ISSN 2219-6749

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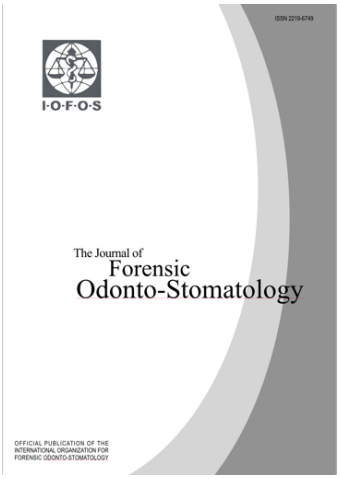
The Journal of Forensic Odonto-Stomatology

Volume 38, n. 1 - May 2020

OFFICIAL PUBLICATION OF THE
INTERNATIONAL ORGANIZATION FOR
FORENSIC ODONTO-STOMATOLOGY

JFOS
ISSN: 2219-6749



THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

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The Journal of Forensic Odonto-Stomatology

Vol 38 n. 1 May 2020

CONTENTS

Risk and limits in dental practice: a Portuguese approach to medical-legal evaluation and professional liability

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KEYWORDS

Forensic Science,
Forensic dentistry,
Law,
Impairment,
Dental practice,
Orofacial

J Forensic Odontostomatol
2020. May;(38): 1-2:7
ISSN :2219-6749

ABSTRACT

Patient safety and quality of healthcare delivery systems are an objective of WHO. This study aims to present and analyse Portuguese clinical data on risk and malpractice in dental practice. Data from the Forensic Dentistry Laboratory (Faculty of Medicine, University of Coimbra) was analysed, between the years of 2013 to 2018. One hundred and seven technical reports were selected, and seventy nine files were included in the iatrogenic sequelae group. Data included the analysis of the performance of dental professionals. Sequelae were divided in descending order of occurrence:1) mandibular dysfunction (53,2%)[(42)79]; 2) neurological deficit (39,2%)[(31)79]; 3) tooth loss (6,3%)[(5)79]; and 4) opening deficit (1,3%)[(1)79].Three major areas with significant expression in the field of expert evaluations were analysed: 1) orthodontic treatment (51,9%), implant rehabilitation (29,1%), and oral surgery. Given the prevalence of malpractice, the need to assess its causes and recognise standards for its prevention is necessary.

INTRODUCTION

In 2002, the World Health Organisation highlighted the problem of patient safety. In the context of public interest, patient safety was associated with the evaluation of the occurrence of good practice.[1,2] The assessment evaluation of professional liability and forensic investigation arises in synergy in obtaining data for a court decision[2]. The data will allow clarification and allocation of medical liability according to the context of medical law: criminal, civil, or labour[2]. An individual´s integrity analysed in a medico-legal context is valued according to the guidelines of each country[2]. To ensure the medical safety of patients, it must consider patient factors as well as professional factors. The European Medical Risk-Related History (EMRRH) is an example of a tool to register medical pathology that interferes with dental treatment and to indicate the degree of medical risks in 10 European countries[3]. The professional factor is an unnerving and sensitive topic! There is a consensus that the medical or surgical procedures may lead to a change in patient's health status, physical and mental integrity[4-10].

Iatrogenic sequelae can be: 1) post-operative complication or risk of the procedure, which means supported by good practice[2] and statistically known[7-12]. In opposition, one can consider malpractice as an incorrect procedure or conduct[2,4,11-15]. Dental malpractice is related to the absence or insufficiency of information between the patient and the health

professional, and it also can be related to the error or delay in the diagnosis and therapeutic failure[11-15].

A retrospective analysis performed in a dental medico-legal Portuguese database aims to highlight various aspects of malpractice, allowing some reflections on the safety and quality of health care delivery systems in the dental-medical context.

MATERIAL AND METHODS

A retrospective analysis was performed using the database of Forensic Dentistry Laboratory of the (Faculty of Medicine, University of Coimbra, Portugal) between 2013-2018. The files were selected according to the inclusion criteria: age between 18 and 65 years old; data of individuals subjected to expert evaluation in the field of post-traumatic body damage, with court decision; victims of road accidents and aggression (traumatic aetiology) that underwent dental rehabilitation. Oncological and genetic pathology were exclusion criteria. The above mentioned oral rehabilitations were performed by different healthcare professionals associated with their Professional Board[17].

The research team, composed of dental doctors with forensic/orthodontic/prosthodontic practice, with medico-legal evaluation experience, proceeded with the individual/objective examination and carefully informed about the objectives of the study. Informed consent was provided regarding the Helsinki Declaration on human subjects and according to the guidelines of the Ethics Committee of Faculty of Medicine (CE-048/2017).

The causal relationship between the sequel and the description of the traumatic event was ensured in each report. The sequelae were identified and categorised according to the table of disabilities[18,19]. The descriptive analysis of the data was performed, according to 1) the sequel category according to direct or indirect relation with the disease[20] and according to excellent practice performance, risk or malpractice[2]; 2) the dental rehabilitation; and 3) the timeline of the dental rehabilitation.

RESULTS

In the database records of Forensic Dentistry Laboratory, 107 cases were selected, between 2013 to 2018. Over 79 cases (73,8%) [(79)107] were judged for iatrogenic sequelae, of which 19 cases (24,1%) [(19)79] were evaluated as malpractice procedures. The mean age of patients filing a complaint was 41 years, with a range from 18 to 65 years, distributed by the female (78%) and male (22%) groups.

Table 1. The chart represents the corporal damage assessment and the type of iatrogenic sequel.

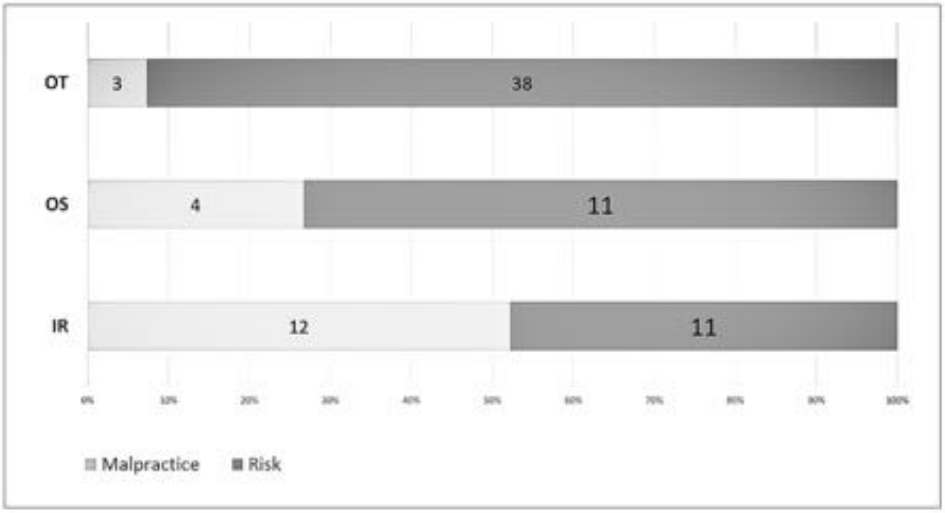
	Clinical findings				N(%)		
Sequelae	**Direct**				28(16.2)		**107**
	Indirect	**Risk/ Malpractice**	**Clinical Description**			79(73.8)	
			Tooth Loss	Incisor/Canine/Premolar	5(6.3)		
			Mandibular Dysfunction	Temporomandibular dysfunction	42(53.2)		
				Maxillary atrophy			
				Non-anatomical reduction of mandibular fracture			
			Opening Deficit	Opening restriction	1(1.3)		
			Neurological Deficit	Asymmetry of lip commissures	31(39.2)		
				Hypoaesthesia, anesthesia, paraesthesia or dysaesthesia			
				Absence of activity			
				Taste alterations			

Sequelae were divided, according to disease criteria, into: 1) direct sequelae, resulting from the normal disease evolution (16,2%), and 2) indirect or iatrogenic sequelae, resulting from the technical or scientific intervention of the health professional (73,8%) (table 1). Iatrogenic sequelae group was divided into: a) risk or complication sequelae, resulting from normal consequences of iatrogenic procedure (75,9%)[(60)79], and b) incorrect sequelae, or malpractice, resulting from incorrect practice procedures or failure to comply with good practice (24,1%)[(19)79]. According to clinical evidence and impairment tables[18,19] sequelae were divided in descending order of occurrence: 1) mandibular disfunction (53,2%) [(42)79]; 2) neurological deficit (39,2%)[(31)79]; 3) tooth loss (6,3%)[(5)79]; and 4) mouth opening deficit (1,3%)[(1)79] (table 1).

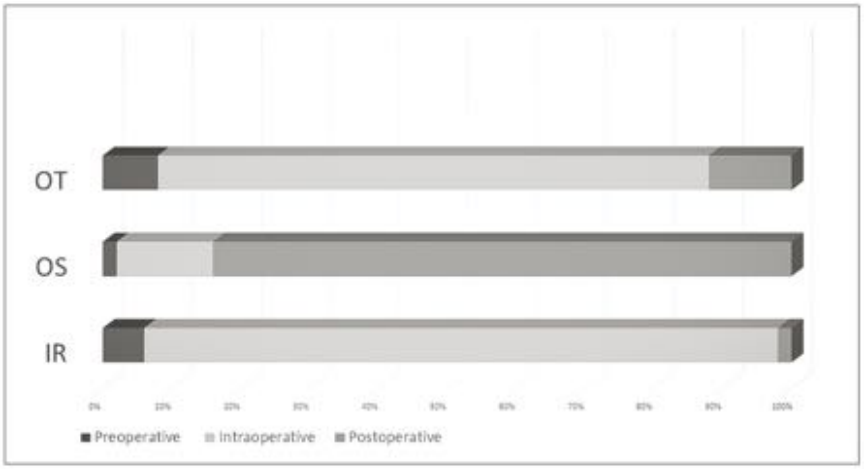
The group of iatrogenic sequelae was divided by rehabilitation fields into:1) orthodontic treatment (OT) (51,9%)[(41)79], implant rehabilitation (IR) (29,1%)[(23)79], and oral surgery (OS) (19%) [(15)79]. According to Graphic 1, the malpractice cases were divided into: 1) IR with 63,2 [(12)19]; OS with 21% [(4)19] and OT with 15,8%[(3)19] (Graphic 1).

