in mouth opening between stature, age, and facial profile of the female and male participants, respectively. No significant relationship was found between mouth opening and the variables for both sexes.

Table 1. Mean difference mouth opening between women and men

Gender	N	Mean of the mouth opening (mm)	Standard deviation	P-value
Female	286	47.94	6.17	<0.001*a
Male	200	51.71	7.07	

*Statistically significant p≤0,05. a Student's t-test

Table 2. Mean difference of mouth opening with stature, age, and facial profile of female patients

Variable		N	Mean mouth opening (mm)	Standard deviation	P-Value
Stature	< 1.58	115	47.89	6.14	0.9152b
	1.59 – 1.62	81	48.17	6.08	
	> 1.63	90	47.79	6.34	
Age	18-35	128	47.98	6.17	0.9113a
	36-60	158	47.90	6.18	
Facial profile	Short Face	22	50.4	4.07	0.1332b
	Mean Face	145	47.89	6.27	
	Long Face	119	47.53	6.29	

*Statistically significant p≤0,05. a Student's t-test. b One-way ANOVA test

Table 3. Mean difference of mouth opening with stature, age, and facial profile of male patients

Variable		N	Mean mouth opening (mm)	Standard deviation	P-Value
Stature	< 1.68	53	50.45	7.57	0.638^b
	1.69 – 1.74	66	51.52	7.01	
	> 1.75	81	52.69	6.72	
Age	18-35	82	52.07	7.17	0.547^a
	36-60	118	51.45	7.01	
Facial profile	Short Face	4	47.97	12.15	0.4797^b
	Mean Face	68	51.38	7.03	
	Long Face	128	52.00	6.93	

*Statistically significant p≤0,05. [a] Student's t-test. [b] One-way ANOVA test

The means found in the present study refer to the 100% normal mouth opening of the studied population. In cases of trauma, three calculations are required to determine the percentage reduction in the mouth opening of the victim. First, it is necessary to establish when the mouth opening remains normal. Subsequently, this value must be reduced by 100% to establish a reduction value. Lastly, this value must be multiplied by 0.3, because the European table determines that the total limitation of mouth opening corresponds to 30% of the corporal damage.

where "x" represents the mean mouth opening of the population to be assessed, "RA" means the reduction in mouth opening, and "A" refers to the mouth opening of the patient at the time of the expert examination, measured in mm.

For men:

$$RA = \frac{\left[100 - \left(\frac{A\ .\ 100}{x}\right)\right]\ .\ 30}{100}$$

$$RA = \frac{\left[100 - \left(\frac{A\ .\ 100}{51.71}\right)\right]\ .\ 30}{100}$$

$$RA = \left[100 - (A\ .\ 1.93)\right]\ .\ 0.3$$

For women:

$$RA = \frac{\left[100 - \left(\frac{A\ .\ 100}{x}\right)\right]\ .\ 30}{100}$$

$$RA = \frac{\left[100 - \left(\frac{A\ .\ 100}{47.94}\right)\right]\ .\ 30}{100}$$

$$RA = \left[100 - (A\ .\ 2.08)\right]\ .\ 0.3$$

DISCUSSION

Any change in the component structures of the temporomandibular joint may cause facial pain, articular noises, headaches in the cervical region, and limitation of mandibular movements.[18] However, the foremost step in determining mouth opening reduction involves defining what is normal and studying how some variables may interfere.[2]

The present study found a statistically significant difference in mouth opening between sexes. Thus, substantiating a major part of the research that assessed the normality of mouth opening, in which the correlation of this variable with gender became clearer.[2,3,5,19] In general, men tend to have a larger mouth opening than women. This is attributed to the difference in mandibular size and strength of the depressant jaw muscles, which are factors that favor a greater and maximal mouth opening in male patients. This is mostly owing to their larger structure and greater strength than women.[3,4] Accordingly, stature is usually studied less than gender. In a study by Sawair et al.[20], the authors found a positive correlation between height and mouth opening in adults. Rakaraddi et al.[5] related height with maximal mouth opening only in patients aged 11 to 25 years; at other ages, height was not found to be significant. This corroborates the findings of the present study, in which no statistical difference was observed in females or males aged 18 to 60 years.

The majority of authors who included age as a variable found a positive, inversely proportional correlation. In other words, as age increased, the range of mouth opening decreased.[1-3,20-23] This can be explained by the dehydration of joints and the reduction in muscle elasticity over the years.[2]

This decrease in mouth opening was observed in the present study, where an opening of 47.98 mm was observed for women aged 18 to 35 years, and a slightly smaller opening (47.90 mm) for women aged 36 to 60 years. Likewise, the opening for men was 52.07 mm and 51.45 mm, respectively. However, this reduction was not statistically significant, as corroborated by the studies by Casanova-Rosado et al.[4], Rakaraddi et al.[5], and Al-Dlaigan et al.[24].

No statistically significant difference was found with regards to facial profile, although the female patients with a short face in this study had a larger mouth opening. Whereas, short-faced male patients had a larger mouth opening in the facial profile. Likely, the variation is more closely related to mandibular growth than the facial profile.[25]

The importance of establishing maximum mouth opening in different populations should also be emphasized, as it differs from one population to another. This can be observed when comparing studies that analyzed mouth opening within the standards of normality.

Four of the six continents have studied mouth opening in relation to the standard of normality of their populations. In the African continent, Chima[1] examined individuals in Nigeria and found a mean mouth opening of 56.1 mm for men and 52.3 mm for women. In the European continent, the research developed in Ireland obtained a mean of 43 mm for men and 41 mm for women[22]. In contrast, the mean in Germany was 52.15 mm and 54.91 mm for male and female participants, respectively.[26]

Four countries were studied on the Asian continent, namely, India,[2] Saudi Arabia,[21] Jordan,[20] and Japan.[25] The results obtained for men and women were a mean mouth opening of 50.3 mm and 49.9 mm, 43.5 mm and 35.5 mm, 45.3 mm and 41.6 mm, 54.46 mm, and 46.9 mm, respectively.

On the American continent, Mexico[4] had a mean of 48.17 mm for men and 44.90 mm for women. Whereas the United States[27] had a mean of 47.4 mm and 50.7 mm, for male and female participants, respectively. In the present study reporting on the Brazilian population, the mean mouth opening was 51.71 mm for men and 47.94 mm for women. Given the disparity in the results, among not only continents but countries, it should be stressed that individualized studies are needed for each population, to determine the real maximum mouth opening.

Regarding personal injury, Ferrara et al. (2016)[28] elaborated the first “International Guidelines on Medico-Legal Methods of Ascertainment and Criteria of Evaluation of Personal Injury and Damage under Civil-Tort Law” which includes a detailed step-by-step personal injury ascertainment. Permanent damage is a frequent finding in cases of facial trauma, and legal claims for damage have increased over the years[29]. Personal injury ascertainment must be performed in the chronic phase once the injured area is healed or stabilised.[30]

Tables of disabilities were created to facilitate the valuation of bodily injury and to unify the language used by examination experts and magistrates to improve mutual understanding.[2,15]

Conceptually, when a table of disabilities is deemed appropriate, similar results should be produced when assessed by different experts. However, when analyzing the main tables used in Brazilian forensics, this has not been case.[2] The tables of disabilities should not be a compendium of all the pathologies or methods for assessing the damage. Instead, they should be minimally provisioned to assist the examination expert in quantifying an injury.[2,15,31] Because of the lack of detail regarding lesions in the stomatognathic system, the DPVAT[32] and SUSEP[33] tables of disabilities are insufficient to assess dental injuries.

The Portuguese table of permanent disabilities has a section just for lesions of the stomatognathic system, which correlates to a greater number of dental sequels.[12] Although this table is more convenient to use, it should be emphasized that it was created in Europe, making its application in Japan incompatible. When analyzing the sequel of the European mouth opening limitation, we noticed that it was assessed with a reduction of only 40 mm.

Real bodily injury is ideally determined by the current state minus the previous state.[14] The problem is evident when we realize that the dental care routine does not normally record a patient's mouth opening. Thus, making it difficult to identify the previous state.

When the previous state is not known by the expert, the definition of the mouth opening due to injury becomes subjective. However, it is extremely important to define the functional defect in an expert examination. Because of this, an attempt is made to turn what is subjective into something objective. To assess the compatibility of a mouth opening limitation due to injury, the previous state must be known; otherwise, the result will be questionable. Thus, the importance of knowing the standard of normality of a population remains unquestionable. Only in this way can a limitation be correctly determined.[2] Since the present study was conducted on the Brazilian population, the values of limited maximal mouth opening should be quantified from the resulting values.

It was suggested that the European table of disabilities be adapted to receive proper valuation by using a method of proportionality. In other words, by drawing up a formula. Although the means of normality of the mouth opening used in constructing the formulas were determined in this study, conducted in Brazil, it should be elucidated that the formulas ($RA = [100 - (A \; . \; 1.93)] \; . \; 0.3$ for males and $RA = [100 - (A \; . \; 2.08)] \; . \; 0.3$ for females) have limitations because they were developed from the means found in this study, corresponding to the population analyzed. However, by using the base formula $RA = \dfrac{\left[100 - \left(\frac{A \; . \; 100}{x}\right)\right] \; . \; 30}{100}$, a specific equation can be determined for each country by merely replacing the letter "x" with the mean of the mouth opening of the desired population.

CONCLUSIONS

The present study concluded that there is a significant difference in mouth opening associated with gender. Namely, the male participants tended to have a larger mouth opening than the female participants.

The table of disabilities of Portuguese legislation must be adapted to the Brazilian reality in order to enable the correct valuation of the mouth opening limitation, and indicates that the formula $RA = [100 - (A \; . \; 1.93)] \; . \; 0.3$ for male patients, and the formula $RA = [100 - (A \; . \; 2{,}08)] \; . \; 0.3$ for female patients was applied in Brazil. The formula used to determine the reduction in mouth opening in other populations was as follows:

$$RA = \frac{\left[100 - \left(\frac{A \; . \; 100}{x}\right)\right] \; . \; 30}{100}$$

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Radiographic changes in endodontically treated teeth submitted to drowning and burial simulations: is it a useful tool in forensic investigation?

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The authors declare that they have no conflict of interest.

KEYWORDS

Burial; Dental Materials; Drowning; Forensic Dentistry; Human Identification; Root Canal Obturation

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ABSTRACT

Dental radiographs, endodontic treatment and materials are a source of useful forensic data. The response of dental materials to death-related events are widely studied and provide forensic evidence for experts. This study aimed to analyze the radiographic images of endodontically treated teeth submitted to burial and drowning simulation, verifying its forensic feasibility, applicability and usefulness. Material and method: n=20 bovine incisor teeth were endodontically treated then divided into two groups: burial and drowning scenarios. Teeth were radiographed two times (before and after scenario) with an aluminium stepwedge, and optical density (OD) was assessed in each root third, in both radiographs, and then compared (ANOVA and Tukey test) for each scenario. Results: Burial scenario did not significantly alter radiopacity. As for the drowning scenario, there was no difference in radiopacity between the root thirds before the test. After drowning, the apical third demonstrated lower OD ($p<.05$) than the other two thirds. Comparing the OD before and after drowning, medium third presented lower and cervical third demonstrated higher means ($p<.05$) after drowning. Conclusion: We concluded that drowning conditions could alter the radiopacity of endodontically treated teeth, more specifically in the medium and cervical thirds. There is no evidence that this also occurs in burial situations. This has the potential to be useful in forensic casework as an initial sign of the type of ambient in which the body was supposedly exposed or set.

INTRODUCTION

The INTERPOL DVI Guide (2018) establishes that fingerprint analysis, dental information, and DNA (so called primary methods of identification) are self-sufficient in the identification process, and do not require further investigations when they establish an identity.[1] When death results from extreme situations, and human remains are severely destroyed or burned, forensic odontology is an essential forensic tool, as teeth are very resistant to hazardous conditions, such as high temperatures.[2]

Dental arches, despite having characteristics that may change throughout life, have a combination of traits that are reproducible and can be compared at any time, granting uniqueness through teeth positioning, anatomical features, treatment provided and pathology in every single person.[3] In this sense, not only clinical examinations but also dental radiographs play an important role in the identification process

by making feasible the comparison of those features at two anatomical levels of complexity: external and internal dental anatomy.[4]
Radiographs are frequently found in dental records, enabling ante-mortem (AM) and post-mortem (PM) comparisons, through the observation of dental anatomy, restorations and endodontic treatment, which are easy to detect radiographically.[3] Consequently, endodontic treatment is valuable for forensic odontology, given the extensive use of post-operative radiographs, thus providing important ante-mortem data.[5]
Although the prevalence of endodontic treatment varies considerably worldwide, usually due to the heterogeneity of populations and endodontic protocols,[6] in Brazil this number is relatively high[7] compared to other countries[8]. Understanding the response of dental tissues and dental materials submitted to hazardous conditions is significant to forensic science as it enhances the identification process.[9-10]
In this context, changes in endodontic materials submitted to hazardous conditions, such as high temperatures, was already studied by analyzing several parameters such as macroscopic,[11] microscopic,[12-13] and radiographic changes.[12,14] However, their effect when set to burial or drowning simulations has not yet been evaluated. The number of other studies in these scenarios (burial and drowning) is scarce.[15-16] These situations are reportedly real conditions in routine forensic odontology and criminal investigations[2,17].
This research aimed to analyze the radiographic images of endodontic treatment, in teeth submitted to burial and drowning simulation, verifying its forensic feasibility, applicability and usefulness.

MATERIAL AND METHODS

For this study, inclusion criteria were bovine incisors with a single root canal – the exclusion criteria were teeth with cracks or fractures, or without complete root apex formation. The final sample number was (n=20) teeth.
The teeth were rinsed in running water, and using periodontal curettes, the tissue remnants adhering to the dental surface were removed. They were then rehydrated in 0.9% saline solution and then stored in a glass vessel with distilled water under refrigeration until use. They were then sectioned perpendicularly to its long axis, above the amelo-cemental junction, separating each tooth in a coronary and a root portion, standardizing the length of the specimens in 30 mm (Fig. 1). In order to establish a default sample size, crown sectioning was done to facilitate the insertion of the K-type files, which had a maximum length of 31 mm and could become lodged at incisal edges, due to the extensive length of bovine teeth. The crown portions were then discarded.
The root portions were then submitted to endodontic treatment using manual instrumentation with K-type files (Dentsply Maillefer Indústria e Comércio Ltda., Petrópolis, Brazil) and irrigation with 0.5 ml of 1% sodium hypochlorite solution (ASFER Indústria Química Ltda, São Caetano do Sul, Brazil). The root canals were filled with gutta-percha cones (Dentsply Maillefer Indústria e Comércio Ltda., Petrópolis, Brazil) and zinc oxide and eugenol endodontic cement (Endofill - Dentsply Maillefer Indústria e Comércio Ltda, Petrópolis, Brazil), being obturated by the cold lateral compaction technique[18].
The sealing of the remnant coronary portion of the root canal was made by composite restoration, using 37% phosphoric acid gel (Maquira Indústria de Odontológicos, Maringá, Brazil) for 15 seconds in dentine in etching phase, then two layers of Adper Single Bond 2 adhesive system (3M ESPE Dental Products, Sumaré, Brazil), and finally Filtek Z250 XT micro-hybrid composite resin (3M ESPE Dental Products, Sumaré, Brazil), photoactivated (FLASH Lite 1401, Vigodent Coltene, Belo Horizonte, Brazil) for 20 seconds. The restoration was then polished and finished out at high speed and under refrigeration with 30-blade multilayer drill bit FF 9714 bur (JET Carbide Burs). Samples were randomly divided into two groups (n=10); each one submitted to a simulation of the two environmental conditions.