The timeline procedure, 1) pre-operative, 2) intra-operative, and 3) post-operative was a criterion for dental practice categorisation. Implant rehabilitation malpractice was mainly related to the intra-operative task (92%/), orthodontics to the intra-operative task (80%), followed by surgery to the post-operative task (84%) (graphic 2).

Graphic 1. Dental rehabilitation [orthodontic treatment (OT), implant rehabilitation (IR), and oral surgery (OS)] and sequel categorisation.



Graphic 2. It represents the correlation between dental rehabilitation type and the procedure's timeline. The dental rehabilitation types were orthodontic treatment (OT), implant rehabilitation (IR), and oral surgery (OS). The timeline of the rehabilitation procedure was pre-operative, intra-operative, and post-operative.



DISCUSSION

As highlighted by Earwaker *et al.*, the legal decision making results from multiple interdependent issues with an emphasis on data sharing and data analysis[21]. According to this study, the community of health professionals can thus adapt and operate with the notion of risk inherent to the healthcare process. In dental malpractice and aesthetic context: Bordonaba-Leiva *et al*[13], present data from Spain; Sarmiento *et al*[12] from the USA; Bordonaba-Leiva[13] and Badenoch-Jones[14] from Australia; Pinchi *et al*[15] from Italy and no data has been published about Portugal.

Iatrogenic dental Portuguese data presented (73,8%)[79(107)], includes iatrogenic risk as complications of the procedure (76%)[60(79)] and clinical findings beyond good practice limits or malpractice (24,1%)[19(79)]. The malpractice data was (24,1%)[19(79)] in line with Bordonaba-Leiva[13] (15,8%).

Our clinical outcomes were categorised according to the sequelae of Portuguese damage assessment tables based on the European guidelines[18-20]. It enables future comparison since there is no uniformity in published studies that allow the correspondence and discussion of these results. The mandibular dysfunction was prevalent in 53,2% of iatrogenic sequelae, taking into account its characterisation (pain with limited mouth opening, temporomandibular dysfunction, maxillary atrophy and non-anatomical reduction of mandibular fractures). The neurological deficit with 39,2% was related to the asymmetry of lip commissures, hypoaesthesia, anaesthesia, paraesthesia or dysaesthesia (inferior dental or lingual nerve), taste alterations, absence of activity of masticatory muscles, and general facial asymmetry[22]. The incisors, canines, and premolars were the type of teeth lost (6,3%). Limitation of mouth opening was reported with 1,3%.

The orofacial neurological changes correspond to sensory or functional deficit and are relevant to the individual's self-esteem[20], socio-professional interactions and, interpersonal relationships. Functions related to the inability to keep food or liquid in the oral cavity, unintentional tongue biting during chewing, difficulty speaking, alterations in phonation affect essential functions in a psychological and social context (e.g., breathing, chewing and communication)[20,22]. The damage assessment of these sequelae established in the medico-legal scope links to the entire trigeminal nerve[18,19]. The mandibular dysfunctions were generally related to pain and degenerative condylar changes. Both sequelae can be associated with early facial ageing, facial asymmetry, and facial disharmony.

Dental risk is well known and classified according to their frequency and importance[6-10,22]. According to Pippi *et al.*[22] , the more invasive a procedure is, the more numerous and relevant the possible sequelae are. Long and complicated treatments could explain our results. A prolonged treatment allows a higher probability of failure in the interaction with the patient, and complex treatment requires a constant scientific update. Our data support that there are three major areas with significant expression in the field of expert evaluations and iatrogenic sequelae: orthodontic treatment (OT) (51,9%)(41)79, implant rehabilitation (IR) (29,1%) (23)79, and oral surgery (OS) (19%) (15)79. Facial asymmetry can be revealed as a frequent risk throughout orthodontic treatment[9,23]. In Thiesen *et al*[23] study facial asymmetry might be concealed by dental compensations and it is related to longer treatment time. External apical root resorption (EARR) is another common pathological side-effect that leads to a permanent apical loss of root structure[24,25]. In most cases of mild EARR the normal function and lifetime of a tooth are unaffected, but in some cases of severe EARR, orthodontic treatment should be stopped, so as not to end in tooth loss [(3)19](graphic 1). We highlight that malpractice is mainly related to surgical staging (IR-63,2%; OS-19%), in line with Sarmiento *et al*[12] study (55%) and Bordonaba-Leiva *et al*[13] study. Pinchi *et al*[15] study identified clinical findings in implant rehabilitation, ending with early or late implant loss. It highlights, as an example, nerve deficit, perforation of the maxillary sinus, pulpal dental necrosis, post-surgical complications and peri-implantitis, and total or partial loss of prosthetic rehabilitation. Oral surgery was related to a neurological deficit in line with Moore *et al*[5] study.

According to the D'Cruz study[6] and the Portuguese Code of Ethics[17] we can highlight some general assumptions of incorrect practice or negligent procedure: the lack of scientific qualification to perform a medical act and the violation of guidelines of therapeutic activity, for non-compliance with ethics and medical

deontology. According to Sarmiento *et al.*[12] the lack of informed consent or coercion in the participation of therapy due to the economic interests of the health professional correspond to 23% of care where the error occurred. Pippi *et al.* [22] emphasise that the professional must inform the patient beforehand by providing a percentage of risk, preventable and non-preventable, before and after the surgery. The literature is consensual to considering the patient´s expectations, adherence to clinical practice guidelines, and complete both verbal and written information, and communication before any treatment are essential for quality and safety of care in preventing malpractice.[23] Despite the guidelines and recommendations of the international scientific community,[2-16,26] the definition of acceptable performance standard does not exist. Therefore, the professional must act with expertise, prudence, and diligence, carrying out the risk management not only within the procedure itself but also before and after it[22]. The timeline procedure, 1) pre-operative, 2) intra-operative, and 3) post-operative was a criterion for dental malpractice categorisation (graphic 2). The intra-operative task was highlighted in implant rehabilitation (92%), in line with Pinchi *et al.*[15] study(82,6%), and orthodontic treatment (80%); followed by surgery in the post-operative task (84%). According to Pippi *et al.* [22] a defensive medicine must be implemented. The use of questionnaires for evaluation of patient risk factors as highlighted by Chandler-Gutiérres *et al.*[22] Gava *et al*,[27] highlighted the use of complementary diagnostic examinations as well for follow-up evaluation and monitoring. Failure at this task may explain the worst results during the procedure in the areas of orthodontics and implantology. The worst results in the context of surgery are attributed to post-procedural follow-up and can be explained in light of the adherence of all elements involved[26].

CONCLUSIONS

This paper presents Portuguese medico-legal data in the field of dentistry. Risk and limits in dental practice reports as an iatrogenic sequel can be a malpractice procedure. It occurred in three significant areas of expertise: implantology, orthodontics, and surgery. Given the prevalence of malpractice, the need to assess its causes and recognise standards for its prevention is necessary.

ACKNOWLEDGMENT

The authors would like to thank the Laboratory of Forensic Dentistry. They would like to thank all those who have helped in carrying out the research, the patients, the physicians, and the support team.

AUTHOR CONTRIBUTIONS

A Corte-Real, Catarina Caetano, and Duarte Vieira conceived and designed the research. Catarina Caetano collected samples and data. Ana Corte-Real, Catarina Caetano, Salomão Rocha, Sónia Alves, André Dias Pereira, and Duarte Vieira analysed and interpreted the data. Ana Corte-Real and Duarte Vieira, also, draft the article and revisit critically for relevant intellectual content. All the authors wrote the paper, and they approve the final version to be published.

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Sex determination in a Brazilian sample from cranial morphometric parameters - a preliminary study

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KEYWORDS

Forensic Anthropology,
Sex dimorphism,
Craniometry,
Forensic Dentistry

J Forensic Odontostomatol
2020. May;(38): 1-8:17
ISSN :2219-6749

ABSTRACT

Sex determination, which is based on the existence of dimorphism between specimens of the same species, plays an important role in the process of human identification. In the absence of pelvic elements, the skull appears to be the best sex indicator, and can also be submitted to quantitative or metric assessments. Eleven measurements were taken for this study, four in the sagittal plane and seven in the horizontal, in two groups of 186 skulls each, with 101 from males and 85 from females for those of the sagittal plane, and 100 and 86, respectively, for those concerning the horizontal, of subjects aged between 18 and 94 years at the time of death. The sample belongs to the Osteological and Tomographic Biobank Professor Doctor Eduardo Daruge of the Piracicaba Dental School of the University of Campinas. The aim of this research was to establish a reliable method to determine sex and elaborate mathematical prototypes capable of assisting in investigation or identification activities, in a preliminary study. Of the measures implemented (Lambda-Nasion, Lambda-Rhinion, Lambda-Nasospinale, Rhinion-Nasospinale, Zygomaxillare-Zygomaxillare, Lambda-Incisive Foramen, Lambda-Right Zygomaxillare, Lambda-Left Zygomaxillare, Basion-Incisive Foramen, Basion-Right Zygomaxillare and Basion-Left Zygomaxillare), only the Lambda-Nasospinale and Rhinion-Nasospinale in the sagittal plane, and the Zygomaxillare-Zygomaxillare and Lambda-Incisive Foramen in the horizontal plane, were significantly dimorphic. Two predictive mathematical models of sex were conceived for each pair of them: one of logistic regression and another of conditional inference trees, displaying accuracy rates of 78.5% and 77.42%, and of 68.28% and 72.04%, respectively. The authors concluded that there is the possibility to apply the aforementioned data in forensic anthropology as an auxiliary tool in investigation or identification tasks.