Environmental conditions

The burial condition was simulated, putting the teeth into a tissue bag filled with soil and placed inside an excavation with a depth of 1.0 m, and covered by the same land, in an area delineated for research. The drowning condition was simulated, putting the teeth in a pouch similar to that used for the burial scenario, placed in a cage immersed in a natural lake. These conditions were simulated for a time-lapse of 90 days. All the bags and pouches had little perforations over their surface, which guaranteed that teeth had full contact with the environment, as well as keeping them from drifting away in the water or losing them in the soil.

Acquisition of radiographic images

Each sample was submitted to two radiographic exposures (Spectro 70X, Dabi Atlante Indústrias Médico Odontológicas Ltda., Ribeirão Preto, SP, Brazil, 70kVp, 8mA, focus/film distance of 20 cm, and exposure time of 0.28s). One exposure was made prior to drowning and burial submission, and the second exposure was made 90 days after being exposed continually to these scenarios.

For the radiographic procedure, the teeth were placed on radiographic films in a standardized way and with an aluminum stepwedge measuring 10 x 32 mm, and scaled in 8 steps, with incremental thicknesses of 2.0, varying from 2.0–16.0 mm in thickness. The purpose of this stepwedge placement was to simulate the densities of the soft and hard tissue structures of the oral cavity, by producing a scale image of radiopacity nuances resulting from radiation exposure and film processing; for assessment of radiographic quality; and to observe the homogeneity of the exposures.[19]

Sample analysis

The optical density (OD) values of the endodontic materials were assessed in the radiographs, in each tooth root third, (cervical, middle and apical) by a photodensitometer (MRA Indústria e Equipamentos Eletrônicos Ltda, Ribeirão Preto, SP, Brazil) with a collimated light beam regulated to a diameter of 1mm. OD reading was made in triplets for each dental third of each radiograph. The stepwedge "steps" had their OD also individually measured. Then, the OD mean values of those triplets were converted to equivalent millimetres of aluminum (mmAlEq), according to Guerreiro-Tanomaru et al. (2009),[20] for statistical analysis. OD mean values of root thirds and aluminum stepwedge were compared. Values below 3mm of mmAlEq were considered as unacceptable or indistinguishable from radiographic imaging.

Mean values of mmAlEq, before and after the environmental conditions, in the different root thirds (cervical, middle and apical) were analyzed by 2-Way ANOVA, and Tukey test, at the significance level of α = 5%.

RESULTS

The mean values for OD readings, on the different thirds, submitted to different conditions, can be seen in Figs. 1 and 2. Before the scenarios, the middle third always presented the highest radiopacity (mmAlEq = 15.55 in both), while the lowest values were observed in the apical third (11.88 and 11.53 for drowning and burial, respectively).

Figure 1. Mean values of mmAlEq (Equivalent Aluminium Milimeters), in the different root thirds, before and after drowning of the samples

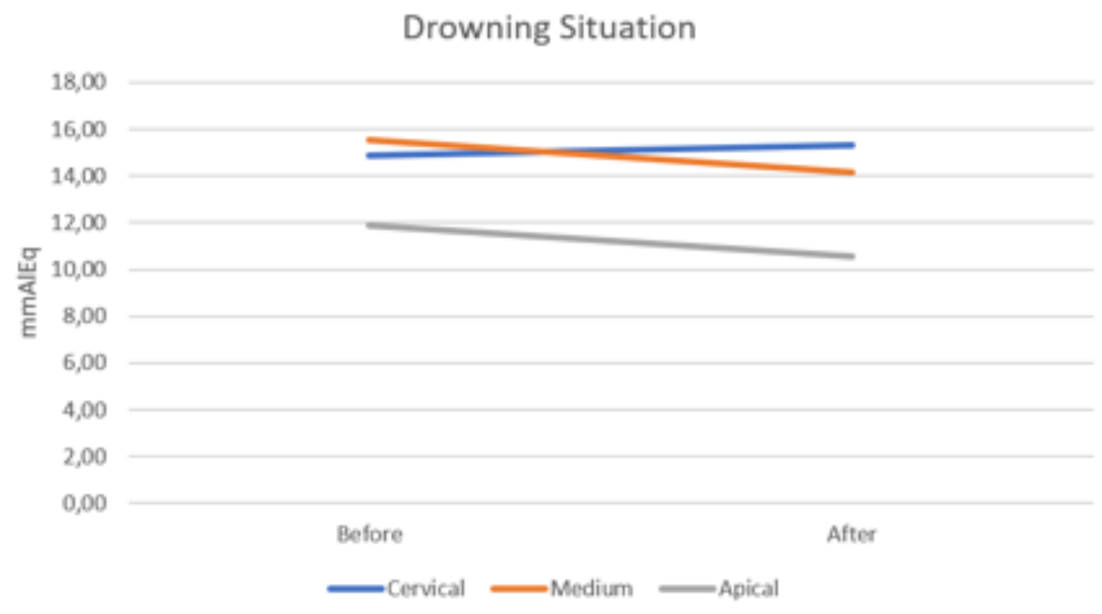
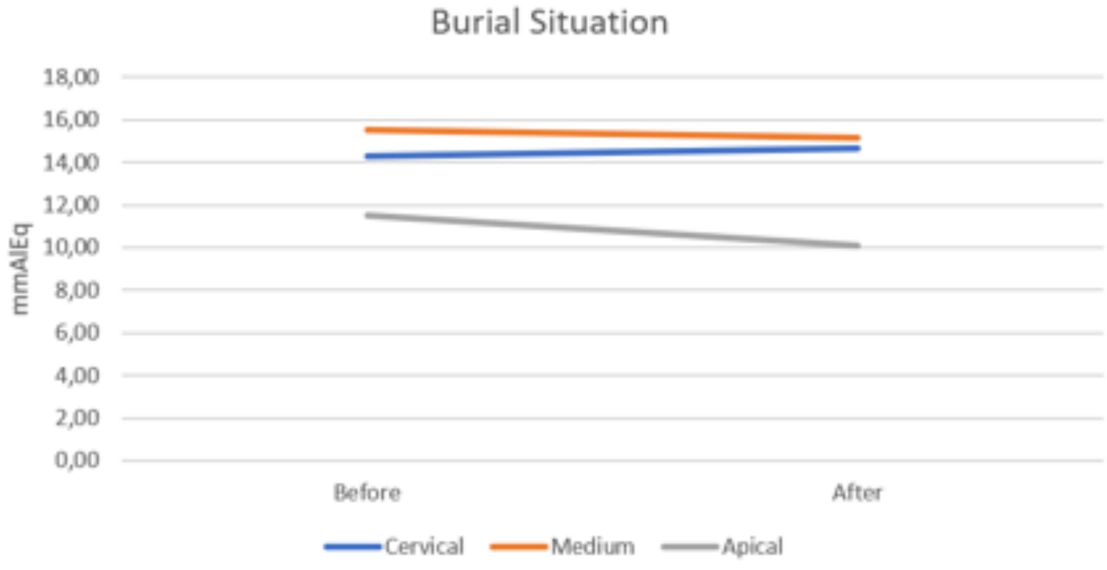


Figure 2. Mean values of mmAlEq (Equivalent Aluminium Milimeters), in the different root thirds, before and after burial of the samples



The groups means comparison (2-way ANOVA, Tukey, p<.05) are shown in Tables 1 and 2. Lower radiopacity (p<.05) was observed in the apical third of the obturation, in comparison with the other two analyzed thirds, before and after burial conditions. Also, no evidence was found that burial alters the radiopacity of the endodontic materials.

Table 1. Mean Values, in mmAlEq, in the different root thirds, before and after burial. Different letters, upper case in column and lower case in line, indicate statistically significant difference (p <0.05).

Burial			
Root Third	Cervical	Medium	Apical
Before	14,27 aA	15,55 aA	11,53 bA
After	14,68 aA	15,13 aA	10,06 bA

Table 2. Mean Values, in mmAlEq, in the different root thirds, before and after drowning. Different letters, upper case in column and lower case in line, indicate statistically significant difference (p <0.05)

Drowning			
Root Third	Cervical	Medium	Apical
Before	14,84 aB	15,55 aA	11,88 aA
After	15,34 aA	14,14 aB	10,53 bA

Prior to submission to the drowning scenario, there was no difference in radiopacity between the root thirds. In the aftermath of this condition, the apical third demonstrated lower OD (p<0.05) than the other two thirds. When comparing the OD before and after the drowning simulation, the medium and the cervical thirds presented lower higher means (p<0.05), respectively.

DISCUSSION

Understanding the influence of catastrophic or adverse events on different dental materials is vital, since some disasters can result in the dispersal of victims in environments with extreme temperatures, and other situations.[21] With this in mind, some studies observed alterations in several restorative materials subjected to such extreme conditions, as the staining and colour stability of different composites submitted to high[22] or low[23] temperatures.

Endodontic treatment is a valuable source of forensic data, because of accompanying radiographs which are suitable for comparison.[5] This analysis is often made as a qualitative comparison between suspects' and the unknown individual's ante-mortem (AM) and post-mortem (PM) information, regarding morphological aspects of the teeth, treatment, obturation shape, and materials used.[24] When endodontically treated teeth are involved, this examination rarely involves an analytical and quantitative approach.

There are several reports in the literature[11-14,25] about endodontic materials submitted to forensic conditions. However, the majority are about the influence of high temperatures. In this study, we analyzed the influence of drowning and burial scenarios in the radiopacity of endodontic materials, using optical densitometry.

Endodontic interventions are composed of several stages, such as: disinfection, modelling, and sealing of the root canals. In the final stage, sealers are employed to obturate the prepared canals, generally using materials that have satisfactory radiopacity, biocompatibility, solubility, and thermoplasticity.[26] Regardless of the technique used, gutta-percha cones are traditionally used for root canal sealing. This material is composed mainly of zinc oxide, gutta-percha itself, waxes, resins, and heavy metal radiopacifiers. Its chemical composition does not vary significantly between different manufacturers.[27]

Nevertheless, endodontic cement can be based on different components, like calcium hydroxide, zinc oxide with eugenol, glass ionomer, silicone, resin polymers, and calcium silicate.[28] In our work, we used the combination of gutta-percha cones added to zinc oxide and eugenol-based cement.

The lowest values of radiopacity were found in the apical third of the root canals, in both groups, before and after drowning and burial. Radiopacity, in general, varies according to the cement thickness around the filling material.[29] Thus, as the apical third usually has a smaller taper and a greater narrowing before the endodontic preparation, smaller amounts of material and cement are found.

In the group submitted to drowning, after the 90 day time-lapse, it was observed lower means of radiopacity in the middle third and a higher value for the cervical third. This finding can be attributed to cement solubility, as eugenol and zinc oxide-based cement have a higher solubility among cement types due to the continuous loss of eugenol from its matrix, diminishing mass and causing the contraction of the material.[28,30] Changes in the plasticity of gutta-percha could also explain this response, despite being considered an innocuous material in water, and low temperatures.[31]

Radiopacity lowering also occurs at high temperatures, as observed in other studies.[12,14] However, in these cases, the endodontic obturation displays unique patterns, macroscopic alterations, along with evident tissue destruction of the tooth remains.[11] By consequence, charred remains of endodontic materials are easily distinguished from those studied here.

Furthermore, we observed no significant radiopacity changes between before and after the burial process. The conservation and stability of dental tissues during burial has been reported by Menon et al. (2011).[15] Despite this, further studies with human teeth are necessary to verify the present results. It is necessary to remark that our study was conducted with bovine teeth, which, despite being routinely used in dental research, can behave differently than human teeth, depending on the analysis done.

The human body has known responses towards death, which involve morphological changes (colour, shape, size, integrity) due to taphonomic events such as decomposition, putrefaction, mummification and skeletalization, which are broadly studied in forensic medicine, given their importance in establishing the *causa mortis*.[32] In forensic odontology, this is no different, and these thanatological findings must be carefully studied and registered as they can potentially help in human identification.[33]

Soil compounds and characteristics are well known to exert influence in these taphonomic changes.[34,37,38] This microenvironment usually triggers or delays the decomposition rate in burial situations, produce signals and leave traces that indicate the type and conditions of the burial site's soil. [34] Similarly, bodies submerged in water during or after death, in a drowning event, also have typical thanatological outcomes, such as the still debatable pink teeth, as a possible dental alteration related to wet or moist environments.[35]

It is also well known that soils, in general, have a significant presence of acids in their composition, originating from several sources.[36] When a soil environment has a high concentration of acids, it may become acidic, and buried bone structures are more prone to changes caused by this condition, as observed by Howes et al. (2012).[37] Highly acidic soils can rapidly decompose bone by dissolving its hydroxyapatite (inorganic matrix)[38], the same substance that composes the majority of dental enamel.

Mazza et al. (2005)[39] observed alterations in dental structures immersed in different types of acid with time and concluded that even when teeth are in direct and complete contact with acid, it is still possible to analyze and recognize its structures until the advanced stages of degradation. A remarkable observation in this study is that one sample tooth studied presented a residual structure after being submitted to acid, which later was identified as gutta-percha bulk. Subsequently, this material was divided into two fragments and immersed again in two different acids, enduring dissolution still, for over 50 hours. As it was already pointed out before, gutta-percha is a stable and firm material that can help immensely in identification processes, which reinforces the importance of endodontic materials in forensic cases.

Although not case-specific nor pathognomonic, all these signs and outcomes discussed here can give initial hints and suggestions to the forensic expert, commencing the investigation of the events surrounding death. Dental and endodontic materials are liable to these mentioned environmental influences. The findings of the present study are relevant, as they indicate that radiographic alterations in the radiopacity of endodontic materials are significant in the drowning scenario. Changes also seems to occur in the burial scenario, but these were found not to be significant, and further research should be conducted.

Therefore, our results may represent an outset forensic finding, specifically in those cases where the cadaver was merged into water, set or left in an aquatic environment. Radiopacity changes are easily detectable with proper equipment and could indicate different conditions to which the body has been exposed, especially in those cases where morphological changes had still not happened nor are visible to the human eye.

Caution must be used when conclusions are drawn from the results of this study. It did not intend to link its results with the certainty of any cause of death, nor to indicate an aquatic submersion of a particular victim during death. More scientifically robust findings must be combined to establish a better conclusion, with larger samples and human teeth, in conjunction with real case reports. However, our results are important, since similar research has not yet been conducted, thus opening paths for researchers to findings related to routine forensic cases.

CONCLUSIONS

Drowning conditions can alter the radiopacity of endodontically treated teeth, specifically in the medium and cervical thirds. There is no evidence that this also occurs in burial situations. These findings have the potential to be useful in forensic cases as an initial sign or suggestion of the type of environment that the body was exposed or set. However, they should not link directly to the cause of death or lead to a case solution, as alternative scientific techniques should be conducted.

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Assessing the probability of having attained 16 years of age in juveniles using third molar development in a sample of South Indian population

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KEYWORDS

Dental age estimation, Third molar mineralization, Demirjian's stages; Probability; 16 years.

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ABSTRACT

Juvenile crime or delinquency has been increasing at an alarming rate in recent times. In many countries, including India, the minimum age for criminal responsibility is 16 years. The present study aimed to estimate the probability of a south Indian adolescent either being or being older than the legally relevant age of 16 years using Demirjian's tooth formation stages. Orthopantomograms (OPG) of 640 south Indian adolescents (320 boys and 320 girls) aged between 12 and 20 years were retrospectively analyzed. In each OPG, Demirjian's formation stage of the mandibular left third molar was recorded and the data was subjected to statistical analysis. Descriptive and Pearsons correlation statistics were performed. The empirical probabilities were provided relative to the medico-legal question of predicting 16 years of age. The distribution of age throughout the 10th, 25th, 50th, 75th and 90th percentile follows a logical distribution pattern horizontally and vertically. Pearson's correlation statistics showed a strong positive correlation between the Demirjian's stages and age for both sexes. Therefore, it can be concluded that stage "F" can be used to predict the attainment of age equal to or older than 16 years with a probability of 93.9% for boys and 96.6% for girls.