INTRODUCTION

Sexual dimorphism, the main pattern of variation among members of population groups, refers to the volumetric, physiognomic, somatic, anatomical, physiological and structural particularities, which manifest themselves differently in males and females of the same species.[1] It is a phenomenon that has undoubtedly aroused the special interest of forensic medical experts and anthropologists due to its presence in most human bones.[2,3]

Some skeletal parts (such as those of hands, feet and scapular

girdle, sternum, first rib, humerus, ulna, radius, femur, tibia, fibula, patella and mandible) are used to determine sex, although the pelvic girdle and skull are considered to be the most reliable for this purpose.[1,3,4] In addition, the skull in particular has enormous forensic potential due to its extraordinary resistance to extreme conjunctures, environmental conditions and natural stages of decomposition, usually being found well preserved and separated from the rest of the skeleton after death.[5]

Several scientific techniques have been used over the course of time to achieve a correct differential sex diagnosis, either through the neuro- and viscero-cranium itself or through their respective images (photographs, radiographs and/or tomographies, preferably digital), ranging from the very classic, common and inexpensive (descriptive and metric of bones and teeth), to the most modern, sophisticated and costly (microscopic observation of pulp tissue or bone architecture, physical and chemical analysis of dental and bone calcified tissues, and DNA examination). In general terms, procedures consisting of qualitative (cranio-scopic), quantitative (cranio-metric) or qualitative-quantitative evaluations are those of first choice, despite the fact that the final decision will depend on the quantity and state of the material questioned, and also the simplicity, practicality, accuracy and cost of those procedures.[1,6-11] However, it should be mentioned that they appear to be much less effective and accurate in the presence of remnants of sub-adults, due to the lack of or minimal expression of secondary sexual characters.[12]

In view of the foregoing, the present work sought to establish a reliable method for sex determination, using linear measurements performed on dry skulls of adult humans, and to create a mathematical model, from the significantly dimorphic ones, capable of collaborating in investigation or identification activities, in a preliminary study.

MATERIALS AND METHODS

This was a descriptive, cross-sectional and quantitative study of dry skulls of Brazilians belonging to the collection of the Osteological and Tomographic Biobank Professor Doctor Eduardo Daruge of the Piracicaba Dental School of the University of Campinas, which are part of 320 skeletons, 179 from males and 141 from females, of individuals with age, origin and cause of death known. The referred material was donated on March 24, 2015 by Parque Nossa Senhora da Conçeição (Amarais) Cemetery, of the city of Campinas, State of São Paulo, as set out on page 44 of case 06-P-1447/16. In regards to ancestry, the collection includes 188 (58.75%) skeletons of Caucasians (whites), 91 (28.44%) of Mulattoes (mixed races), 40 (12.5%) of Afro-descendants (blacks) and 1 (0.31%) of Amerindian (aborigine).

Of the 320 skulls, only 194 (105 from males and 89 from females) were selected, of subjects who died in the second half of the 20th century, aged between 18 and 94 years at the time of death, with no morphological or pathological abnormalities, neither traces of extensive trauma nor surgical interventions that might interfere with their assessment. The study consisted of eleven measurements (four in the sagittal plane and seven in the horizontal plane) in two groups of 186 skulls each (n=186), with 101 male skulls and 85 female skulls for measurements in the sagittal plane, and 100 and 86, respectively, for those concerning the horizontal plane, because of the impossibility of materialising them on the 194 skulls, as some of the anthropometric points used would be damaged.

These measurements were carried out by means of a digital sliding calliper with a resolution of 0.01mm (150 mm - Digimess®, São Paulo, Brazil), or a digital spreading calliper with a resolution of 0.01mm (203 mm - Igaging®, California, United States of America), by a single operator as follows: in the first 25 skulls on three different occasions, with an interval of not less than two weeks between them, a requisite for intra-examiner calibration (intra-examination agreement), and in the remaining 161, merely once. Three of the four measurements in the sagittal plane [Lambda-Nasion (La-Na), Lambda-Rhinion (La-Rhi) and Lambda-Nasospinale (La-Ns)] were performed with the aid of the spreading calliper, and one, Rhinion-Nasospinale (Rhi-Ns) of the sliding calliper, while four of the seven in the horizontal plane [Zygomaxillare-Zygomaxillare (ZgM-ZgM), Lambda-Incisive Foramen (La-IF), Lambda-Right Zygomaxillare (La-RZgM) and Lambda-Left Zygomaxillare (La-LZgM)] made with spreading calliper, and three [Basion-Incisive Foramen (Ba-IF), Basion-Right Zygomaxillare (Ba-RZgM) and Basion-Left

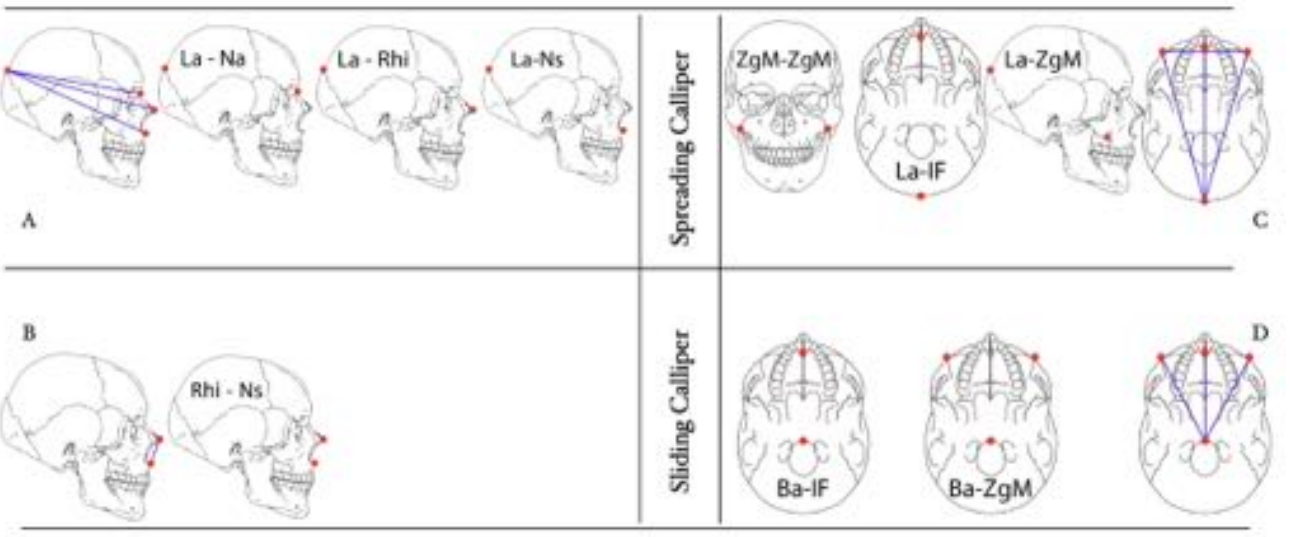
Zygomaxillare (Ba-LZgM)] with sliding calliper (**Figure 1, Table 1 and Figure 2**).
It should be noted that the corresponding project was submitted to the Ethics Committee in Research of the Piracicaba Dental School, and finally approved (protocol nº 138/2014, CAAE nº 38522714.6.0000.5418), complying with Resolution 466/12 about guidelines and regulatory norms of research involving human beings.[13]

Figure 1. Instruments for measuring and Rhi-Ns and ZgM-ZgM measurements

Table 1. Definition of the cranial landmarks (adapted from Pereira & Mello e Alvim, 1979)

Lambda (La)	Point at the intersection of the sagittal and lambdoid sutures, in the midline.
Nasion (Na)	Point at the intersection of the internasal and nasofrontal sutures, in the midline.
Rhinion (Rhi)	Most inferior and anterior point of the internasal suture.
Nasospinale (Ns)	Most inferior and anterior point of the inferior margin of nasal aperture, at the anterior nasal spine base.
Zygomaxillare (ZgM)	Most inferior point of the zygomaticomaxillary suture. Bilateral (RZgM and LZgM).
Incisive Foramen (IF)	Midpoint on the posterior margin of the incisive foramen.
Basion (Ba)	Midpoint on the anterior margin of the foramen magnum.

Figure 2. Cranial measurements in the sagittal (A and B) and horizontal (C and D) planes



STATISTICAL ANALYSIS

The data obtained were entered into a spreadsheet and analysed using R statistical programme. Intra-examiner reliability was evaluated by the intra-class correlation coefficient, with a range from 0.985 to 0.997 for sagittal plane measurements, and from 0.993 to 0.999 for the horizontal values, thus proving the absence of a statistically significant difference amongst the three series of measurements taken.

A scatter plot matrix of a set of independent variables, quantitative (measurements in the sagittal or horizontal planes), and one response or dependent, dichotomous, with only two categories ("male" and "female"), in which each element consisted of a scatter plot of one of the first and the response, allowed to select the most significantly dimorphic variables.

Then, two kinds of statistical models were proposed and customized for this survey: one parametric of logistic regression and one more non-parametric of conditional inference trees (Ctree).

Afterwards, two logistic regression models were elaborated, consisting of a constant (x_0=1) and two explanatory variables - La-Ns and Rhi-Ns for the measurements in the sagittal plane, and ZgM-ZgM and La-IF for those in the horizontal - whose mathematical formulae are the result of the substitution of the respective parameters or coefficients ($ß_0$, $ß_1$ and $ß_2$) by the appropriately estimated values. In these equations, results greater than zero are indicative of feminine sex and less than zero of masculine sex.

In turn, two models of conditional inference trees were developed, one for each group of measurements, with the same variables as the previous ones, evaluating the corresponding final nodes. Each of these was assigned one of the two categories of the response variable, being designated as category 1 to the one with the highest number of components, and 2 to the one with the lowest number of them.

Hence, the logistic regression model for the group of measures in the sagittal plane had the succeeding expression, as well as the conditional inference trees, the configuration represented in Figure 3.

$$logit(p) = Ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k$$

$$logit\ (p) = ß_0 + ß_1 \text{ x La-Ns} + ß_2 \text{ x Rhi-Ns}$$

$$logit\ (p) = -29.5393 + (0.1057 \text{ x La-Ns}) + (0.2985 \text{ x Rhi-Ns})$$

On the other hand, the logistic regression model for the group of measurements in the horizontal plane was formulated as follows:

$$logit(p) = Ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k$$

$$logit\ (p) = ß_0 + ß_1 \text{ x ZgM-ZgM} + ß_2 \text{ x La-IF}$$

$$logit\ (p) = -24.4233 + (0.1089 \text{ x ZgM-ZgM}) + (0.0777 \text{ x La-IF})$$

The conditional inference trees model for the aforementioned acquired the configuration reproduced in Figure 4.