INTRODUCTION

Age disputes arise when a child or a person fails to prove their age by providing documentary evidence which is legally accepted by a court of law. The question of age usually arises in civil and criminal proceedings. Forensic experts are often confronted with the conceptually simple medico-legal question, to determine whether an individual has attained legal age threshold or not.[1] According to Indian law and the Juvenile justice act, there are three different age thresholds to consider i.e., 14, 16 and 18 years. Similar to many countries, 18 years of age is one threshold with important ramifications in India. The minimum age for criminal responsibility is 16 years. According to the amendment bill of Indian juvenile justice act, 2015, juvenile offenders aged between 16 and 18 years will be treated as an adult if they are accused of committing a heinous crime.[2] Any such crimes involving individuals between 16 and 18 years of age must then be established using different criteria.

The physiology of human age estimation can be evaluated by the degree of maturation of the different tissue systems.[3] Age assessment using skeletal and dental anthropological methods is useful mainly in children and adolescents because of the development of several teeth and bones in parallel during childhood.[4] Dental maturation in particular is a helpful indicator due to its high reliability, less affected by variation in nutritional and endocrine status.[5,6] Several dental age assessment methods based on radiographs have been described in the literature. One of the most widely applied methods is the maturity standards proposed by Demirjian et al. in 1973 which was based on a sample of French-Canadian children.[7] Theoretically, it is based on eight developmental stages (A to H), ranging from crown initiation, root formation until the apex closure of the seven left permanent mandibular teeth.

Late in adolescence i.e., after the formation of the second molars, third molars are the only tooth that continues to form. It is a well known fact that third molars are far from ideal developmental markers of age, as they are considered the most variable tooth in the dentition. However, they still remain of prime medico-legal interest due to the unavailability of alternative reliable biological indicators. Mincer et al.[1] first studied the discriminatory ability of Demirjian's grading of third molar development with a view to predicting the attainment of 18 years. Later many researchers have tested the accuracy and precision of Demirjian's stages and reported varying probabilities for predicting the age of 18 years.[8-12] However, the effectiveness of these stages in predicting the attainment of 16 years, i.e., age of criminal responsibility was never tested within a south Indian sample. Therefore, the present study was aimed at determining the accuracy of Demirjian's classification of lower third molar in predicting the attainment of the age threshold of 16 years in a south Indian population.

MATERIAL AND METHODS

Sample

A sample of 640 orthopantomograms (OPG) were collected retrospectively from the archives of the radiology department, Panineeya Institute of Dental Sciences, Hyderabad, India and from private dental clinics. Of these, 320 were boys (50%) and 320 were girls (50%) of south Indian origin, aged from 12 to 19.9 years. Table 1 shows the age and gender distribution of the sample. All the radiographs were coded with unique identification to ensure that the observers were blinded to the demographic details of the subjects. A prior approval from the institutional research and ethics committee was obtained (PMVIDS&RC/IEC/OP/PR/0352-19). The need for obtaining informed consent was waived due to the retrospective nature of the study.

Table 1. Age and gender distribution of the total sample

Age groups	Boys	Girls	Total
12-12.9	40	40	80
13-13.9	40	40	80
14-14.9	40	40	80
15-15.9	40	40	80
16-16.9	40	40	80
17-17.9	40	40	80
18-18.9	40	40	80
19-19.9	40	40	80
Total	**320**	**320**	**640**

Radiographs of the individuals who were healthy, with no apparent history of disease or developmental anomalies were included. Radiographs exhibiting obvious pathology, deformities affecting appearance of third molars and showing major variations in tooth eruption or tooth morphology were excluded. All the radiographs evaluated were pre-treatment in nature. Chronological age of each individual was calculated by the difference between the date of birth and the date on which the radiographic examination was carried out.

Method

Developmental stage of each mandibular third molar was rated according to the grading system described by Demirjian et al.[7] The observers scored the stage of third molar development by comparing the radiographs with representative sketches of each stage. All OPGs were analysed by a single examiner, a forensic odontologist (SBB), who had six years of experience in evaluating radiographic images and in age estimation analysis. The second examiner was a dentist with a master's degree. In case of disparities while allotting stages to the same tooth by the two observers, the earliest formation stage was chosen. Intra- and inter-observer reliability was tested by evaluation of 100 orthopantomograms selected randomly after an interval of two months.

Statistical analysis

Statistical analysis was performed using the SPSS 20.0 statistical package (IBM SPSS Inc, New York, USA). The level of significance was set at 5% ($p<0.05$).

Cohen's kappa statistics were performed to calculate intra- and inter-observer reliability. For statistical analysis, the letter stages of Demirjian et al.[7] were converted to numerical values as follows: A=1, B=2, C=3, D=4, E=5, F=6, G=7 and H=8. Descriptive statistics and percentile distribution for each stage of tooth development for both genders were calculated. Pearson's correlation statistics were performed to test the correlation between the age and the Demirjian stages of tooth development.

Chi-square analysis was conducted to test the association between the developmental stage and age. For this purpose, the chronological age is dichotomized as < 16 or ≥ 16 years, instead of being continuous and open-ended. The performance of the stages was tested by 2×2 contingency table. The output of contingency table displays the number of true positives, true negatives, false positives and false negatives.[13] The performance was assessed using accurate classification, sensitivity or true positive rate (refers to the measure that correctly detect individuals who are above 16 years), specificity or true negative rate (measure the ability to correctly detect individuals who are below 16 years), positive (LR+) and negative (LR-) likelihood ratios. Likelihood ratios combine the sensitivity and specificity into a single value that indicates which cut-off is best in discriminating the age threshold. Values of LR+ >1 increase the likelihood of the subject being older than 16 years, while values of LR- <0.1 decrease the likelihood of age being above 16 years.[14]

The Bayes posterior probability (Bayes PTP) of being 16 years or older may help to discriminate between those who are or are not aged 16 years or more.[15] Briefly, it refers to the conditional probability of a hypothesis being correct given the value of the observed information.[16] According to Bayes' theorem, post-test probability may be written as[17]

$$\text{Post-test probability} = \frac{\text{Pre-test probability} \times \text{sensitivity}}{(\text{Pre-test probability} \times \text{sensitivity}) + (1-\text{Pre-test probability}) \times (1-\text{specificity})}$$

Pre-test probability is the probability that the subject in question is 16 years old or older, given that he or she is aged between 12 and 20 years, which represent the target population. It was calculated as the proportion of subjects between 16 and 20 years of age who live in the Andhra Pradesh and Telangana according to demographic data from the 2011 census (http://www.censusindia.gov.in/2011census/C-series/C-13.html) and those between 12 and 20 years which was evaluated from data from the same website source. This proportion was considered to be 51.3% for boys and 51.9% for girls.

RESULTS

Cohen's kappa statistics revealed values of 0.891 for intra-observer and 0.863 for inter-observer, indicating almost perfect agreements. The results of repeated scoring of 100 radiographs did not reveal statistically significant intra- or inter-observer differences ($p>0.05$), indicating substantial consistency of evaluation. The final sample analysed consisted of 609 orthopantomograms, 310 (48.4%) were boys and 299 were (46.7%) girls. Of the total sample, 4.8% subjects were excluded from analysis since they presented with no mandibular third molars. The mean ages of 320 boys and 320 girls were 15.99 ± 2.33 years and 15.99 ± 2.34 years, respectively ($p=0.742$). Prior to the evaluation of the main sample, mineralization of the lower wisdom teeth was compared between the right and left sides using Wilcoxon test. No difference in terms of the mineralization between the sides was observed ($p=0.639$). Pearson's correlation statistics showed a strong positive correlation between the Demirjian's stages and age for both sexes i.e., 0.785 ($p<0.05$) and 0.733 ($p<0.05$) for boys and girls, respectively.

Table 2 displays the output of descriptive statistics, i.e., mean, standard deviation, median, minimum and maximum ages of left mandibular third molar crown- root formation for the eight stages of tooth development. The sample sizes for stage "A" were too small to consider, and therefore, not included in the analysis. The mean ages at each developmental stage showed that the third molar genesis in boys attained the formation stages "C", "D" and "E" earlier than in girls. However, approximately a six month delay was recognised in boys for the formation stages "F" and "G" except the stage "H". Table 3 shows the percentile distribution at each developmental stage for both genders. This illustrates the variation of each stage in the age span. The distribution of ages throughout the 10th, 25th, 50th, 75th and 90th percentile follows a logical distribution pattern horizontally and vertically.

Table 2. Age distribution by sex and Demirjian stage for tooth 38

Stage	Sex	N	Mean (SD)	Median	Minimum	Maximum
B	Boys	5	12.45 (0.3)	12.39	12.08	12.8
	Girls	4	12.47 (0.2)	12.51	12.19	12.68
C	Boys	34	12.81 (0.9)	12.56	12	15.71
	Girls	25	12.78 (0.7)	12.3	12.01	14.44
D	Boys	67	14.25 (1.2)	14.16	12.01	19.03
	Girls	80	14.01 (1.1)	14.13	12.1	18.17
E	Boys	56	15.51 (1.5)	15.49	12.73	19.35
	Girls	46	15.26 (1.3)	15.33	13.1	19.39
F	Boys	53	16.63 (0.9)	16.61	13.82	18.48
	Girls	59	17.07 (1.1)	16.88	14.38	19.98
G	Boys	28	17.81 (1.1)	17.83	13.77	19.86
	Girls	28	18.23 (0.9)	18.08	16.23	19.67
H	Boys	67	18.96 (0.6)	18.99	17.33	19.91
	Girls	56	18.83 (0.7)	18.75	17.41	19.96

SD Standard deviation

Table 3. Age distribution in percentile by stage and sex

Percentiles						
Stage		10th	25th	50th	75th	90th
B	Boys	12.08	12.13	12.39	12.8	-
	Girls	12.19	12.23	12.51	12.67	-
C	Boys	12.06	12.19	12.56	12.84	14.47
	Girls	12.09	12.15	12.3	13.26	13.91
D	Boys	13.08	13.3	14.16	14.72	15.4
	Girls	12.39	13.13	14.13	14.78	15.2
E	Boys	13.45	14.57	15.49	16.31	17.49
	Girls	13.42	14.44	15.33	15.96	16.58
F	Boys	15.4	16.19	16.61	17.35	17.72
	Girls	16.12	16.27	16.88	17.58	19.01
G	Boys	16.72	17.39	17.83	18.43	19.31
	Girls	17.07	17.57	18.08	19.11	19.53
H	Boys	17.98	18.52	18.99	19.43	19.79
	Girls	18.01	18.34	18.75	19.47	19.87

Table 4 displays a cross-tabulation of the data undertaken on the basis of the age group (whether < 16 or ≥ 16 years) and developmental stage. The chi-square test showed that the relationship between the age and stage attainment is statistically significant for both sexes ($p<0.05$). According to our data, 100% of the subjects, both boys and girls, who were marked stages "B" and "C" were found in the age group under 16 years. A total of 95.5% boys and 98.8% girls who were rated as stage "E" were below 16 years of age. For stage "F," approximately 84.9% boys and 91.5% girls were in the age group above 16 years. 100% subjects, who were categorized stages "G" and "H" were older than 16 years of age.

Table 5 shows the output of contingency table for Demirjian stages "D", "E" and "F". Table 6 displays the performance measures. Among the tested stages, Stage "F" showed better performance. For boys, the values of sensitivity, specificity LR+, LR-, accuracy and Bayes PTP 88.2%, 93.9%, 14.52, 0.12, 90.9% and 93.9%. For girls, they were 92.3%, 96.5%, 26.4, 0.08, 94.3% and 96.6% respectively. LR+ values of 14.52 and 26.4 in boys and girls indicate that when Demirjian stage "F" was attained, then a boy is almost 14.52 times and a girl is 26.4 times more likely to be above 16 than under 16 years. LR- values of 0.12 and 0.08 in boys and girls indicate that when Demirjian stage "F" was not attained, then a boy is almost 8 times and a girl is 20 times more likely to be below 16 than above 16 years.

Table 4. Distribution of the sample (percentage), by sex and age group, according to the stage of mineralization

Sex	Age groups	Formation stages						
		B	C	D	E	F	G	H
Boys	<16 years	5 (100)	34 (100)	64 (95.5)	40 (71.4)	8 (15.1)	1 (3.6)	0 (0)
	≥16 years	0 (0)	0 (0)	3 (4.5)	16 (28.6)	45 (84.9)	27 (96.4)	67 (100)
Girls	<16 years	4 (100)	25 (100)	79 (98.8)	35 (76.1)	5 (8.5)	0 (0)	0 (0)
	≥16 years	0 (0)	0 (0)	1 (1.2)	11 (23.9)	54 (91.5)	28 (100)	56 (100)

Table 5. Criterion validity (chronological age ≥ 16 years) according to tooth staging for boys and girls

Stage	Sex	TP	TN	FP	FN
D	Boys	39	158	113	0
	Girls	30	150	119	0
E	Boys	103	155	49	3
	Girls	109	149	40	1
F	Boys	143	139	9	19
	Girls	144	138	5	12

TP True positive; TN True negative; FP False positive; FN False negative

Table 6. Performance measures of Demirjian's stages for legal age threshold over 16 years

Measures	Boys	Girls
Stage D		
Sensitivity	100 (90.9- 100)	100 (88.4- 100)
Specificity	58.3 (52.1- 64.2)	55.7 (49.6- 61.7)
LR+	2.4 (2.08- 2.76)	2.26 (1.98- 2.59)
LR-	0.00	0.00
Accuracy	63.5 (57.9- 68.9)	60.2 (54.4- 65.7)
Bayes PTP	71.7 (68.7- 74.4)	70.9 (68.1- 73.6)
Stage E		
Sensitivity	97.1 (91.9- 99.4)	99.09 (95.04- 99.9)
Specificity	75.9 (69.5- 81.6)	78.8 (72.3- 84.4)
LR+	4.05 (3.16- 5.17)	4.68 (3.55- 6.17)
LR-	0.04 (0.01- 0.11)	0.01 (0.00- 0.08)
Accuracy	83.2 (78.5- 87.2)	86.2 (81.8- 89.9)
Bayes PTP	81 (76.9- 84.5)	83.5 (79.3- 86.9)
Stage F		
Sensitivity	88.2 (82.2- 92.7)	92.3 (86.9- 95.9)
Specificity	93.9 (88.7- 97.1)	96.5 (92.03- 98.8)
LR+	14.52 (7.69- 27.41)	26.4 (11.15- 62.53)
LR-	0.12 (0.08- 0.19)	0.08 (0.05- 0.14)
Accuracy	90.9 (87.2- 93.9)	94.3 (91.05- 96.6)
Bayes PTP	93.9 (89- 96.7)	96.6 (92.3- 98.5)

LR Likelihood ratio; PTP Post-test probability

DISCUSSION

It is a well known and widely accepted fact that the third molars are by far the most variable teeth in the dentition. However, their protracted formation in adolescence and into early adulthood with completion often beyond the second decade of life made them the subject of interest in many studies.[18] In the present study, we set out to determine the accuracy of Demirjian's classification of the lower third molar in discriminating between individuals of 16 years of age threshold in a south Indian population. We observed bilateral agenesis in 4.8% cases of total sample, with no significant differences between genders.

When analysing the probability of an individual being under 16 years of age based on Demirjian's stages of third molar mineralization, the accuracy is higher in earlier stages (B & C). More than 95% of subjects who were classified as stage "D" are under 16 years of age. Subsequent to stage "E", there is a sharp decline in the proportion of times that chronological age is estimated to be less than 16 years of age.

One of the measures to test the ability of the model to discriminate the subjects 16 years of age or older is through finding the percentage of correct classifications i.e., sensitivity and specificity. According to Cardoso et al.[6] the model has good predictive capabilities if sensitivity and specificity are greater than or equal to 80%. The capacity of the model is called reasonable, if the values are between 50% and 80%, and is called mediocre model when they are below 50%. From a legal point of view, it is important to enable a subject to be judged as accurately as possible to confirm if they are of legal age. Therefore, methods that have better sensitivity and specificity should be used, with errors kept to a minimum. When the performance of stage "D" as a cut-off value for predicting 16 years was tested, sensitivity of 100% in both sexes and specificity of 58.3% and 55.7% in boys and girls was observed. This difference between the sensitivity and specificity for stage "D" could be due to the fact that the authors tested attainment of stage "D" for predicting age over 16 years. However, the distribution data according to stage of mineralization (Table 4) showed that more than 95% of subjects who were classified as stage "D" are under 16 years of age. Specificity values will be improved when attainment of stage "D" is tested to predict age under 16 years.

The sensitivity and specificity percentages for stage "E" were 97.1% and 75.9%, 99.09% and 78.8% for boys and girls respectively. A total of 24% and 21.2% of false positives, 2.8% and 0.9% false negatives were seen in boys and girls. In the criminal context, the issue of specificity is of special importance as it represents the number of false positive attributions.[19] Only methods or cut-off values with high specificity index can fulfil the legal requirements. In the present study, when stage "F" was tested as a cut-off value, a sensitivity percentage of 88.2%, 92.3% and specificity percentage of 93.9% and 96.5%, indicating only 6.1% and 3.5% false positive attributions for boys and girls, respectively. Similar to our findings, Caldas et al.[20] also reported better specificity values for stage "F" then stages "D" and "E". However, their sensitivity values were much less.

Mincer et al.[1] believed that third molar development may provide better accuracy for prediction of attainment of adulthood, instead of estimation of exact chronological age. So far, most authors chose to determine the likelihood of attainment of 18 years using Demirjian stages of tooth development.[21,22] According to their results, Demirjian's developmental stage "H" could be a reliable developmental marker for indicating age over 18 years. Comparatively, stage "H" is easily recognizable, fully mineralized tooth with apex. Therefore, the probability of a subject being 18 years or older can be easily determined. However, in the present study we chose 16 years age for assessment, as it is also an age with legal relevance in India. In our opinion, the diagnosis of age equal to older than 16 years can be made with accuracy using stage "F" of the radiological development of the third molars, with an accuracy of 90.9% for boys and 94.3% for girls. Few authors have reported lower accuracy between stages "F" and "G" (due to a span of 3-3.5 years) particularly while representing 16 year cut-off.[23] They believed that fewer Demirjian's root stages might affect the accuracy of age estimation. Harris[18] recommended that finer gradations would be an advantage especially in root stages where differences of a fraction of a year can have considerable medico-legal consequences. Solari and Abramovitch[8] modified Demirjian's method and introduced two extra root stages at "F" and "G" to improve the precision of this method. Future studies might require to adopt these extra stages to improve the accuracy of age estimation especially in the 16 year cut-off in the studied population.

Probabilistic assessments are crucial in a forensic setting because they provide a measure of uncertainty about the correlation between the real age and dental maturation.[24] Although, our study findings offer a probabilistic approach using Demirjian's tooth developmental stages, one should bear in mind that this approach may perhaps be seen as more representative of dental maturation and, thus, may not perform accurately in all populations. One of the main concerns is the representativeness of the sample which is comprised of healthy south Indian children. However, age estimation in forensic and legal settings does not typically involve such children, but children who grow under impoverished environments. In particular, dental and skeletal maturation tend to be delayed in malnourished children, and they may appear younger than they really are.[6] Therefore, proper care must be taken while using these models in malnourished children as it is more likely that false negatives may increase. Finally, we believe that the described data may provide south Indian references for third molar examination for the purpose of forensic investigation, especially in 16 year olds.

CONCLUSIONS

To the best of the present investigators' knowledge, this is the first study to address the issue of the minimum age of criminal responsibility, i.e., 16 years from third molars using Demirjian's tooth developmental stages in south Indian children. Our findings concluded that stage "F" can be used to predict the attainment of age equal to or older than 16 years with probability of 93.9% for boys and 96.6% for girls. These determined probabilities might be valuable in future forensic practice for the prediction of age over 16 years in the studied population. However, additional studies with larger samples should be conducted concerning the larger number of crimes by juveniles of 16 to 18 years.

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Morphological dental trait examination of Ajnala skeletal remains and their possible population affinity

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KEYWORDS

Dental anthropology, Ajnala skeletal assemblage, non-metric traits, population affinity, identification

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ABSTRACT

Objectives: The metric and non-metric features of the fragmented, badly damaged and commingled human remains play a significant role in their identity establishment in forensic anthropology. The main objective of the present study was to assess the population specific attributes of the unknown human dental remains excavated from an abandoned well, found underneath a religious structure at Ajnala (Punjab, India). Written accounts mentioned that Ajnala skeletal remains belonged to 282 Indian origin soldiers of the British army who had revolted against the colonial rulers in 1857, who were killed after capture, and their bodies dumped in an abandoned well.

Materials and Methods: Eleven non-metric dental traits were investigated in a sample of 1527 teeth (1200 dislodged teeth comprising of 300 canines, 300 premolars and 600 molars along with 93 jaw fragments having 327 teeth of different types) collected from this non-scientifically exhumed skeletal assemblage. These selected traits were examined with adequate magnification and lighting, using a flexible arm illuminated magnifier (Lensel Optics Pvt. Ltd., Pune, India), identified and scored in accordance with the descriptions provided in the Arizona State University Dental Anthropology System (ASUDAS).

Results: Eight of the eleven features examined (Carabelli trait, Hypocone absence, Cusp 5, Cusp 6, Tomes root, mesial canine ridge, Y-groove pattern, and four-cusp mandibular second molar) exhibited frequencies that were distinctly higher or lower than the three major continental ancestries.

Discussion: Some amateur historians doubted the authenticity of the written versions and argued that these remains might belong to the non-Indians. Present study results revealed that the studied dental trait frequencies were not similar to any major continental ancestry and were relatively unique in Ajnala teeth like previous Indian studies. While this in itself does not guarantee that these teeth belong to individuals of the Indian subcontinent, it endorses previous scientific analyses and supports the written accounts that the majority of Ajnala teeth were probably Indian in origin. However, the determination of ethnicity from dental morphological features remains debatable and can be used only as suggestive than diagnostic tool owing to possible bias in recording morphological features of teeth. Although determining the racial affinity from teeth is very difficult, caution must be exercised in concluding the racial identity of an individual from the teeth.

INTRODUCTION

Forensic anthropological identification of commingled human remains recovered from diverse contexts is a more challenging and complex process due to material obstacles like poor preservation, severe damage or fragmentation, missing elements, and lastly the inadequate historical records about past armed conflicts, natural or mass disasters which produced such human remains. The expert-mediated retrieval of intact skeletal remains from burial sites like trenches, abandoned wells, *nallahs*, etc., and use of advanced scientific methods and techniques becomes essential for successful identification of disarticulated inter-mixed human remains. Such remains belong either to civilian victims of violent conflict or combatants who sacrificed on behalf of a nation. A multidisciplinary approach is essential to resolve the issue of identification of large commingled assemblages using multiple strategies like osteometric pair-matchings, exclusions and associations, simulations, DNA sequencing strategies.[1] The recovery, segregation, inventory and articulation of commingled human remains from mass burial sites pose a difficult challenge. In case of the present study, skeletal remains resulting from state-sponsored violence, the commingling was further exacerbated by their non-scientific excavation which discouraged the establishment of social identity of the remains and nature of violence committed against the victims. Each commingled assemblage is unique in its history, challenges, and context, and no single identification approach is universally applicable to the diverse situations under which CHR are recovered.[2] Restoring individual identity to remains and repatriation of these badly damaged remains to their relatives for last rites is the ultimate purpose of almost all forensic anthropological analyses. Recovering, reassembling, and naming individuals who died in war or war-related violent conflicts is itself a form of honouring and remembering the victims.[1]

The badly damaged, skeletonized and commingled human remains present a serious challenge, whose identification has been attempted by the application of different physical anthropological methods and techniques [3-4]. The identity establishment of an unknown badly damaged skeleton remains requires a collective multi-disciplinary approach involving forensic anthropologists, odontologists, pathologists and DNA scientists. When such human remains are retrieved by forensic anthropologists, or bio-archaeologists, the first step is to establish the identity affiliations (i.e., sex, age, stature, ethnicity) of the remains wherein the experts from diverse scientific disciplines can contribute significantly. Identifying commingled human remains is a sophisticated task that requires significant time, energy, resources, expertise, and application of innovative methods[1]. Individualizing identification of war dead, or victims of violent conflict, natural disaster, or whatever incident of mass fatality, is a complex phenomenon and is a primary objective for forensic anthropological analyses in medicolegal and humanitarian contexts.[1] When a human skeleton or its elements are recovered from the site of mass disasters or are unearthed during archaeological excavation or construction activities (road, canal or building), a forensic anthropologist is generally warranted to identify them, to ascertain the minimum number of individuals, to detect signs of any traumatic or pathological injuries in the bones and teeth or to identify any kind of alteration/damage inflicted to the skeleton(s). Forensic anthropological or archaeological methods play a significant role in biological profiling of unknown human remains retrieved from complex recoveries.

Teeth are the strongest part of human body which have served as crucial evidence in mass fatality incidents to establish the biological identity of severely mutilated bodies or badly damaged skeletal remains.[5,6] Teeth can provide significant information about the cultural practices, dietary behaviour, lifestyle and occupation (such as electrician, carpenter, shoe maker, tailor and musician) of an individual.[7] The teeth of shoe makers, electrician, upholsterers, glass blowers, dress designers/makers and seamstresses have abrasive modifications resulting from holding nails, tacks, needles and glass tube between the teeth. Wind instrument players or musicians develop dental defects.[8-11] The existence of a notch in the incisal edge of a central or central incisor is common in dress makers, seamstresses and electricians whereas grooving may be present in the incisor teeth of carpenters and roof tilers.[12] The radiation damage to teeth in X-ray technicians, radiographers and watch dial painters generally results in radiation caries, gingival recession, periodontitis, alveolar bone damage and causes loosening of teeth and root resorption.[13] Various morphological characteristics of teeth are specifically present in certain population groups and can be used to differentiate ethnicity affiliations of an individual. Dental characteristics include the curves, bulges, cusp pattern, cusp frequency, cusp position, groove pattern, number of roots, number of teeth, bends, furrows, joining (linkage), that appear in various sizes, shapes on the crown and root for assessment of ethnicity.[14] Dental anthropologists have classified human dental variations (non-metric dental traits) into two basic types; the first type features involve major deviations (dental anomalies) from the normal dental blueprint (e.g., extra tooth or fused teeth). The other types of dental variation are minor and more delicate ones that involve variation in secondary cusps, fissure patterns, and supernumerary roots (amongst others).[15] The various unique characteristics/features associated with the human dentition are those which can be visually recorded from the teeth. The dental anomalies can be in the form of the defects, abnormalities of the size, shape, numbers, structure, eruption, or position of the teeth. The structure and shape of teeth and details of their arrangement in the dental arches provides information that can be unique to an individual. Even in identical twins, the slight variations in tooth form and position can enable the twins to be separated on the basis of their dentition.[16] Therefore, dental anomalies are special features of the teeth which are helpful in distinguishing and identifying the sex and ancestry of an individual. Dental anthropologists have been studying the dental variations since the 19th century as they are more common and vary within and between populations, and thus, are largely considered to be useful in evolutionary and forensic contexts.[15]

About the Study Material:
The written accounts mentioned that human cadavers of 282 Indian 'mutineer' soldiers were dumped into an abandoned well at Ajnala (Amritsar, India) and then a religious structure was built over its periphery. About 500 soldiers of the 26th Native Infantry regiment of the British army in India had revolted against colonial rule in July 1857, who were, subsequently captured, killed and their cadavers were intentionally concealed underneath a religious structure at Ajnala.[17-19] The sanitary concerns were cited as the immediate reason for their disposal in a nearby deserted well. It was only in late 2013 that awareness of the remains was created following a chance reading about it in the book written by the British administrator of Amritsar at that time.[17] The most emergent reason advocated for their excavation was to scrutinize the authenticity of the written records about presence of any remains underneath a religious structure in Ajnala. Some amateur historians doubted the truthfulness of the written versions and argued that the said excavated remains might belong to non-Indians.[20] The bundles of severely damaged and commingled human remains were retrieved from the Ajnala well by amateur excavators which presented a tough challenge for their identification owing to their non-scientific excavation. Thousands of human skeletal remains along with the associated personalized items like colonial-era coins, army medals, hand bracelets, iron and copper rings, metalloid arm-bands, gold beads and amulets, etc., dug out from the well sediments corroborated the self-congratulatory but harrowing narrations proclaimed by the author of the book "The Crisis in Punjab: From 10th of May Until Fall of Delhi".[17-18] The application of multiple forensic anthropological methods like osteological, odontological, radiological, and elemental examinations have revealed that the excavated remains belonged to adult males who were non-local to the site, ate mixed diet, and shared a common geographical area during their childhood.[20-24] The main objective of the present investigation was to assess the population affinity of the unknown human dental remains excavated from the Ajnala well based on the frequency distribution of some selected morphological dental traits. The estimated trait frequencies were compared with the published global and Indian data to make the ancestry estimations about the Ajnala victims.

MATERIAL AND METHODS

A total of eleven morphological non-metric dental traits were investigated in a total sample of 1527 teeth (1200 dislodged teeth comprising of 300 canines, 300 premolars and 600 molars along with 93 jaw fragments having 327 teeth of different types) collected from the Ajnala site skeletal assemblage (Figure 1 and 2). Each tooth and jaw fragment was cleaned of any extraneous material like soft tissue, tartar/ calculus; rinsed with distilled water, cleaned and dried, before attempting the morphological trait analysis. In spite of this, tenacious debris, calculus and tooth wear precluded assessment of several teeth, reducing the sample further. The dental non-metric traits were examined and analyzed by first author (AA) with adequate magnification and lighting, using a flexible arm illuminated magnifier (Lensel Optics Pvt. Ltd., Pune, India), identified and scored in accordance with the descriptions provided in the Arizona State University Dental Anthropology System (ASUDAS) referring to Turner et al. (1991)[25] as well as Scott and Turner (1997).[15] Some traits were also examined by the corresponding author, and negligible inter-observer error was noticed. Accordingly, the ordinal scale was converted to a nominal scale to categorize the non-metric trait for each assessed tooth as being present or absent.[15]

Figure 1. Abandonded well unearthered beneath a religious structure at Ajnala

Figure 2. Fragmented, badly damaged and commingled human skeletal remains exhumed from Ajnala well

The description for each morphological characteristic examined is as under:

1) *Cusp of Carabelli:* The Carabelli cusp is an additional tubercle on the first maxillary molars. It is situated at the mesio-palatal site of the tooth (anthropologically, on the 'protocone') (Figure 3).
2) *Three-cusp upper second molar*: The upper second molar usually has four cusps, but one of the cusps (the distolingual/ distopalatal cusp, anthropologically referred to as the 'hypocone') may be missing, resulting in a three-cusp feature (Figure 4).
3) *Four-cusp lower first molar:* The lower first molar usually has five cusps, however, when the distal cusp is missing, it results in the four cusp trait (Figure 5).
4) *Four-cusp lower second molar*: The lower second molar usually has four cusps, however, the distal cusp is present at times, it results in five cusps (Figure 6). In such cases, the tooth is graded as 'absent' for the four-cusp feature.
5) *Cusp 5 or distal accessory tubercle*: It is also referred to as the 'distal accessory tubercle' as the trait is seen as an occlusal tubercle on the distal marginal ridge of the upper first molar (Figure 7).
6) *Cusp 6:* This feature is characterised by the presence of an additional cusp

between the distal and distolingual cusps of the lower first molar.