Figure 3. Conditional inference trees model for measurements in the sagittal plane

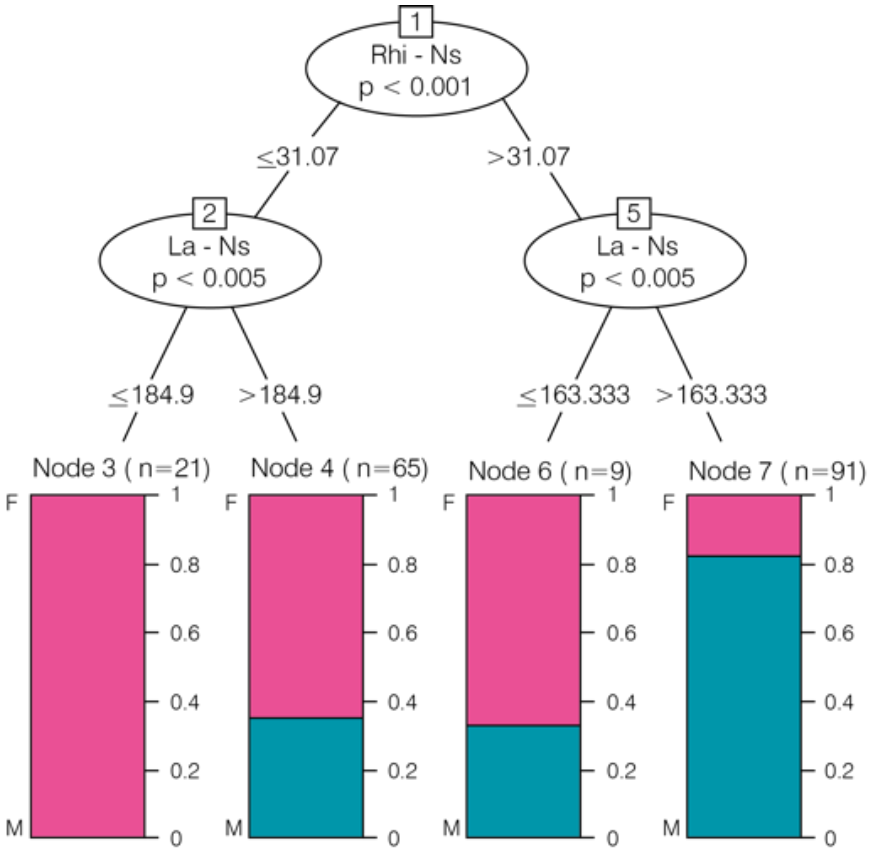
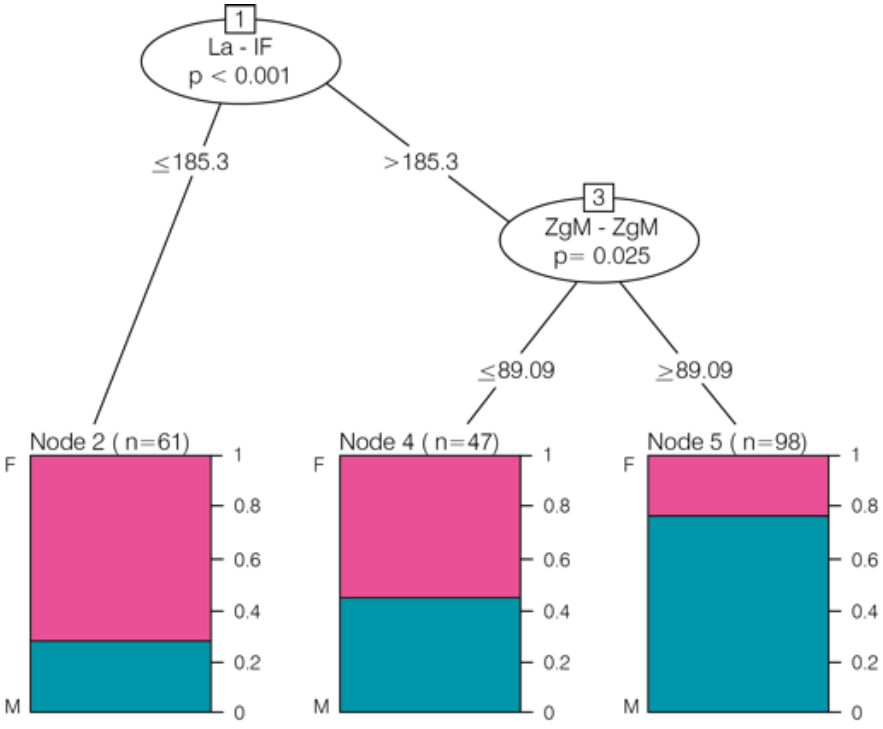


Figure 4. Conditional inference trees model for measurements in the horizontal plane



RESULTS

Once the measurements in the sagittal plane were evaluated through logistic regression model (Table 2), it was possible to verify that:

- 83 male and 63 female skulls were correctly classified (success rate of 78.5%);
- the model identified 105 of the 186 skulls examined as male and the remaining 81 as female, with an accuracy of 79.04% (83 of 105) and 77.77% (63 of 81), respectively;
- the success rate by sex corresponded to 82.18% of males and 74.12% of females; and
- the total classification error was 21.5%, whereas the error by sex reached 17.82% in males and 25.88% in females.

Table 2. Cataloguing of skulls for measurements in the sagittal plane, by means of the logistic regression model

		Predicted		
		F	**M**	**Total**
Observed	**F**	63	22	85
	M	18	83	101
	Total	81	105	186

When these measurements were submitted to the conditional inference trees model (Table 3), the subsequent outcomes were obtained:

- 75 male and 69 female skulls were correctly categorized (success rate of 77.42%);

91 skulls were typified as male and 95 as female, with an accuracy of 82.4% (75 of 91) and 72.6%
(69 of 95), respectively;

- the success rate by sex corresponded to 74.26% of males and 81.18% of females; and
- the total classification error was 22.58%, whilst that considered by sex reached 25.74% in males and 18.82% in females.

Table 3. Categorization of skulls for measurements in the sagittal plane, according to conditional inference trees model

		Predicted		
		F	**M**	**Total**
Observed	**F**	69	16	85
	M	26	75	101
	Total	95	91	186

After evaluating the measurements in the horizontal plane, taking advantage of the logistic regression model (Table 4), it became feasible to assert that:

- 74 male and 53 female skulls were correctly catalogued (success rate of 68.28%);
- the model predicted that 107 of the 186 skulls examined were masculine and the remaining 79 feminine, with an accuracy of 69.1% (74 of 107) and 67.1% (53 of 79), respectively;
- the success rate by sex corresponded to 74% of males and 61.63% of females; and
- the total classification error was 31.72%, 26% in males and 38.37% in females when considered by sex.

Table 4. Classification of skulls for measurements in the horizontal plane, using the logistic regression model

		Predicted		
		F	**M**	**Total**
Observed	**F**	53	33	86
	M	26	74	100
	Total	79	107	186

Finally, the measurements were analyzed through conditional inference trees model (Table 5), with the following results:

- 65 male and 69 female skulls were correctly classified (success rate of 72.04%);

- 82 skulls were characterized as male and 104 as female, with an accuracy of 79.2% (65 of 82) and 66.3% (69 of 104), respectively;
- the success rate by sex corresponded to 65% of males and 80.23% of females; and
- the total classification error was 27.96%, while that considered by sex reached 35% in males and 19.77% in females.

Table 5. Cataloguing of skulls for measurements in the horizontal plane, by means of the conditional inference trees model

		Predicted		
		F	**M**	**Total**
Observed	**F**	69	17	86
	M	35	65	100
	Total	104	82	186

DISCUSSION

It could be stated that the need and eagerness for identification can be traced back to the beginnings of civilisation, since man has always been careful regarding his property and identity rights, in order to differentiate himself from his peers.[14] In view of these precepts, nowadays, the legal scope, social significance and media repercussions of this are no longer discussed.[14,15]

On this treadmill, sex determination, which is based on the existence of dimorphism between specimens of the same species, arises as a key step to reconstruct the biological profile of human remains,[1,3,8,16] by enabling the reduction of the search universe by 50%.[3,9,17] It is also one of the cardinal scopes of anatomists, bioarchaeologists, anthropologists and criminal, forensic medical and odontology experts, when responsible for the anthropological examination of unscathed or fragmented bones.[2,3]

These contrasting features are evident in most parts of the skeleton, and markedly so in the pelvic girdle and skull, which are considered as reliable sex indicators.[1,3,4] It is added that, because of its formidable resistance, the skull is often recovered from crime scene or crash site in a good condition to undergo a detailed appraisal.[3,5]

Some cranial and mandibular traits must be analyzed in an attempt to arrive at a precise differential sex diagnosis, not forgetting that their degree of expression - usually higher in males - will be influenced by diachronic, mesological, geographical, evolutionary, ontogenic, occupational, socio-economic, behavioural, nutritional, genetic, ethnic, age-related, constitutional, physiological, hormonal, biomechanical, traumatic, pathological and/or surgical factors.[1,3-5,9,10,15,16,18] Therefore, forensic anthropology, a sub-speciality of physical, anatomical or biological anthropology, comes on scene as a supporting science for forensic medicine and justice, especially in cases related to identification.[15]