7) *Cusp 7*: This feature is characterised by the presence of an additional wedge-shaped cusp between the mesiolingual and distolingual cusps of the lower first molar (Figure 8).
8) *Y-groove pattern*: When the mesiolingual and distobuccal cusps of the lower molar are in contact at the central fossa, the resultant groove pattern is referred to as the Y-pattern, which is usually observed on lower second molars.
9) *Mesial canine ridge*: This is a non-metric dental trait characterised by the coalescence of a large cingulum with the mesial marginal ridge of upper canine teeth.
10) *Tomes root*: This is characterised by the presence of an additional root on the lower first premolar, with the tooth exhibiting a buccal and lingual root (Figure 9).
11) *Three-rooted lower first molar*: This is characterised by the presence of an additional root on the lingual surface of the distal root of lower first molar.

The research outline of present study was duly approved by the Institutional Ethics Committee of the Panjab University, Chandigarh (India), vide letter nos. PUIEC/2018/99/A/09//01 dated: January 28, 2018 and PUIEC/201/41/20//05 dated: August 18, 2016

Figure 3. Carabelli trait on an upper right first molar is at least Grade 5 as per the ASUDAS and hence Carabelli cusp (arrow) is considered 'present'

Figure 4. The upper right second molar has three cusps only, with the hypocone missing

Figure 5. In this lower jaw specimen, the four-cusp lower molar is 'present' in the right first and second molar since the distal cusp is absent in both

Figure 6. The four-cusp lower molar feature is 'absent' in the lower left first and second molar of this jaw specimen since the distal cusp (arrows) is present

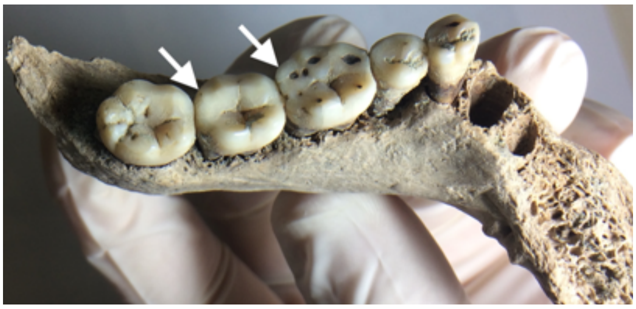


Figure 7. Cusp 5 (arrow) or the 'distal accessory tubercle' on an upper first molar

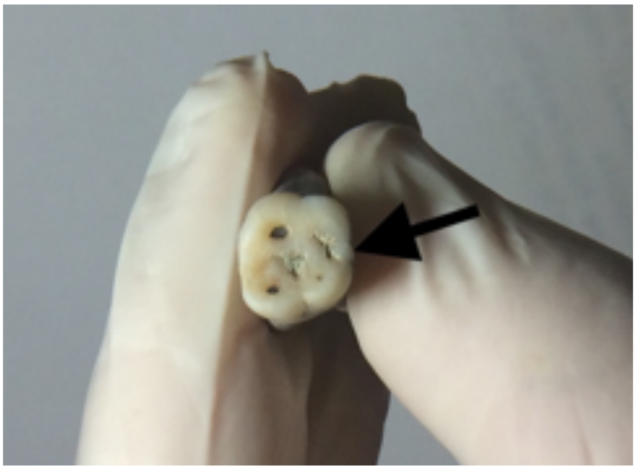


Figure 8. Cusp 7 (arrow) viewed between the two lingual cusps of a lower first molar

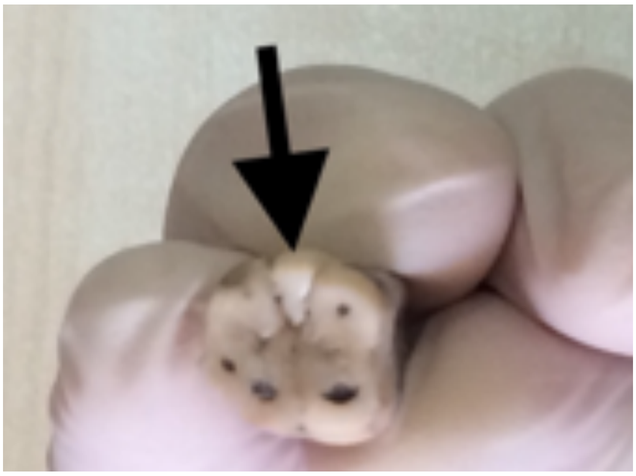
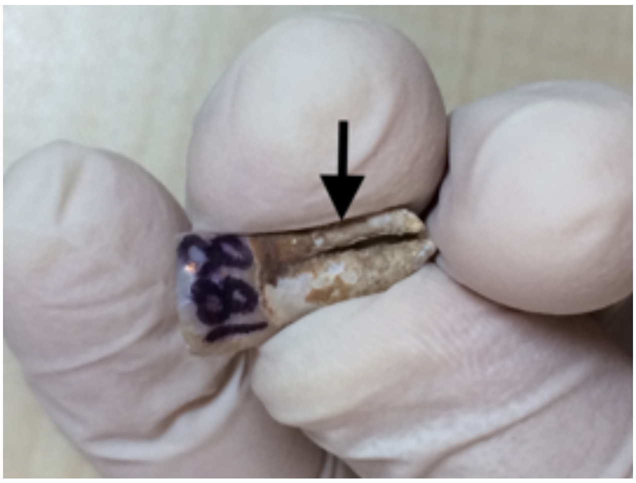


Figure 9. Tomes root seen on a lower first premolar



RESULTS

Out of 125 maxillary first molars assessed, 44 (35.2%) exhibited the Carabelli cusp (≥ Grade 5, as observed in the ASU plaques) (Figure 3). Three-cusp upper second molars were observed in 40 out of 122 (32.8%) upper second molars (Figure 4). Four-cusp lower first molar was a feature in the 10 out of 172 (5.8%) teeth assessed in the present investigation (Figure 5). Four-cusp lower second molar trait was observed in 156 out of 192 (81.3%) teeth (i.e., 36 of the mandibular second molars exhibited the distal cusp and, thereby, five cusps) (Figure 5). The distal accessory tubercle or Cusp 5 was observed in 4.2% (5/120) of the maxillary first molars (Figure 7). Cusp 6 was observed in 3 out of 173 (1.7%) mandibular first molars assessed in present study, while 'Cusp 7' was observed in 14 out of 173 (8.1%) teeth (Figure 8). The Y-groove pattern was observed in 6 out of 192 (3.1%) lower second molars. Out of the 122 upper canine teeth available, wear and debris on the lingual/palatal surface allowed the assessment of 46 tooth specimens of which 14 (30.4%) exhibited the mesial canine ridge. Of the 293 premolars, 34 were mandibular first premolar of which only 2 (5.9%) exhibited the Tomes Root (Figure 9). Three-rooted lower first molar trait was observed in 2 out of the 122 (1.6%) teeth.

DISCUSSION

Identification of the deceased becomes crucial from ethical, legal, social and sometimes political perspectives. Victim identification from the decomposed, skeletonized, incinerated and disintegrated body parts play an important role in forensic death investigations when the traditional methods of visual recognition or dactylography fail to do so. If a human body is found completely or partially skeletonized, identification of victim becomes an uphill task and in such situations, the forensic anthropological and odontological methods play a decisive role in identification.[26] Forensic odontologists glean information from the teeth to decide about their peri-mortem fate, age, sex, dietary status, migration history, geographical affiliations, pathological or traumatic conditions, ancestry etc.[27-28] Attributing population affinity to unknown remains can play a crucial role in establishing their biological identity. The main aim of the present analysis was to estimate the population affinity of the Ajnala skeletal remains from non-metric dental traits to authenticate (endorse or refute) the written versions about geographical affiliations of the victims.[17-19] For this purpose, eleven morphological/non-metric dental features were assessed in the randomly selected dislodged and jaw-located teeth collected from Ajnala skeletal collection.

The frequency distribution of the studied non-metric traits in different teeth types of the Ajnala dental sample and their comparisons with other major continental ancestries has been presented in Table 1. In the present analysis, Carabelli Cusp (≥ grade 5 in the ASU plaques) was present in 35.2% first maxillary molars (Figure 3). The prevalence of this trait was higher than the data available for three major continental ancestries namely, Western Eurasians (22.6%), Sub-Saharan Africans (14.5%), and North-eastern Asians (14%).[15] Angadi and Acharya (2008)[28]—who also used ASU plaques—reported a similarly higher frequency of this non-metric dental trait (26%) in a sample of 100 subjects from India, predominantly from the southern and western regions of the country. While this appears closer to the frequency of Western Eurasians, it is recognizably higher, akin to the present investigation. It is also plausible that the frequency is inherently different here because the sample originates from India. In fact, Carabelli trait is shown to occur in approx. 48% of subjects in another study from India which looked at populations from the southern and south-eastern regions of that country.[29] A study that looked at root traits in a heterogeneous group of South-western Indians observed that the frequency of dental traits for this population "were not similar to any specific geographic subdivision".[30] In fact, they observed the Tomes root in 5% of the mandibular first premolars from their sample, which is near identical to the 5.9% observed in the present investigation (Figure 9). This frequency was lower (and different) than that reported for three major continental ancestries i.e., Western Eurasians (7.3%), Sub-Saharan Africans (6.8%), and North-eastern Asians (8.8%).[15] However, we acknowledge that the Tomes root frequency reported in the current investigation (n = 34) and in Yeli and Acharya (2013) (n = 40) is based on a small sample.

With regards to the three-rooted mandibular first molar, the 1.6% frequency in the present investigation is in between the frequencies for Western Eurasian (0.7%), Sub-Saharan African (2.3%), and North-eastern Asian (20.4%).[15] Yeli and Acharya (2013) found a relatively high 9.3% frequency for the three-rooted first molar trait in a sample of 144 South-western Indian subjects. Therefore, it is likely that Indians may exhibit a frequency for this trait that is in between the frequency of major world populations.

Three-cusp upper second molar (Hypocone *absence*—Figure 4) was observed in 32.8% of the Ajnala teeth is similar to that in Angadi and Acharya (2008)[28], who reported 34% of upper second molars exhibited hypocone absence in their aforementioned Indian sample (n = 100). Again, this frequency is different (and higher) than that has been reported for Western Eurasians (17%), Sub-Saharan Africans (6.7%), and North-eastern Asians (12.7%).[15]

The 5.8% incidence of four cusp lower first molar (Figure 5) in the teeth assessed here is lower than Western Eurasian (9.3%) but higher than Sub-Saharan African (1%) and North-eastern Asian (0.2%) [15]. While this is in between the frequencies of three major human subdivisions and different to them, Angadi and Acharya (2008)[28] reported it in a relatively high 11% of the Indian dentitions. The same trait's frequency of 81.3% in mandibular second molars (Figure 5) compare well with the findings of 90% in the Indian sample of Angadi and Acharya (2008)[28], both of which are higher than for Western Eurasians (72.5%), Sub-Saharan Africans (23.6%), and North-eastern Asians (20.3%).[15]

'Cusp 5' (Figure 7) was observed only in 4.2% of maxillary first molars considered in the present investigation, which is recognizably lower than the frequency data available for Western Eurasians (17.8%), Sub-Saharan Africans (26.7%), and North-eastern Asians (23.6%).[15]

'Cusp 6' was observed in 1.7% of mandibular first molars assessed in the current analysis. This frequency, again, is much lower than the frequency in three major continental ancestries, where it was reported to be present in 9.6% Western Eurasians, 18.8% Sub-Saharan Africans, and 40.1% North-eastern Asians.[15]

'Cusp 7' (Figure 8) was observed in 8.1% mandibular first molars and this frequency falls in between the frequency data available for Western Eurasians (6.9%), Sub-Saharan Africans (28.7%), and North-eastern Asians (6.3%)[15], although the trait frequency observed herein is comparable to Western Eurasians and North-eastern Asians.

Table 1. Frequency distribution (%) of different non-metric features in Ajnala skeletal remains and their comparison with three sub-divisions of humankind

Sr. No.	Dental Trait	Location and definition of feature	Present sample	Western Eurasians	Sub-Saharan Africans	North east Asians
1	Cusp of Carabelli	A tubercle on mesiolingual or lingual aspect of upper 1st molar	35.2	22.6	14.5	14.0
2	Three-cusp upper 2nd Molar	Distolingual cusp (hypocone) missing on upper 2nd molar	32.8	17	6.7	12.7
3	Four-Cusp Lower 1st Molar	Lower 1st Molar	5.8	9.3	1.0	0.2
4	Five-Cusp Lower 2nd Molar	Lower 2nd Molar	81.3	72.5	23.6	20.3
5	Distal accessory tubercle (Cusp 5)	An occlusal tubercle on the distal marginal ridge of upper 1st molar	4.2	17.8	26.7	23.6
6	Cusp 6	Additional cusp between distal and distolingual cusps of lower 1st molar	1.7	9.6	18.8	40.1
7	Cusp 7 (Wedge-shaped)	Additional cusp between mesiolingual and distobuccal cusps on lower 1st molar	8.1	6.9	28.7	6.3
8	Y-groove pattern	Lower 2nd molar (mesiolingual and distobuccal cusps are in contact at central fossa)	3.1	26.6	48.4	14.6
9	Mesial canine ridge	Upper canine characterized by coalescence of a large cingulum with mesial marginal ridge	30.4	4.2	17.6	2.8
10	Tomes root	Additional root on lower first premolar with the tooth having a buccal and lingual root	5.9	7.3	6.8	8.8
11.	Three-rooted lower 1st Molar	Additional root on lingual surface of distal root of lower 1st molar	1.6	0.7	2.3	20.4
12.	Taurodontism					
13.	Shovelling					
14.	Premolar Tuber-apex					
15.	Dilaceration					
16.	Tuberculum Dentale in maxillary canines					
17.	Multiple lingual cusp					

Y-groove pattern trait was observed in 3.1% of mandibular second molars, which is again much lower than the Western Eurasians (26.6%), Sub-Saharan Africans (48.4%), and North-eastern Asians (14.6%) as per the compilation of Scott and Turner (1997).[15]

Lastly, the mesial canine ridge was observed in 30.4% maxillary canines considered here. This frequency was much higher than the three major continental ancestries, namely Western Eurasians (4.2%), Sub-Saharan Africans (17.6%), and North-eastern Asians (2.8%).[15] It is likely that the low number of maxillary canines assessed in our investigation (n = 46) impacted the results and the actual frequency could be lower and comparable to other human subdivisions since findings in another study from India (n = 1042) show that its frequency is 14.3% (which it must be noted is towards the higher end of the spectrum of incidence amongst the three major continental ancestries).

Out of eleven features evaluated, eight exhibited frequencies which were recognizably or distinctly higher or lower than three major continental ancestries namely Western Eurasian, Sub-Saharan African and North-eastern Asians. Three features namely Cusp 7, four cusp lower first molar and three-rooted lower first molar show frequencies which were in between the frequencies of the three major continental ancestries and relatively close to at least one of the them. Two of the non-metric traits with 'distinct' frequency were based on a relatively small sample size (Tomes root of mandibular first premolar and the mesial canine ridge). Still, six out of eleven features—accounting for a majority of the non-metric traits applied herein—that were assessed on relatively large sample size exhibited distinctiveness in their frequencies.

The non-metric dental features of the Ajnala teeth samples could be compared with only a limited number of Indian studies that previously made use of the ASUDAS; some of the recent Indian research that utilized ASUDAS [31-33] comprise of non-Indians and therefore the authors here relied on the studies headed by Acharya and colleagues[29, 30] and another.[29]

A previous work undertaken on a skeletal collection in Denmark showed that relatively accurate population categorization was possible.[34] We believe that the findings herein have parallels to that work wherein a blinded analysis of a skeletal collection (of known provenances in that study) revealed that population attribution was largely accurate and showed expected dental non-metric traits. In fact, those authors found that the results for the dental non-metric traits followed the anticipated frequencies, in general.[34]

From the non-metric dental traits examined in the current sample for estimating their population affinity, it can be concluded that trait frequencies were relatively unique. This in itself does not guarantee that these teeth belong to individuals of the Indian subcontinent but it endorses the findings from previous research that allude to Indians exhibiting a relatively unique frequency for non-metric dental traits.

The results of our analysis in turn are in agreement with written records which mentioned that the remains belong to the Indian-origin soldiers killed in 1857 whose cadavers were dumped into the said disused well at Ajnala,[17-19] Preliminary dental health status [22, 35], odontometrics [36], elemental analysis [21], radiocarbon dating [23] and radiological ageing [22, 24] and molecular[37] results have corroborated that the excavated teeth belonged to adult individuals most likely killed in 1857.

CONCLUSIONS

Bones and teeth have been increasingly used to determine the biological profile of individual(s) as they retain crucial markers of human form and identity after death. Teeth protect invaluable information to answer forensic anthropological queries of provenance, biological identity, taphonomic/traumatic insults, estimation of dietary practices and subsistence patterns, paleopathology, etc. In the present investigation, out of eleven dental features evaluated for which population frequency data existed for comparison, eight traits (comprising almost three-quarters of the assessed features) exhibited frequencies recognizably higher or lower than three major continental ancestries. Although results of two of the features may be undermined by a relatively small sample size, the non-metric dental traits examined in this sample, by and large, have frequencies that are relatively unique. While this per se does not imply that these remains positively belong to the Indian subcontinent, it reaffirms the findings from previous research that allude to Indians exhibiting a relatively unique frequency for non-metric dental traits compared to other major continental ancestries.[28, 30] The present investigation's observations also endorse the previous scientific analyses with these remains that the majority of Ajnala teeth were probably Indian in origin to support the written accounts.[17-19]

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Sex estimation by maxillary sinus using computed tomography: a systematic review

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KEYWORDS

Maxillary sinus; Forensic dentistry; Sex determination analysis; Forensic anthropology

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ABSTRACT

Sex estimation is an important part of forensic human identification, and when primary methods cannot be applied, forensic anthropology becomes an important auxiliary method of identification. The maxillary sinus may remain intact even if the skull is severely damaged and could thus be used in forensic investigations. The objective of this study was to verify the effectiveness of the evaluation of the maxillary sinus as a technique for sex estimation. Articles published in the past 10 years were searched using PubMed, Web of Science, Scopus, and Cochrane databases, including those that used computed tomography to perform maxillary sinus measurements with the purpose of sex estimation. Studies that used radiographs to perform the measurements were excluded because they do not allow for a three-dimensional analysis. Studies that did not provide information regarding the origin of the analyzed population were also excluded. The selected articles were evaluated for methodological quality according to the indication of the objective, studied population, inclusion and exclusion criteria, parameters for obtaining the computed tomography image, measurements evaluated, bias discussion, and ethical authorization. From the 52 articles found, 18 were included. The populations studied included Indians, Iraqis, Iranians, Egyptians, Brazilians, French, Dutch, and Turkish. Height, width, length, and volume of the maxillary sinus were measured. The maxillary sinus can act as an auxiliary tool for sex estimation in forensic investigations.

INTRODUCTION

Human identification can be applied to living individuals, intact corpses, or human remains, and may employ several appropriate methods.[1] Evidence based on forensic science is accepted in a judicial environment, playing an important role in identifying individuals who cannot be recognized visually.[2]

The primary methods of identification recognized by the International Criminal Police Organization (INTERPOL) are fingerprint analysis, DNA, and forensic odontology. Secondary methods of identification include a personal description of the missing person, anthropological information, circumstantial evidence, documentation, and medical procedures performed.[3] Thus, in cases where the

remains are decomposed or charred, when the DNA is destroyed, and when there are no previous dental records, forensic anthropology becomes the method of choice to assist the identification process.[4]
Sex estimation is the first step in anthropological examination,[5] since it represents an important stage in the *post-mortem* profile.[6] In this sense, the evaluation of the skeleton and its remnants can provide useful information. However, acquisition of the entire body is often impossible, with only isolated parts of the body being recovered.[7] Additionally, there are situations where the skull itself is only partially recovered and not all conventional markers may be available for sex estimation.[8] In such situations, evaluation of the maxillary sinus can be useful because its structure can remain intact even if the skull and other bones are severely damaged.[9]
The maxillary sinuses are two cavities located in the maxillary bone that are filled with air. They have thin walls and reach maturity in humans at approximately 20 years of age when most permanent teeth are fully developed.[9] These sinuses may have variations in size, shape, and position, not only in different individuals but also on both sides of the same individual.[10] Thus, this systematic review aims to answer the following question: is the use of maxillary sinus measurements effective in estimating an individual's sex?

MATERIAL AND METHODS

Searches were conducted without language restriction for articles published in the last 10 years (2009-2018) in the following electronic databases: PubMed, Web of Science, Scopus, and Cochrane. The descriptors used were: “maxillary sinus”, “paranasal sinuses”, “maxilla”, “forensic anthropology”, “forensic dentistry”, “sex”, “sex characteristics”, “sex differentiation”, and “sex determination analysis”, as shown in Table 1.
The initial selection was carried out by reading the titles and abstracts of the articles. When these did not provide enough information or if the given abstract was not available, the articles were downloaded and read in full to assess their eligibility. Studies found in more than one database were considered only once. Manual searches were also carried out on the reference lists of the selected articles to determine if any studies were not found by previous searches of the databases. The search and selection processes of the articles were carried out by two researchers and, in case of uncertainty regarding the inclusion or exclusion of any particular article, a third evaluator was consulted until a consensus was reached.
To be selected, studies should have been conducted in accordance with the following PICO format: P (population): subjects underwent maxillary sinus computed tomography (CT); I (intervention): measurements of the maxillary sinuses were performed; C (comparison): comparison between the measurements was recorded; and O (outcome): identification of sex was conducted. The following were excluded from this review: duplicates, literature reviews, studies that did not provide information regarding the origin of the population analyzed, and studies that used radiographs to perform the measurements, since they do not allow three-dimensional observation of the maxillary sinus.
This is because the measurement of the volume of the maxillary sinus is obtained from three dimensions (Fig. 1).
To evaluate the methodological quality of the included articles, a score was applied according to Capitaneanu et al.[11] (Fig. 2). The articles were evaluated according to the objectives, definition of the examined population, established inclusion and exclusion criteria, defined parameters, measurements recorded, study bias, and ethical authorization. When the information requested in each item was answered with "yes", it was scored as "1", while the questions where "no" was obtained as an answer were scored as "0". The maximum score that could be achieved for each article was 11 points.

Table 1. Electronic databases, research strategies, and number of results.

Electronic databases	Research strategies	Total
PubMed	((("maxillary sinus" [MeSH Terms] OR "paranasal sinuses" [MeSH Terms]) OR "maxilla" [MeSH Terms]) AND ("forensic anthropology" [MeSH Terms] OR "forensic dentistry" [MeSH Terms])) AND ((("sex" [MeSH Terms] OR "sex characteristics" [MeSH Terms]) OR "sex differentiation" [MeSH Terms]) OR "sex determination analysis" [MeSH Terms]))	28
Web of Science	TS=((maxillary sinus OR paranasal sinuses OR maxilla) AND (forensic anthropology OR forensic dentistry) AND (sex OR sex differentiation OR sex characteristics))	19
Scopus	(TITLE-ABS-KEY (maxillary AND sinus OR paranasal AND sinuses OR maxilla)) AND (TITLE-ABS-KEY (forensic AND anthropology OR forensic AND dentistry)) AND (TITLE-ABS-KEY (sex OR sex AND differentiation OR sex AND characteristics))	5
Cochrane	"forensic dentistry" in Title Abstract Keyword OR "forensic anthropology" in Title Abstract Keyword AND "maxillary sinus" in Title Abstract Keyword OR "paranasal sinus" in Title Abstract Keyword AND "sex determination" in Title Abstract Keyword	0

Figure 1. Flowchart to identify studies in which maxillary sinus measurements were carried out to determine sex.

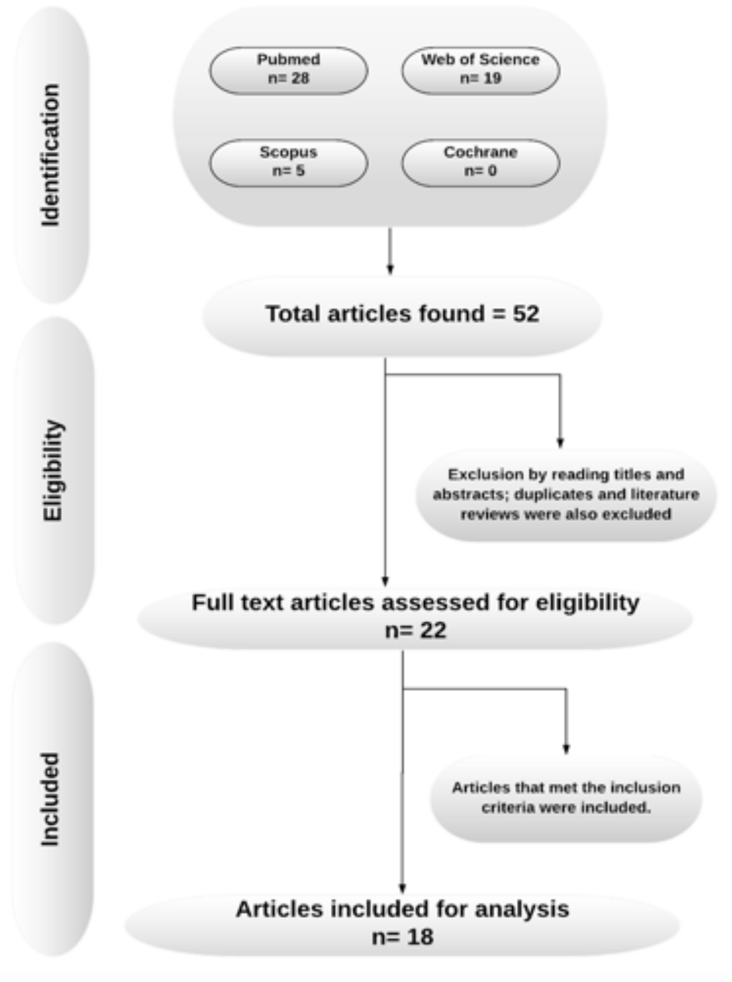

Figure 2. Criteria for research quality assessment. Source: Original article of Capitaneanu et al.(11) (Capitaneanu C, Willems G, Thevissen P. A systematic review of odontological sex estimation methods. *J Forensic Odontostomatol*. 2017;35(2):1–19.)

I. Was the research question or objective clearly stated?
II. Was the study population clearly specified and defined?
III. Were inclusion and exclusion criteria for subjects included in the study sample:
- **A. pre-specified?**
- **B. applied uniformly to all participants?**

IV. Were the studv parameters clearly defined?
V. Were the outcome measures:
- **A. clearly defined?**
- **B. validated?**
- **C. reliable (intra / inter observer)?**

VI. Were the study bias discussed, related to:
- **A. selection bias**
- **B. analytical bias**

VII. Did the study have ethical clearance?

RESULTS

The searches in the electronic databases found 52 articles, of which 22 were considered eligible after reading the titles and abstracts. After removing duplicates and applying the inclusion and exclusion criteria, 18 articles were included in this review (Fig. 1).
Table 2 illustrates that the results of the methodological assessment and the scores received by the studies varied between 5[12–14] and 8[15–17] points. All the studies were scored in questions about the objective, population, and inclusion and exclusion criteria. However, six studies[13,15,16,18–20] did not indicate the parameters used, and therefore, did not receive a score in this regard. The discussion of bias was also not mentioned by any of the authors, and this item was not scored in the methodological scoring. Regarding the ethical approval of the research, nine articles[7,15–17,20–24] addressed this item and were scored.
Information about the sample size, age range, populations studied, measurements recorded, and accuracy rate was extracted from the studies included in this review (Table 3). The sample size of the studies was varied from 30[2,19] to 288[12] individuals. Most authors conducted research in more than one age group, and only Kanthem et al.[19] did not specify that point.
Regarding the studied population, there was a geographical diversity among the surveys, which included participants with Indian,[2,13,14,19,22,23] Iraqi,[18,21,24] Iranian,[12,16,26] Egyptian,[17,25] Brazilian,[15] French,[20] Dutch[27] and Turkish[7] origins. Concerning the measurements recorded, some studies[20,26] used only the volume of the maxillary sinus as a measure, although the height, length, and width of the maxillary sinus were the most frequently recorded measurements.
The accuracy of sexual estimation was not reported by three authors;[13,18,26] nevertheless, Jehan et al.[13] explained that the bizygomatic distance is considered a strong parameter to be used. Some studies have established the anteroposterior diameter of the maxillary sinus[7,14] and width of the left maxillary sinus[21,23] as possible choices for sexual estimation, obtaining the maximum values of accuracy of 75.7% and 61.3%, respectively. Other gender-discriminating variables were also defined, such as the height of the maxillary sinus[16,24] and maximum distance between the left and right sinuses,[12] with respective precision of 77.7% and 65.6%, respectively.