In general, macroscopic qualitative, non-metric or visual inspection techniques continue to be widely used as a consequence of their universality, practicability, replicability, speed, efficiency and simplicity (not requiring sophisticated equipment), despite demanding capable, experienced and trained professionals, and of their high subjectivity load.[1,3-5,8,9-11,15,17,18] To overcome these disadvantages, minimise the chances of inter-observer error[11] and favour the admissibility of findings in court,[11,17] an attempt was made to quantify the morphological attributes using ordinal scales with three or five levels,[10,19] or special devices such as coordinate callipers,[11] which are not always effective in increasing the accuracy rate. In contrast, quantitative or metric techniques rely on standardised linear or angular measurements of distances delimited by landmarks, weighted individually or as indices, as well as in spatial references of superior objectivity, statistical value, population specificity and susceptibility to secular changes, and of lesser challenge in the judicial sphere.[3-5,7,8-11,15,17,18] Nevertheless, their use may be restricted in view of the three-dimensionality and natural irregularity of the various body units in question,[20] for which Mandelbrot[21], in 1982, proposed resorting to fractal geometry for morphometric analysis of these structures. In this same goal, Bookstein[22] utilized a geometric morphometric approach with the aim of recording 2D and 3D coordinates on landmarks and semi-landmarks. This is based on the application of new photographic (photogrammetry, digital cameras, microlenses, illuminators, reflectors, tripods, etc.) and computational (images digitization, scanners,

computers, and specific software) technologies and accessories, which make it possible to visualise, measure and reproduce the variations in shape and size of biological components.[1,4,5,10,18] Its main contribution consists of the possibility of practising a more objective, realistic, complete and accurate evaluation,[10] although hampered by the eventual unavailability or lack of economic, operational and/or human resources.[18]

It is worth remembering that Brazilian anthropology directed its first efforts towards the validation (for the national samples) of findings of successive and celebrated European scientific works, capturing the inconveniences that generated to interpolate them and the urgency to coin their own paradigms and tables. For these reasons, in the midst of the uninterrupted and impetuous expansion of statistics in the mid-twentieth century, mathematical archetypes began to be developed that were adapted to the reality of the country.[2]

In a nutshell, adequate statistical analysis requires the application of flexible methodologies capable of leading to easily understandable results. Using this as a guide, it should detail the selection process of variables and indicate the performance prediction of mathematical models. On this occasion, two multivariate tools of uncontroversial utility in health sciences - one of logistic regression and another of conditional inference trees - were expressly designed for the assorting of the skulls studied. The first is a valuable statistical appliance for forecasting a binary dependent variable such as sex, albeit still insufficiently explored in forensic dentistry.[23] In essence, it represents a generalised alternative that has proven to be very adaptable in its hypotheses and able of manipulating both discrete and continuous variables, which request not to be normally distributed, linearly related, or of equal variance within each ensemble.[24] Furthermore, the second is a predictive and exploratory instrument that facilitates the explanation of a numerical response variable (regression) or categorised (classification), through a group of covariables and their possible relations. In fact, it is a versatile scheme with a simple interpretation, with no restrictions as to the type and distribution of the considered variables, either dichotomous (as in this case) or not.

The sample in question consisted of dry skulls of Brazilians belonging to the Osteological and Tomographic Biobank Professor Doctor Eduardo Daruge of the Piracicaba Dental School of the University of Campinas. The collection comprises of 320 skeletons, 179 from males and 141 from females, 188 (58.75%) of Caucasians (whites), 91 (28.44%) of Mulattoes (mixed races), 40 (12.5%) of Afro-descendants (blacks) and 1 (0.31%) of Amerindian (aborigine), of individuals with age, origin and cause of death known. This material was donated by Parque Nossa Senhora da Conceição Cemetery, of the city of Campinas, State of São Paulo, South East Region of Brazil. As to the ancestry, it would be inappropriate to forget that the nation is home to a multi-ethnic society with European, indigenous and, above all, African and Asian influences,[9,14,15] since 46% of its population (one of the most miscegenated in the world) are descendants of former slaves from the African continent,[14] and the largest Japanese community in the world is located in and around São Paulo.[14] Given the aforementioned information, there appears to be no physical or genetic stereotype of the Brazilian citizen, which symbolises the synthesis of a long, intricate and very rich ethnocultural history.[25]

The present research focused on cranial measurements performed in the sagittal and horizontal planes in order to verify the presence of sexual dimorphism. Statistically significant linear distances were Lambda-Nasospinale and Rhinion-Nasospinale in the sagittal plane, and Zygomaxillare-Zygomaxillare and Lambda-Incisive Foramen in the horizontal. For each pair of them, a mathematical prototype of logistic regression and one of conditional inference trees were created, with success rates of 78.5% and 77.42%, and of 68.28% and 72.04%, respectively.

The bizygomatic breadth has been referred to as the most dimorphic measurement in conglomerates of white[7] and black[17] South Africans, Greeks,[4] Americans,[8] Indians,[3] Romanians,[26] Scots[27] and Brazilians.[15,27,28] Nonetheless, it was excluded from this evaluation because it appears in the measurement battery of Ulbricht et al.[28] for a sample of the same osteological collection.

The Basion-Incisive Foramen distance was statistically significant in two subsets of Brazilians, one in São Paulo[29] and the other in Mato Grosso,[9] but not in this study. In contrast, the Zygomaxillare-Zygomaxillare distance was significant in this, but not in Western Australians[18] and South Eastern Brazilians,[10] whereas the Rhinion-Nasospinale was

significant for two of this bone collection, as expected: that of Ulbricht et al.[28] and the present study.

Saini et al.[3] reported the dimorphic character of the Nasion-Nasospinale distance in Indians, which was not measured in this survey, but the Rhinion-Nasospinale (which is known to be included in the former) was, and it was shown to be significant. It should be noted that an analogy has been drawn between the two, since they are associated with the sagittal plane and in the same anatomical region.

For Green & Curnoe[5] and Saini et al.[3], the aforesaid results would be attributed to a succession of biological phenomena and mechanisms, as well as to the applied methodology, sample size and/or ancestry of the human sets assessed. Indeed, the singularities of organic growth and development, which are fundamentally conditioned by genetic and hormonal factors (hereditary patterns, beginning and end of puberty), are responsible for the onset and magnitude of sexual dimorphism in the cranial unit. The traits considered more dimorphic are located in the face and vault, due to the fact that they reach their final volume and shape relatively late in ontogeny. The superior facial region (orbital region) is the first to complete its maturation and is consequently less dimorphic than the nasal, zygomatic, maxillary and mandibular areas.[1,3,5] The vault is closely related to cranial capacity (on average 10% higher in men) and should adapt to the progressive increase in brain mass until the age of forty, from which it will decline at a rate of 5% per decade,[16] as the base will grow and mature earlier to support the brain and protect the vital nerves and vessels.[1] Taking all of this information into account, it should be possible to explain the reason for the dissimilar success rates of predictive models of the present study and the lesser reliability of the measurements in the horizontal plane. On the other hand, it is essential to emphasise that modern population groups do not exhibit appreciable or palpable phenotypic or genotypic discrepancies, as is evident from cladistic analyses that seek to trace the genealogy of our species.[14] In essence, they constitute authentic, temporary, dynamic and changeable ethnic crucibles, with certain ancestral peculiarities that are periodically repeated, in total harmony with increasing degrees of human variation and miscegenation for which it is necessary that every procedure of identification is tested and validated for each of them.[14]

In addition to the limitations and controversies inherent in osteometric methods in general and craniometric methods in particular, the one used in this opportunity, although not infallible,[9] proved to be reliable, valid, simple, feasible, accessible, rapid, practical, economical, standardised and reproducible. Likewise, it can achieve results comparable to those of much more abstruse and costly ones, and be useful in cases of fractured skulls.[17]

At last, it should be pointed out that sex determination and human identification are conceptualised as fundamental individual rights. Besides, these activities are the responsibility of Brazilian dental surgeons when invested with the role of odontology experts (items IV and IX of article 6 of the Law 5081/66), who will have the possibility to contribute and expedite these processes, in the pursuit of providing better and more efficient services to the community and to be subject to current legislation.[30]

CONCLUSIONS

Quantitative assessments of the different cranial structures emerge as suitable, useful, valuable, pragmatic, inexpensive and complementary resources for the reconstruction of the individual biological profile. Sex determination as a major step in this arduous and relevant task, is one of the most common and critical problems faced by anatomists, bioarchaeologists, anthropologists and criminal, forensic medical and odontology experts.

A positive identification should never be based on a single technique, according to the anthropological premise of making use as many available means as possible. Additionally, it will be necessary that the above mentioned be tested and validated for each population sample, in light of the notorious level of variation and miscegenation of contemporary human beings.

The Lambda-Nasospinale and Rhinion-Nasospinale distances in the sagittal plane and the Zygomaxillare-Zygomaxillare and Lambda-Incisive Foramen in the horizontal revealed themselves to be significantly dimorphic. For each pair of them, two predictive mathematical models of sex were developed, one of logistic regression and one of conditional inference trees, with success rates of 78.5% and 77.42%, and of 68.28 % and 72.04%, respectively.

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Frequency and degree of inter-trait association of maxillary Non-Metric Dental Crown Traits in the permanent dentitions of two states of India

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The authors declare that they have no conflict of interest.

KEYWORDS
Forensic science,
Forensic dentistry,
Non-metric dental crown traits,
Bushman canine,
Cusp of Carabelli,
Shovelling, parastylid

J Forensic Odontostomatol
2020. May;(38): 1-18:25
ISSN :2219-6749

ABSTRACT

Non-metric Dental Crown Traits are a principal source of information in forensic dentistry. However, inadequate data on the prevalence of these traits prompted this study to determine the frequency, sexual dimorphism and degree of inter-trait association in two different populations of India. Dichotomized data on the existence of non-metric features were recorded among individuals from Odisha (n=506) and Kerala (n=536) between 15 to 30 years of age. Cusp of Carabelli is the most common trait to occur (48 %) followed by shovelling of incisors (15%) and Bushman canine (14%). Bushman canine (p=0.045) and Cusp of Carabelli (p = 0.041) were found to be significantly expressed in Odisha and Kerala populations respectively. A strong association between shovelling of central incisor and Bushman canine with a likelihood ratio of 14.041 (p=0.001) was observed. This study will help in characterizing the Indian dentition and post-mortem dental profiling.