Table 2. Analysis of methodological scores

AUTHOR	I	II	III	IV	V	VI	VII	Total
Ahmed et al. (2015)[21]	1	1	2	0	2	0	1	7
Akhlaghi et al. (2017)[12]	1	1	2	0	1	0	0	5
Amin & Hassan (2012)[25]	1	1	2	1	1	0	0	6
Ekizoglu et al. (2014)[7]	1	1	2	1	1	0	1	7
Etemadi, Seylavi, & Yadegari (2017)[26]	1	1	2	1	2	0	0	7
Gamba et al. (2017)[27]	1	1	2	1	1	0	0	6
Gomes et al. (2018)[15]	1	1	2	1	2	0	1	8
Jasim & Al-Taei (2013)[18]	1	1	2	1	2	0	0	7
Jehan et al. (2014)[13]	1	1	2	0	1	0	0	5
Kanthem et al. (2015)[19]	1	1	2	0	2	0	0	6
Paknahad, Shahidi, & Zarei (2017)[16]	1	1	2	1	2	0	1	8
Prabhat et al. (2016)[2]	1	1	2	1	1	0	0	6
Radulesco et al. (2017)[20]	1	1	2	1	1	0	1	7
Sharma, Jehan, & Kumar (2014)[14]	1	1	2	0	1	0	0	5
Sherif et al. (2017)[17]	1	1	2	1	2	0	1	8
Tanushri et al. (2015)[22]	1	1	2	1	1	0	1	7
Urooge & Patil (2017)[23]	1	1	2	1	1	0	1	7
Uthman et al. (2011)[24]	1	1	2	0	2	0	1	7

Table 3. Summary of selected articles

Study	Subjects	Age (years)	Population's origin	Measurements evaluated	Sex estimation accuracy (%)	Conclusion	QAS *
Ahmed et al. (2015)[21]	119 (M: 57 F: 62)	20–75	Iraqis	Length Width Height	61.3	The diameters of the maxillary sinus can be used as a complement for sex estimation.	7
Akhlaghi et al. (2017)[12]	288 (M: 144 F: 144)	Older than 20 years	Iranians	Maximum height Anteroposterior diameter Width Distance between sinuses	56.2–65.6	In young adults, the parameters of the maxillary sinus had considerable value in sex determination, while in the age group above 50 years, the sinus was small and insufficient for sex determination.	5
Amin & Hassan (2012)[25]	96 (M: 48 F: 48)	20–70	Egyptians	Anteroposterior, transverse, and cephalo-caudal measurements Size	66.7	The cephalo-caudal measurement and the size of the left maxillary sinuses can be useful to support sex determination.	6
Ekizoglu et al. (2014)[7]	140 (M: 70 F: 70)	18–63	Turkish	Anteroposterior, transverse, and cephalo-caudal diameters Volume	77.15	The dimensions of the maxillary sinus will be useful in sex determination.	7
Etemadi, Seylavi, & Yadegari (2017)[26]	70 (M: 35 F: 35)	Older than 18 years	Iranians	Volume	Uninformed	The maxillary sinus volume does not serve as a definite and reliable indicator of sex.	7
Gamba et al. (2017)[27]	160 (M: 80 F: 80)	20–60	Dutch	Height Length Width	75	The developed formula can be applied for sexual prediction as an auxiliary method for human identification in the Dutch population.	6

Table 3. Continuation

Study	Subjects	Age (years)	Population's origin	Measurements evaluated	Sex estimation accuracy (%)	Conclusion	QAS *
Gomes et al. (2018)[15]	94 (M: 45 F: 49)	20–35	Brazilians	Height Length Width Distance between the maxillary sinuses Distance between the infraorbital foramina Volume	84	The formula presented an accuracy of 84% for sex estimation and can be applied as a complementary method of human identification in the Brazilian population.	8
Jasim & Al-Taei (2013)[18]	120 (M: 60 F: 60)	40–69	Iraqis	Volume Width Depth Height	Uninformed	The volume and dimension of the maxillary sinus were greater in males then females. Maxillary sinus measurements with CT could be useful to support sex and age estimation.	7
Jehan et al. (2014)[13]	191 (M: 106 F: 85)	20–70	Indians	Bizygomatic distance Intermaxillary distance Anteroposterior dimension Width	Uninformed	Bizygomatic distance measurements and dimensions of the maxillary sinus can be useful to support sex determination in forensic medicine when other methods are inconclusive.	5
Kanthem et al. (2015)[19]	30 (M: 17 F: 13)	-	Indians	Height Depth Width Volume	15–85.4	The dimensions of the maxillary sinus, especially the volume on the right side, are valuable in the study of sexual dimorphism.	6
Paknahad, Shahidi, & Zarei (2017)[16]	100 (M: 50 F: 50)	20–54	Iranians	Height Width Length	76	Maxillary sinus measurements can be used as an additional tool for sex determination.	8
Prabhat et al. (2016)[2]	30 (M: 15 F: 15)	20–50	Indians	Length Width Height Volume	83.3	The length, width, height, and volume of the maxillary sinus can be used to predict sex.	6

Table 3. Continuation

Study	Subjects	Age (years)	Population's origin	Measurements evaluated	Sex estimation accuracy (%)	Conclusion	QAS *
Radulesco et al. (2017)[20]	103 (M: 50 F: 53)	13–97	French	Volume	68	The volume of the maxillary sinus can be useful for sex estimation in forensic medicine	7
Sharma, Jehan & Kumar (2014)[14]	102 (M: 61 F: 41)	20–60	Indians	Anteroposterior dimension Width Height Volume	67	The volume and dimensions of the maxillary sinus can be useful for sex identification	5
Sherif et al. (2017)[17]	100 (M: 50 F: 50)	18–60	Egyptians	Maximum craniocaudal diameter (CCD) Maximum width Average width Maximum depth Intermaxillary distance	72-76	The maxillary sinus shows the highest level of accuracy in estimating sex.	8
Tanushri et al. (2015)[22]	40 (M: 20 F: 20)	20–60	Indians	Width Height Length	67	The length, width, and height of the maxillary sinuses predict an individual's sex with a reasonable degree of accuracy.	7
Urooge & Patil (2017)[23]	100 (M: 50 F: 50)	20–50	Indians	Width Length Height Area Perimeter Volume	71	The female group showed statistically significant higher values for the width of the left maxillary sinus, but the other parameters showed no significant difference between sexes.	7
Uthman et al. (2011)[24]	88 (M: 43 F: 45)	20–49	Iraqis	Width Length Height Distance between both sinuses	73.9	The CT images can provide adequate measurements for the maxillary sinuses.	7

DISCUSSION

Sex estimation is an important part of forensic human identification. However, there may be situations in which primary techniques cannot be employed as a result of *post-mortem* degeneration of the human body[20] in addition to the absence of dentition. Evaluation of the maxillary sinus can emerge as a viable methodology for sex estimation in such situations.[28]

Measurement of the dimensions of the maxillary sinus can be performed with great speed and accuracy with a CT scan[29]. One of its advantages is that there is no overlapping of structures outside the plane of interest. Additionally, the availability of *ante-mortem* tomographic films can offer useful elements for the exact reproduction of the examination after death.[30]

When different age groups are studied, different results are obtained. According to a previous study, younger individuals may have larger maxillary sinus dimensions,[22] while another study reported no difference in the sinus dimensions related to age and the measurements evaluated.[20] The maxillary sinus is known to increase in size throughout childhood and adolescence, but there are still very few detailed descriptions of subsequent changes in adults and the elderly.[31] Moreover, advanced age is not necessarily related to tooth loss, but studies usually consider only the patient's age, forgetting an important factor, which is edentulism. Thus, it is relevant that the status of the dentition is cited, since sex estimation through evaluation of the maxillary sinus without the presence/absence of teeth being included in the analysis, is not entirely possible.[32]

Regarding the measurements evaluated in patients with dentate and edentulous jaws, different values can be found in the literature for each dimension analyzed. Some studies[18,33] claim that there are no differences in values for the volume, width, and depth of the maxillary sinus, while greater measurements were obtained for the height in individuals with tooth loss.[18] It is known that the extraction of a posterior dental unit stimulates more severe resorption of the alveolar crest promoted by the absence of masticatory function.[34] In addition, the initial bone remodeling that occurs during alveolar ossification promotes new bone organization in the edentulous region, whereas an increase in tooth loss and consequent less bone stress drives its degradation. Thus, dentition affects the pneumatization of the maxillary sinus and is also related to the presence of oral atrophy.[32]

The studies selected for this review analyzed several measurements of the maxillary sinus to determine the accuracy of each of them in estimating sex. The vast majority of the results demonstrated that the dimensions of the maxillary sinuses were larger in males than in females.(2,7,20–22,24–27,12–19) This was explained initially by the fact that men require larger lungs to support their relatively larger muscles and body organs. Moreover, physiological changes in the size and shape of the nasal cavity occur because of the necessity of breathing, such as heating and humidifying the inhaled air. Thus, by occupying the remaining space within the nasomaxillary complex, the maxillary sinus also increases in size.[14]

The variations in the identified accuracy rates (15%,[19] 61.3%, and[21] 84%[15]) can be motivated by different aspects. First, the great geographical diversity in the studies included in this review can be noted, as populations from India, Iraq, Iran, Egypt, Brazil, France, the Netherlands, and Turkey were analyzed. In a more heterogeneous population, for example, the Brazilian population, sexual dimorphism is easier to identify when compared to more homogeneous population groups, such as the Europeans. Thus, it should be borne in mind that each population has distinct morphological characteristics, being influenced by both genetic and environmental factors.[27] In addition, the accuracy values can also be impacted by elements, such as bone fragment conditions,[33] the methodological and statistical analyses adopted, different radiographic techniques, and unequal sample sizes.[16]

CONCLUSIONS

The measurement of the maxillary sinus dimensions can serve as an auxiliary method for estimating sex, but should be applied with caution, since its efficacy may vary because of various factors, such as the population, conditions in which the maxillary sinus was found, and radiographic techniques adopt

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Human identification through DNA analysis of teeth using powder-free method – A case study

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KEYWORDS

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ABSTRACT

Change is the universal law of nature, and human bodies after death cannot be an exception for a long time. In forensic science, the tissue from the hardest part of the human body is the only hope to establish the identity, and maternity/paternity of unidentified dead bodies. In this case, a foreign national on a tourist visa to one of the Himalayan states went missing when passing through a dense forest. His relatives could not trace him despite the best efforts of the search team, because of inaccessible hilly terrain. Later on, shepherds while grazing their livestock in the forest area accidentally came across the fragmented remains of a human skeleton. They informed the villagers, and then the police. Teeth collected during the autopsy and blood samples of the putative son, and wife of the missing foreign national on FTA (Flinders Technology Associates) cards were sent to DNA Division, State Forensic Science Laboratory, Junga, Shimla, Himachal Pradesh to establish the identity. DNA profiles obtained from the blood samples of the putative son, wife of missing foreign national, and teeth showed a complete, and concordant match, which established the identity of the skeleton. Moreover, the probability of paternity (>99.99%) between unidentified deceased person and the putative son also assessed the identity of the deceased. Hence, human teeth from unidentified dead bodies can establish the identity of unidentified deceased persons.

INTRODUCTION

Human identification based on teeth is needed if the possibility of achieving identification is to be maximised.[1] Forensic disciplines use teeth in establishing identity and maternity/paternity of unidentified human skeletal remains because of easy access, availability, and resistance of useful tissue to degradation even in extreme environmental conditions.[2,3] Further studies over time in this area suggest that the preservation of DNA in teeth is better than in bones.[4,5] Moreover, DNA of high quality can be isolated from teeth.[6,7] There is also less chance of contamination of DNA isolated from teeth.[8] Teeth were used as a source of DNA from mass graves resulting from wars, and armed conflicts.[9,10] Hence, teeth play an important role in forensic science. In the present case, human identity was established from the teeth of a foreign national found dead in a remote Himalayan area. According to investigating officers, the wife of the deceased reported her missing husband at the local Police station. She made a statement that her husband went trekking in the forest and did not return. Police and search teams, despite their best efforts, could not find the person. After a few days, local shepherds, while grazing their livestock in the forest found a partially decomposed and skeletonized human body, eaten by animals. They first informed the villagers and then the police. Based on torn pieces of clothing, found at the site, the wife of the deceased recognized the clothing as her husbamd's. To confirm the identity, the teeth of the deceased person, blood samples of the putative son, and wife of the deceased on FTA cards were received in the DNA Division, State Forensic Science Laboratory, Junga, Shimla, Himachal Pradesh. By comparing the DNA profiles of the deceased person with putative son, the probability of paternity confirmed the identity of the unidentified deceased person.

MATERIAL AND METHODS

Materials

Seven intact and healthy teeth (molar and canine) of the unidentified deceased person, blood samples of putative son and deceased's wife on FTA cards were received for routine casework analysis at DNA Division, State Forensic Science Laboratory, Junga, Shimla, Himachal Pradesh and labelled as A, B, C, respectively. Since molar teeth contain optimum DNA, they are selected for DNA retrieval using the EZ1 DNA Investigator Kit from QIAGEN, Hilden, Germany. The PowerPlex® 21 PCR amplification kit was procured from Promega Corporation, Wisconsin, United States.

Methods

DNA isolation

The powder-free method was used for DNA isolation from the tooth with Qiagen EZ1 Advanced XL BioRobot.[11,12] In brief, the molar tooth was cleaned properly by scraping with a sterilized blade. The tooth was vortexed with absolute alcohol in a falcon tube and kept for two hours to remove microbial contamination. The tooth was drained of alcohol and dried at room temperature. After complete drying, the tooth was fractured with a hammer and all the pieces, with the root, were placed in an autoclaved micro vial (1.5 ml). Added to this were, buffer G2 (500 µl), proteinase K (25 µl) and lysed at 56°C for 72 hours in a NB 20 water bath (Nuve, Ankara, Turkey). The lysate containing the extracted DNA was poured into a sample tube (2 ml) and the remaining tooth pieces were discarded. Then inserted into the elution tube, was a tip holder containing filter-tip, and reagent cartridge in an EZ1® Advanced XL BioRobot (QIAGEN, Hilden, Germany) as per instructions given in the handbook. The DNA isolation was done with "Large-Volume Protocol" without adding MTL buffer, as there was sufficient lysate. The isolated DNA was stored at -20°C in a refrigerator (Celfrost, India) for further use. The DNA from FTA cards was purified as per method with slight modifications[13]. The FTA cards bearing blood samples of putative son and deceased's wife were punched with the help of Harris 1.2 mm micro punch. Punches were added in two separate micro vials containing FTA purification reagent (200 µl) and proteinase K (25 µl). The micro vials containing punches were incubated at 56°C in a NB 20 water bath (Nuve, Ankara, Turkey) for two hours, then washed twice with autoclaved distilled water and dried in a digital dry bath (Labnet International, U.S.A.). The dried FTA card punches were stored at -20°C in a refrigerator (Celfrost, India) for further use.

Pcr amplification

The isolated and purified DNA were subjected to PCR amplification with PowerPlex® 21 System kit[14]. In brief, master mix (5 µl) and primer mix (5 µl) was added in three separate PCR tubes followed by isolated DNA (15 µl) from the tooth (A) and one punch from FTA cards of the putative son (B) and deceased's wife (C). To complete reaction mixture in PCR tubes containing FTA card punches (B and C), 15 µl nuclease-free water was also added. The contents in the PCR tubes were mixed thoroughly and spun in a SPINWIN microcentrifuge (Tarsons, India). The PCR tubes were put into GeneAmp®PCR System 9700 thermocycler (Applied Biosystems, U.S.A.) and amplification was performed according to the manufacturer instructions for the PCR amplification kit. The amplified products were quantified using agarose gel electrophoresis (2%). The appropriate dilutions were made for further capillary electrophoresis.

Capillary electrophoresis
Capillary electrophoresis of amplified products was carried out with ABI 3130 Genetic Analyzer with 4-capillary using POP-4 at a current of 15 Ampere (Applied Biosystems, U.S.A.). The genotyping was carried out using GeneMapper® ID Software Version 3.2.

Biostatistical calculations
The paternity index (PI) for each marker was calculated using the following equation:

$$PI = X/Y$$

Where, X = chances of alleged father is the biological father

Y = any randomly selected person from concerned population is the biological father

The combined paternity index (CPI) was also calculated by multiplying PI values. Using CPI value, probability of paternity was calculated using following formula:

Probability of paternity = CPI/CPI + 1 x 100

These values help in inclusion or exclusion of the alleged father to be biological father of putative son.

RESULTS

The Punnett square table of autosomal STR DNA profiles generated from the PowerPlex®21 kit is shown in table 1. Amelogenin is a gender determining marker, and help in the identification of the sex of the individual. As shown in the table, amelogenin displayed "XY" alleles in the profile of the unidentified deceased person (A), which confirmed that the person was male. The electropherogram also showed amplification at all the 21 autosomal STR loci (Fig. 1). A complete DNA profile with amplification at all the 21 autosomal STR loci was also obtained from the putative son (B) with "XY" alleles at amelogenin (Fig. 2). By comparing these DNA profiles, it was observed that one of the two alleles in the genotype of the unidentified deceased person (A) showed a match with one of the non-maternal alleles found in the genotype of the putative son (shown as bold and underlined) at 20 loci except for amelogenin. This data included that unidentified deceased person as the biological father of the putative son. In addition to this, the genotype of deceased's wife (C) at amelogenin showed "XX" alleles, confirming female individual (Table 1). The electropherogram of the deceased's wife showed amplification at all the 21 autosomal STR loci (Fig. 3). One of the two alleles in the genotype of the deceased's wife also showed a match with one of the two maternal alleles in the genotype of the putative son (B) at 20 autosomal STR loci except for amelogenin (non-underlined). This data included that the deceased's wife as the biological mother of the putative son. The transfer of parental alleles (unidentified deceased person and his wife) to son followed the law of Mendelian inheritance, which assessed the identity of the deceased. Moreover, the combined paternity index (CPI) in this case was calculated to be 1,880,0922,263,36 (1.88009E+12), which resulted in the probability of paternity of >99.99%. This data also suggested that unidentified deceased person cannot be excluded as the biological father of putative son. Hence, identity of unidentified deceased person was established.

Teeth play an important role in forensic science to confirm the identity and maternity/ paternity of the unidentified dead body. The identification of such dead bodies is important for socio-legal purposes. Teeth are used for identification purposes for unidentified dead bodies. Kaur et al.[15] in a case, developed DNA profile from the teeth of an unidentified decomposed human body, who was murdered and established paternity by comparing the DNA profiles with putative children. Sweet and Sweet[16] solved a female homicide case in which the victim was completely burnt with gasoline and it became difficult to identify the person. However, the teeth of the victim tolerated the extreme temperature of gasoline and the authors were able to isolate high molecular weight DNA from the dental pulp of molar teeth. DNA profiling was done and the victim was identified. Dutra Correa et al.[17] developed full DNA profiles from the teeth of a carbonized and a skeletonized human body using the non-powder method. In another report, Xavier et al.[18] evaluated the possibility of DNA extraction from primary teeth in disaster victim identification with probative value. Recently, Dutra Correa et al.[3] performed human identification by DNA typing of healthy and restored teeth of exhumed remains. These findings suggest that teeth are less prone to degradation and contamination.[19] Further, conventional method for teeth samples has a scope for loss of DNA yield besides contamination issues. But powder-free method involves all parts of tooth viz. enamel, dentine, pulp, cementum, and root which result in high quality DNA yield leading to complete DNA profiles for comparative analysis. Hence, teeth are a good source of DNA from decomposed human bodies for identification purposes.

Table 1. Punett square table of the analysed samples

Genetic markers	Unidentified deceased person (A)		Putative son (B)		Deceased's wife (C)	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
Amelogenin	X	Y	X	Y	X	X
D3S1358	13	18	17	**18**	17	17
D1S1656	14	17.3	12	**14**	11	12
D6S1043	12	19	12	**19**	12	13
D13S317	11	14	8	**11**	8	8
Penta E	12	14	11	**12**	10	11
D16S539	11	11	**11**	12	8	12
D18S51	12	16	16	**16**	12	16
D2S1338	22	23	**23**	24	20	24
CSF1PO	11	12	11	**11**	11	12
Penta D	9	9	**9**	10	10	13
TH01	8	8	**8**	9.3	6	9.3
vWA	17	17	**17**	18	14	18
D21S11	27	33.2	**27**	30	30	31
D7S820	11	12	8	**12**	8	8
D5S818	13	13	13	**13**	12	13
TPOX	11	11	11	**11**	8	11
D8S1179	10	15	**10**	16	14	16
D12S391	18.3	23	21	**23**	18	21
D19S433	14	16	14	**16**	13	,14
FGA	22	24	**24**	25	23	25

Figure 1. Electropherogram of DNA from teeth of unidentified deceased person (A)

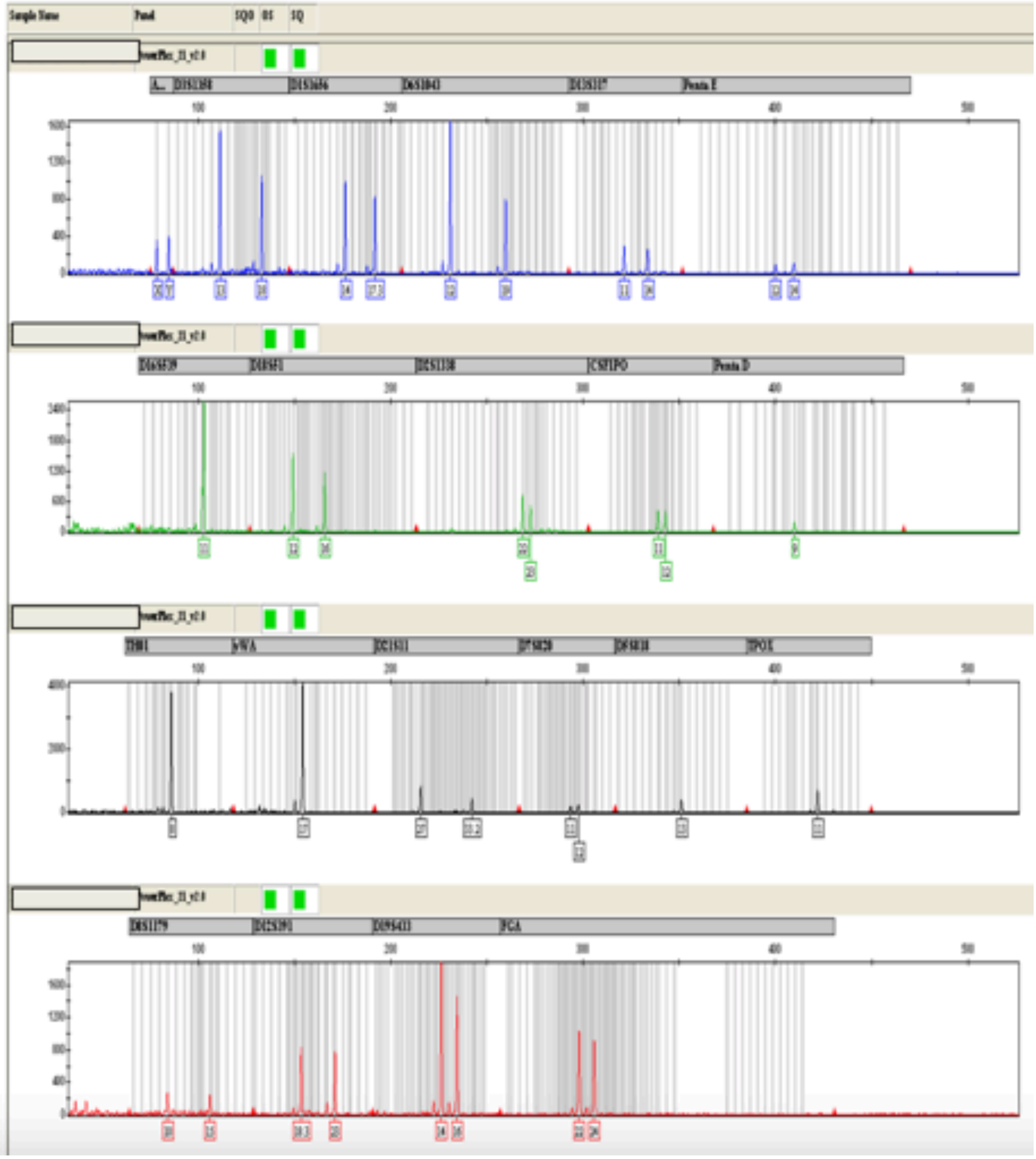


Figure 2. Electropherogram of DNA from FTA card of putative son (B)

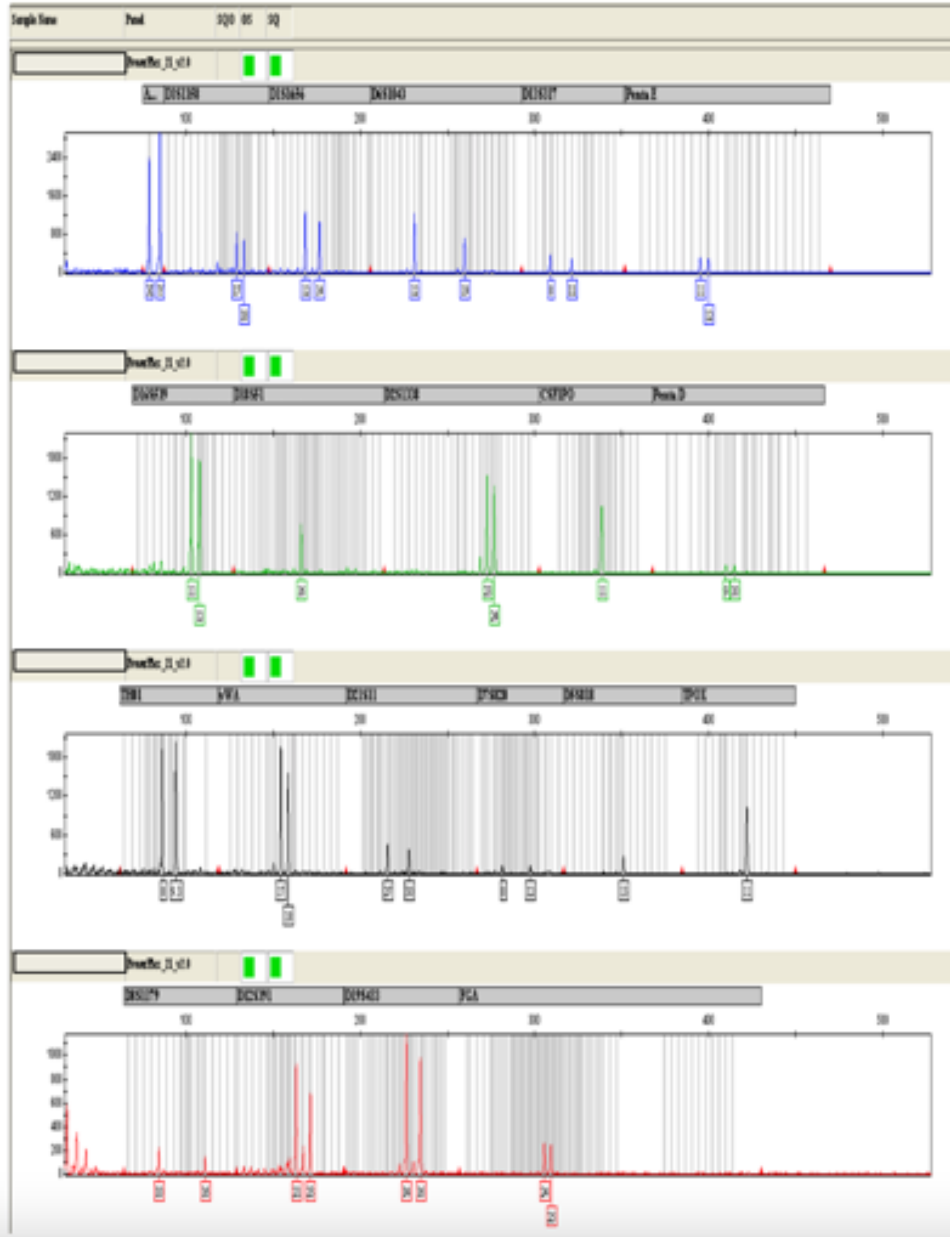
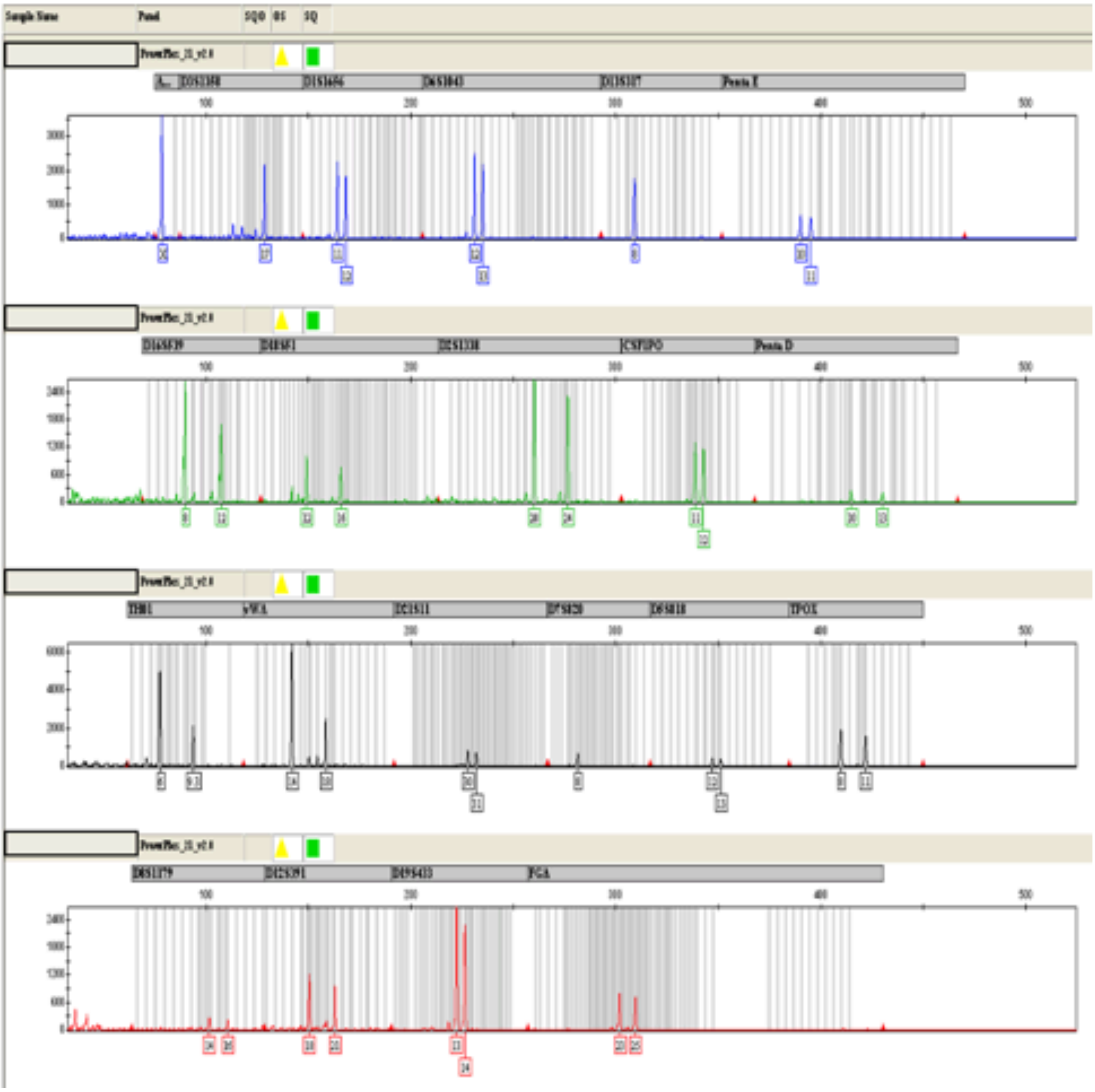


Figure 3. Electropherogram of DNA from FTA card of deceased's wife (C)



CONCLUSIONS

DNA profiling plays an important role in the identification of decomposed human bodies. In this scenario, teeth are important exhibits for DNA profiling, since teeth protect DNA from harsh environmental conditions as compared to other body parts. DNA profiles generated from teeth can be used to establish identity, maternity, and paternity. However, there are few studies available on this topic. There should be more studies on the establishment of identity from the teeth of unidentified dead bodies. In some cases, medical practitioners send broken teeth for DNA profiling after post-mortem to forensic laboratories. Such types of teeth yield degraded DNA, which results in partial DNA profiles. Full DNA profiles generated from teeth can be helpful to make DNA databases at the state and national levels.

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