INTRODUCTION

Morphological characteristics of teeth are informative indicators for the study of human populations, serving as the basis for classifying human groups in taxonomic, phylogenetic and evolutionary categories. This is possible because teeth are commonly preserved even in the extreme conditions in which skeletal remains are found. Although metric and non-metric features of teeth solely cannot identify an individual, they may expedite personal identification by narrowing down the ethnic origin and gender (1). Non-metric dental crown traits (NDCTs) are phenotypic forms of the enamel that are inherited and controlled in their location, growth and orientation; they result from indirect processes of mineral secretion mediated by proteins during tooth development, and they are expressed and regulated by the human genome of each individual (2). Unlike metric traits, non-metric features can be recorded exclusively by visual observation without any specific equipment, even in fragmented remnants. NDCTs can be described as positive (cusps) or negative structures (pits, furrows and grooves) that have the potential to be present or absent in a specific place (frequency), in a different form or grade (variability), and in one or more members of a population group. To date, there are more than 100 non-metric dental crown and root traits described in the human dentition (3). Winging, shovelling, cusp of Carabelli, parastylid, accessory cusp and Bushman canine are a few of the readily definable and

identifiable NDCTs which may help in the assessment of differences in their frequency and degree of expression. NDCTs may also differ from generation to generation because of the ability to become extinct or vestigial. According to the Clonal model theory each trait is the result of interaction between genetic and environmental factors. This may hold true for NDCTs too; therefore, population differences of NDCTs are likely to occur. Although several studies exist in Chinese [4, 5], Japanese [4], Hungarian [6], Nigerian [7], Jordanian [8, 9] and Malaysian [10] populations, there are only a handful of studies evaluating the frequencies of several NDCTs in the Indian population [11-13]. In fact, this study is the first to compare two Indian populations from two distant states, namely Odisha, the eastern state and Kerala, the southern state.

The objective of the present study was to determine the frequency, sexual dimorphism, and degree of association of eleven maxillary NDCTs, namely winging of central incisor (I1), shovelling of central incisor (I1), shovelling of lateral incisor (I2), double shovelling of central incisor (I1) and lateral incisor (I2), peg lateral, Carabelli's trait, parastylid, accessory cusp, Bushman's canine and three cusp first molar (M1) among the age group of 17 to 25 years in a current Indian population comprised of individuals of Odisha, an eastern state and Kerala, a southern state. The objective was also to compare these frequencies in both populations with the purpose of understanding the developmental behaviour of these features, the dominant ethnic influence, and the dental morphological characters of the sample. These findings may contribute to discussions of the usefulness of dental morphology in dental anthropology and forensic dentistry. The findings may also provide statistically useable reference data of the prevalence of these non-metric coronal features in the populations of Odisha and Kerala. This study will test the hypothesis that the development of tooth based anthropological standards differ among generations and populations [14-17].

MATERIAL AND METHODOLOGY

Sample collection

This is a descriptive cross-sectional study evaluating the frequency of eleven NDCTs among 506 maxillary casts (329 female and 175 male) of 17 to 25 years of age recruited from the Department of Orthodontia, Institute of Dental Sciences, Siksha 'O' Anusandhan deemed to be University, Odisha. 536 maxillary casts (293 female and 243 male) of a similar age group recruited from Kerala population. Inclusive criteria are subjects' belonging to Odisha/Kerala, sound dental health without any congenital anomaly, without regressive alterations, and subjects with upper and lower first permanent molars. Each NDCT feature can be categorized into several patterns according to the Arizona State University dental anthropology system (ASUDAS) [18]. The ASUDAS system of recording the expression of NDCTs is based upon the principle of physical representation of minimal and maximal trait expression and various gradations between these two points[19]. Although this method can record the finest level of distinction of any non-metric trait, we have dichotomized the traits into two types as existing and non-existing traits for ease and convenience in recording.

Statistics

We performed the analysis of the obtained study casts manually evaluating eleven maxillary NDCTs in the permanent dentitions. The data were processed using the SPSS® software version 21. Chi-square test was applied for each of the NDCTs. The p value of ≤ 0.05 was considered to be statistically significant.

OBSERVATIONS AND RESULTS

One hundred casts were randomly selected and scored again by the same observers as well as an independent observer. The intra-class correlation coefficient analysis for intra-observer and inter-observer reliability are expressed as r values presented in Table 1. Approximation of r-values towards 1 indicated that the data were highly reliable on multiple observations.

Prevalence analysis

Prevalence analysis of NDCTs in the populations of the two states combined to represent the Indian population (table 2) demonstrated that cusp of Carabelli (figure 1) is the most common trait to occur (48 %) followed by shovelling of incisors (figure 2) (15%) and Bushman canine (figure 3) (14%).

Evaluation of frequency of NDCTs in Odisha (table 2) exhibited an increase in the frequency of cusp of Carabelli (39%), followed by shovelling of upper incisors (22 % for lateral incisor and 16 % for central incisor), and Bushman canine of maxilla (21%). Evaluation of the frequency of NDCTs in Kerala (table 3) presented an increase in frequency of cusp of Carabelli (56%). Chi square test applied to compare the prevalence of traits between Odisha and Kerala showed a statistically significant increased frequency of expression of Bushman canine (p=0.045), parastylid (figure 4) (p=0.024) and three cusp molar (figure 5)

(p=0.021) in Odisha along with increased expression of cusp of Carabelli (p = 0.041) in Kerala. Expression of double shovelling in lateral incisor is similar in both populations.

Table 1. Reliability of inter- and intra-observer assessments

Reliability	R value
Intra-observer	0.900-0.954*
Inter-observer	0.976-0.990*

Figure 1. Photograph of maxillary cast shows cusp of Carabelli (accessory cusp on lingual surface of mesio-lingual cusp) on maxillary first molar

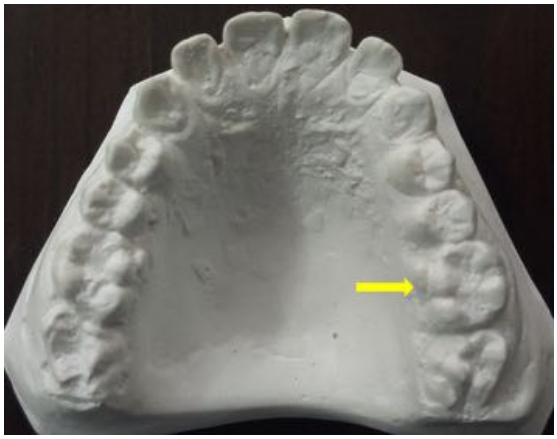


Table 2. Frequency of NDCT in maxilla in Odisha and Kerala population (n=1042)

Jaw	NDCT	Frequency (n=1042)
Maxilla	Winging	74
	Shovelling I1	121
	Shovelling I2	156
	Double Shovelling I1	67
	Double Shovelling I2	38
	Peg lateral	56
	Cusp of Carabelli	498
	AC	1
	BC	149
	Parastylid	8
	Three cusp molar	9

Figure 2. Photograph of maxillary cast shows shovelling (accentuated marginal ridges) of central (a) and lateral (b) incisors

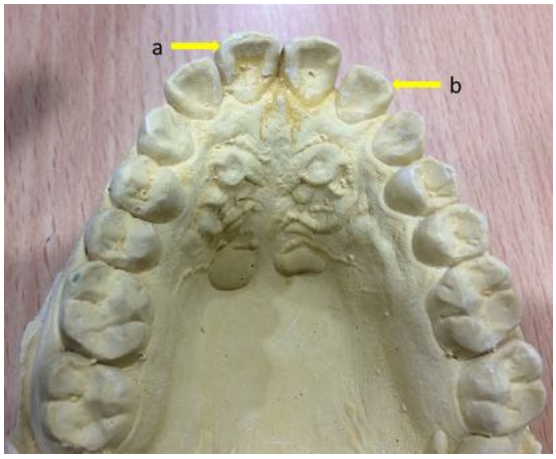


Figure 3. Photograph of maxillary cast shows Bushman canine of left maxillary canine (prominent marginal ridges and cingulum resembling premolar)

Figure 4. Photograph of maxillary cast shows Parastylid (accessory cusp on buccal surface) in maxillary second molar

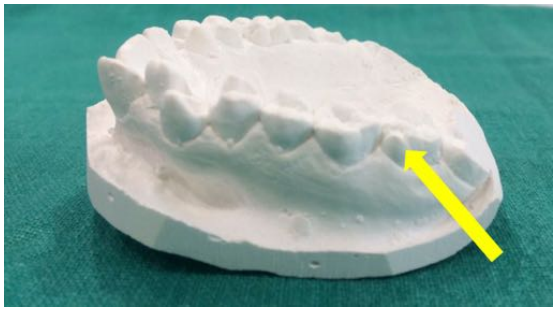
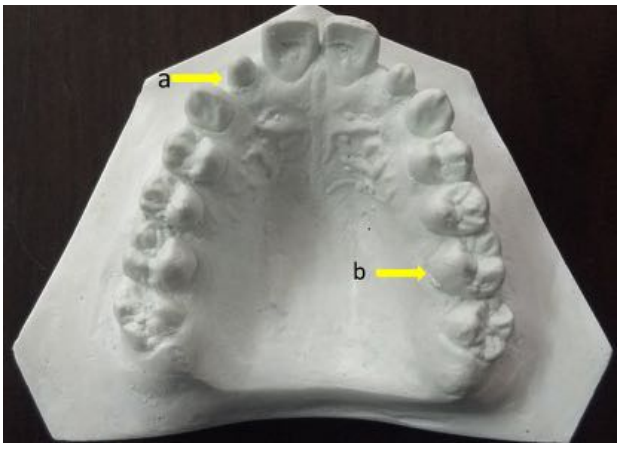


Table 3. Statistical comparison of frequency of NDCT of maxilla between Odisha and Kerala population (both genders combined)

	NDCT	Frequency Odisha (n=506)	Frequency Kerala (n=536)	P value
Maxilla	Winging	21	53	0.453
	Shovelling I1	80	41	0.631
	Shovellin g I2	113	43	0.229
	Double Shovelling I1	23	44	0.248
	Double Shovelling I2	20	18	0.524
	Peg lateral	32	26	1.000
	Cusp of Carabelli	148 - Bilateral 37- Unilateral	215- Bilateral 86- Unilateral	0.288
	Accessory cusp	1	0	.....
	Bushman Canine	104	45	0.045
	Parastylid	6	2	0.024
	Three cusp molar	9	0	0.021

Figure 5. Photograph of maxillary cast shows peg shaped lateral incisor (a) and three cusps of maxillary first molar (b)



Among Odisha and Kerala males (table 4) there was no statistically significant difference in expression of NDCTs whereas there was statistically significant higher prevalence of winging of incisors (0.05) followed by cusp of Carabelli (0.004) in Kerala females compared to that of Odisha females (table 5)

Table 4. Statistical comparison of the frequency of non-metric NDCT between Odisha and Kerala (Male)

Jaw	NDCT	Frequency Odisha (n=175)	Frequency Kerala (n=243)	P value
Maxilla	Winging	11	20	0.604
	Shovelling I1	31	22	0.469
	Shovelling I2	42	26	0.780
	Double Shovelling I1	0	10	
	Double Shovelling I2	0	8	-do-
	Peg lateral	8	7	1.000
	Cusp of Carabelli	60- Bilateral 15- Unilateral	103- Bilateral 35- Unilateral	
	Accessory cusp	1	0	ACKe a constant
	Bushman Canine	35	28	0.478
	Parastylid	1	1	1.000
	Three cusp molar	4	0	

Sexual dimorphism

Chi square test and discriminant function analysis were used in assessing sex differences with respect to individual NDCTs using the SPSS 21 version. Both tests did not show any significant sexual dimorphism with regard to any of the NDCTs.

Degree of association

There was a strong association between shovelling of central incisor and Bushman canine with a likelihood ratio of 14.041 (p=0.001) in the Indian population. No other variables are associated significantly.

Table 5. Statistical comparison of the frequency of non-metric NDCT between Odisha and Kerala (Female)

	NDCT	Frequency Odisha (n=331)	Frequency Kerala (n=293)	P value
Maxilla	Winging	10	33	0.092
	Shovelling I_1	49	19	1.000
	Shovelling I_2	71	17	0.233
	Double Shovelling I_1	23	34	0.088
	Double Shovelling I_2	20	10	0.516
	Peg lateral	24	17	1.000
	Cusp of Carabelli	96-Bilateral 22-Unilateral	112-Bilateral 51-Unilateral	0.016
	Accessory Cusp	0	0	.....
	Bushman Canine	69	17	0.474
	Parastylid	5	1	1.000
	Three cusp molar	5	0	

DISCUSSION

A clue to the evaluation of racial variations attributed to micro-evolutionary processes may be obtained by the analysis of NDCTs which in turn possess a high taxonomic value and forensic importance. The importance is more noticeable when there is a need to focus the investigation of personal identification into potential gender and race from unknown remains. Several bioarchaeological studies have established consistencies in the manifestation and occurrence of non-metric dental traits among various ethnic groups in ancestry determination in the context of forensic dental anthropology [20]. NDCTs have the potential for physical profiling of skeletonised remains of different populations in forensic and anthropological scenarios. This will be possible when population specific data exists on the frequency of NDCTs. This research aims to determine the frequency of several NDCTs in the Indian population.

The world renowned palaeontologist William King Gregory (1922) opined that tooth crown morphology varied scarcely among the major races of mankind. However in the recent past, variations have been noted with respect to non-metric dental crown traits like shovelling, double shovelling, cusp of Carabelli etc. [21-23]. Although there are hundreds of NDCTs, cusp of Carabelli and shovelling have remained as the important pointers with almost no studies on other NDCTs primarily because of their extremely rare occurrence. This research is the first of its kind to evaluate the frequency, sexual dimorphism and degree of inter-trait association of eleven maxillary NDCTs in the permanent dentition of 1038 Indians. Because of the reported regional variations, we have attempted the comparison of these NDCTs in 506 individuals of Odisha and 536 individuals of Kerala, two states of India.

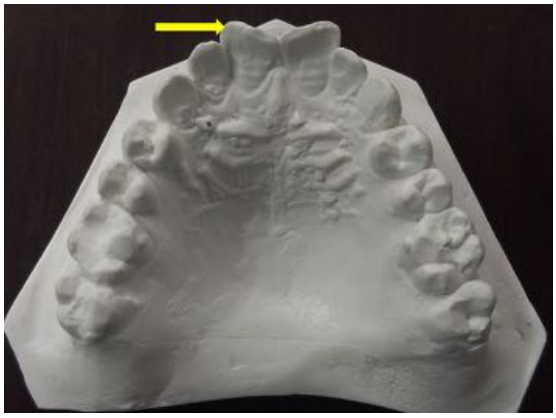
Elevation of the mesial and distal lingual ridges on the incisors produces a unique but quite variable NDCT known as shovel-shaped incisor. Carabelli's traits are found on the lingual aspect of the mesio-lingual cusp of the upper first molar teeth on which the traits may take the form of a pit, fissure or cusp. The presence or absence of Carabelli's trait was recorded for the upper right first molar. The higher frequency of cusp of Carabelli has been reported in Chinese [4, 5, 24], Japanese [4], Hungarian [6], Nigerian[7], Jordanian [8, 9], Malaysian [10] and other Caucasoid [25] populations. Only two studies [11, 26] conducted on an Indian population, restricted to southern Indian states opine a higher frequency of cusp of Carabelli whereas another two southern Indian studies [12, 13] stressed the higher frequency of shovelling and double shovelling. Our finding indicated that the frequency of cusp of Carabelli among the studied NDCTs are highest (48%) followed by shovelling of lateral incisor (15%) and Bushman canine (14%). According to Zubov (1973), evolutionary tendencies, too, are quite different: while in the Eastern groups the trait remained stable or tended to become more common, the frequencies of the shovelling gene in the West decreased quite markedly and in a regular fashion. At present, the frequency of the

shovelling gene in the West appears to continue dropping, making the East-West differences even more pronounced. Two features of the Mongoloid dental complex, namely a high frequency of shovel incisors and a low frequency of Carabelli's trait molars, were reported by Dahlberg in 1951 and by Hanihara in 1968. The regional variations among NDCTs are significant with respect to cusp of Carabelli being high in Kerala population whereas Bushman canine, parastylid and three cusp molar being higher in Odisha population. A larger representative sample size involving different regions of India would be an important area of further research to assess the overall frequency of NDCTs in India and perhaps throwing light on existing regional variations of NDCTs, if any, in different parts of India. High frequency of shovelling and low frequency of Carabelli's trait are significant Mongoloid features which individually may predict inter-population ethnic differences and may aid in personal identification; the degree of association of these two traits may further facilitate the process. Multiple studies opine a positive [27] as well as a negative [28] association of these traits. There is preliminary evidence of a positive association [26] between shovel and Carabelli's traits in Indian populations but the degree of association is obscure. This study demonstrates no significant association of these two traits in the Indian population although a very significant degree of association is found between shovelling of incisor with Bushman's canine. This noteworthy finding will definitely aid in inter-population analysis and personal identification.

Due to the greater development of the cingulum, canines may resemble premolars and are referred to as Bushman's canine.[29] This trait is most common in African populations especially the bushmen but has also been reported in other geographical locations.[29] We have seen a reasonable frequency of grade 1 of Bushman canine in an Indian population (10%) with a significantly higher level in Odisha compared to Kerala. Because of lack of studies on this trait in Indian as well as other populations, studies of this kind should be encouraged to make use of these traits in the forensic identification process.

A wing-like appearance formed by maxillary incisors initially observed among American Indians due to the rotation of distal margins of the incisors in a labial direction has been termed winging by Dahlberg. Mesio-lingual rotation of incisors bilaterally is considered as distinctive of Native American populations while the rotation of a single tooth or both in a disto-lingual direction is attributed to crowding [30]. Frequency of winging in the Indian population, according to our study (Figure 6) was found to be 7.2 % which is considerably lower than Colombia [2]. The lack of recent studies evaluating frequency of winging makes this particular study worth presenting.

Figure 6. Photograph of maxillary cast shows winging (mesio-lingual rotation) of maxillary central incisors



A maxillary molar has four major cusps and one minor cusp named as disto-lingual cusp or hypocone. Because it is the smallest and latest to develop odontogenetically [31] it is most likely to reduce with evolution leading to the three cusped maxillary molar. Therefore, with the objective of evaluating hypocone reduction in modern humans we estimated the frequency of the three cusp maxillary first molars in the Indian population. Our study showed a frequency of 9 bilateral three cusp maxillary permanent first molars among 504 individuals of Odisha (1.8%) with none found in Kerala population which contradicts an Indian study [32] conducted in Madhyapradesh, India showed a prevalence of (0.32%) four cases of permanent maxillary first molars with three cusps. The four cases having three cusp permanent maxillary first molars were present unilaterally and only in females. This trait is showing significant regional variation and rarity of this trait may be important in personal identification.

Paramolar tubercles have long been recognized as non-metric dental traits influenced by ethnic and racial background for its occurrence. They are presented as anomalous cusp, supernumerary

inclusion or eminence occurring on the buccal surfaces of both upper and lower premolars and molars.[33] Dahlberg [33] in 1945 introduced paleontological nomenclature when he referred to this structure as "parastyle" when present in the upper molars and as "protostylid" when present in the lower molars. This study emphasizes the rare incidence of paramolar tubercle or parastylid as 6 out of 504 in the Odisha population and 2 out of 536 in the Kerala population. Bilateral occurrence of all cases is worth reporting which contradicts the unilateral occurrence reported previously.[32, 34] The occurrence of this structure is very low in upper first molars (0% to 0.1%) as compared with upper second molars (0.4% to 2.8%) or upper third molars (0% to 4.7%) in all the given populations [34, 35].

Sexual dimorphism of any trait impacts the personal identification process to a great extent. If significant sexual dimorphism exists for a trait, pooling of two genders influences the identification process and individuals of unknown sex should not be used whereas in the absence of sexual dimorphism gender may be ignored for inter-population comparisons. Sexual dimorphism is not demonstrated by any of the NDCTs taken up in this study indicating that these traits may be conveniently adopted for personal identification process for the Indian population.

Broadly, the world may be divided into three geographical evident groups namely 1) Europe/ Mediterranean (Europe, West Asia, North Africa), 2) Northeast Asia/New World (South Siberia, China-Mongolia, Northeast Asia, American Arctic, North and South Native Americans), and 3) Australia/Oceania (Southeast Asia, Australia, Melanesia, Micronesia, Polynesia). Dental characterization could be conducted using these types of study eventually assisting in personal identification as well as inter-population comparison. With all the relevant data of this study, the Indian population may be characterized by 48% cusp of Carabelli followed by almost equal prevalence of shovelling of lateral incisors and Bushman canine with considerable low prevalence of shovelling central incisor, bilateral winging of central incisors, peg lateral, three cusp first molars and negligible prevalence of double shovelling, accessory cusp and parastylid. This characterization is between the Caucasoid and Mongoloid dental characterization. The study of the influence of evolutionary process upon NDCTs would measure the distance of modern human from ancient human that may be deduced from such types of prevalence study. However maxillary NDCTs must be looked for as a group rather than as isolates for personal identification. It is hoped that this paper helps fill, to some extent, the great void in Indian dental characterization.

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Evaluation of mandibular third molar for age estimation of Filipino population age 9 – 23 years

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The authors declare that they have no conflict of interest.

KEYWORDS

Age estimation,
Chronologic Age,
Dental Age,
Filipino Population,
Mandibular Third Molar

J Forensic Odontostomatol
2020. May;(38): 1-26:33
ISSN :2219-6749

ABSTRACT

Objectives: The study aims to determine the correlation between dental age and chronologic age in the assessment of third molar development among Filipino patients aged 9 to 23 years seen in the Paediatric Dentistry Division (PDD) of the Philippine Children's Medical Centre (PCMC) between 2012-2017.

Materials and Methods: 384 digital panoramic radiographs of Filipino patients (215 males and 169 females) were gathered. Right mandibular third molars were assessed using Modified Demirjian Scoring System. Mean Absolute Error, Percentage error and Correlation is determined between dental age (DA) and chronologic age (CA) of the population.

Results and Conclusion: Initial development of the third molars is observed to begin at approximately 9 years of age and root completion commences at around 19 years of age. Using Spearman Rank-Order Correlation, a strong positive correlation between CA and DA was observed among the overall population (r=0.9518). The observed correlation was stronger among females (r=0.9595) compared to males (r=0.9445). All correlation coefficients were significant (p-value<0.0001). Low percentage error among males and females is observed indicating no significant difference between the CA and DA values of the population.

INTRODUCTION

Age estimation in forensic medicine aims to determine the chronologic age of a person in the most accurate way.[1] Following legal proceedings, age estimation is usually used in cases of orphaned or abandoned children, of determining legal liabilities as stated in the law[2] and of identifying an unknown body or corpse.[3] There are many tools available to aid in age estimation; these include use of radiographic examination of hands and wrists,[4] epiphyseal fusion of long bones,[5] cervical vertebrae,[6] pubic symphysis[7] and skull suture's fusion.[8] Along with these, studies have also pointed out the associations of dentition with age estimation[9,10] and was found to be an even more reliable indicator of biological maturity in children since it is less affected by an individual's nutritional and endocrine condition.[11] Throughout the decades, methods of age estimation using the dentition have been developed which include morphological, biochemical and radiographic methods. Morphological methods assess extracted teeth prepared microscopically. Biochemical methods assess teeth based on specific amino acids that are at an increased level with age.

Radiographic methods assess teeth through different radiographic images.[12,13] Of these methods, radiographic assessment is the simplest, least invasive, most reproducible and cheapest means to estimate age.[14] But when all permanent teeth have erupted, age estimation becomes challenging leaving the third molars as the only basis for age estimation. Noting its varied formation, eruption and calcification stages, a study on its correlation with chronologic age is necessary.

The purpose of the study is to assess the difference and determine the correlation between dental age and chronologic age in the assessment of third molar development among Filipino patients of the Philippine Children's Medical Centre aged 9 to 23 years. Since studies have shown racial differences[15,16] in tooth formation and development, it is important to establish if such a correlation exists within the population.[17]

MATERIALS AND METHODS

The study is a quantitative analytical cross-sectional type aiming to observe the difference and correlation of dental age with chronologic age in the assessment of third molar development among Filipino patients aged 9 to 23 years seen in the Paediatric Dentistry Division (PDD) of the Philippine Children's Medical Centre (PCMC) between 2012-2017

Sample and Sampling Design

The study involved Filipino patients who had their digital panoramic radiograph taken by the Paediatric Dentistry Division of the Philippine Children's Medical Centre between 2012-2017. All panoramic radiographs of the sample population were gathered from the database of the said institution.

A total of 384 panoramic radiographs, with 215 males and 169 females were included in the study. Table 1 shows the summary of distribution of samples according to age and sex while Table 2 shows distribution according to age with Mean ± Standard Deviation.

Table 1. Distribution of Samples According to Age and Sex

Chronologic Age	Male	Female	Total
9	17	20	37
10	31	14	45
11	22	14	36
12	19	14	33
13	12	12	24
14	18	8	26
15	12	10	22
16	13	9	22
17	17	9	26
18	10	10	20
19	12	7	19
20	8	7	15
21	9	17	26
22	7	8	15
23	8	10	18
Total	**215**	**169**	**384**

Table 2. Distribution according to sex and age with mean ± standard deviation

	Total (N=384) Mean ± SD
Chronologic Age	14.81. ± 4.35
Dental Age (Using Modified Demirjian Scoring System)	82. ± 2.55

Description of the Study Procedure

Approval from PCMC Institutional Research Board – Ethics Committee and UP Manila Research Ethics Board to conduct the study

was obtained. After which, the records custodian of the Paediatric Dentistry Division of the Philippine Children's Medical Centre was asked to gather soft copies of the patients' digital panoramic radiographs taken between 2012-2017 (using Vatech Pax-C Digital Panoramic X-ray Machine) from the patient database and its corresponding patients' charts. Assistant researcher 1 (AR1) kept all soft copies of the digital panoramic radiographs in a USB Flash drive. Following the inclusion and exclusion criteria, AR1 identified the radiographs and labeled each from 1 to 384. Inclusion and exclusion criteria are as follows:

Inclusion criteria:
- Filipino patients aged 9 to 23 years
- Patients without history and/or pre-existing medical condition
- Radiographically sound tooth #48

Exclusion criteria:
- Patients with recorded or history of serious medical illness
- Patients with congenitally missing, either one or both, mandibular third molars
- Those in whom tooth #48 has a restoration or cavity or presence of periapical infection as seen on the digital panoramic radiograph
- Children with existing or with history of physical trauma on the face

Rotated tooth #48 were also excluded from the study due to difficulty in assessing crown and root development. Information from the panoramic radiographs included in the study were encoded in an MS Excel Sheet including: assigned radiograph number, sex, date of birth, nationality and date the radiograph was taken. Chronologic age (CA) was also encoded by subtracting the date of birth from the date the radiograph was taken. After encoding, AR1 removed all personal information visible from the digital panoramic radiographs in such a way that only the panoramic image was left for the Primary Investigator (PI) to analyze.

All gathered data were saved in AR1's laptop that was secured with a password, copied to a USB Flash drive and a duplicate was sent to AR1's electronic mail. Data were kept with utmost care by AR1.

Calibration

AR1 gave 100 randomly selected digital panoramic radiographs to the PI and Assistant Researcher 2 (AR2). The PI and AR2 separately assessed tooth #48 using the Modified Demirjian Scoring System[18,19] viewed in a MacBook Air laptop with 100% brightness. Data were encoded in an MS Excel Sheet and were sent separately to AR1 for safekeeping. Data gathered were then sent to the statistician for analysis of inter-rater agreement through Cohen's Kappa.

Panoramic Radiograph Interpretation

After calibration, the PI scored all Tooth #48 with a maximum of 100 radiographs a day, to avoid fatigue. The developmental stage of third molars was determined using the Modified Demirjian Scoring System. Each stage had its numerical value for statistical analysis. The assessment took four days in total. Data collected were recorded in an MS Excel Sheet and then sent to the biostatistician for analysis.

Ethical Considerations

Ethical approval and authorization to conduct the study and exemption to obtain informed consent were sought from the independent local Institutional Review Board-Ethics Committee (IRB-EC) of PCMC.

Good Clinical Practice (GCP) / Principles of Helsinki was observed throughout the study. All data gathered were treated with utmost confidentiality and were safely stored to ensure data privacy.

All data gathered will be discarded, in accordance to the Philippine Data Privacy Act of 2012 and NEGHRR 2017, in a secure manner to prevent unauthorized access by any party or the public.

Data Processing and Analysis

Data gathered were summarized in tables for interpretation of results. A biostatistician assisted in the statistical analysis of the data obtained. Kappa Analysis was used to determine inter-rater agreement. Spearman's Rank-Order Correlation was used to determine the relationship between chronologic age and dental age. Level of significance was set at 0.01. Correlation analysis was conducted using Stata MP ver. 12. Mean Absolute Error and Percentage Error of the population were also computed and tabulated.

Table 3. Distribution according to sex and age with mean ± standard deviation

Two examiners	Intra-class correlation	95% Confidence Interval		F test with true value= 0			
		Lower Bound	Upper Bound	Value	df1	df2	Significant
Single measures	0.995	0.993	0.997	409.27	94	95	p-value <0.001
Average measures	0.998	0.996	0.998				

RESULTS

100 of the 384 radiographs were randomly selected by AR1 for calibration between the PI and AR2. Inter-rater agreement was analyzed through Kappa Analysis and an agreement of >93% with a p-value <0.0001 was achieved (Table 3). Inter-rater correlation coefficient is shown in Appendix C.

Table 4 shows the summary of ratings of the raters and Cohen's Kappa. A minimum of 90% inter-rater agreement was targeted in the study. Calibration results achieved a >93% inter-rater agreement that demonstrates a high agreement between the PI and AR2.

Table 4. Summary of Ratings of the Raters and Cohen's Kappa

	Rater 1									
Rater 2	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Total**
1	7	0	0	0	0	0	0	0	0	7
2	0	9	0	0	0	0	0	0	0	9
3	0	0	11	0	0	0	0	0	0	11
4	0	0	0	13	0	0	0	0	0	13
5	0	0	0	1	12	1	0	0	0	14
6	0	0	0	0	4	0	0	0	0	4
7	0	0	0	0	0	0	11	0	0	11
8	0	0	0	0	0	0	0	14	0	14
9	0	0	0	0	0	0	0	0	12	12
Total	7	9	11	14	16	1	11	14	12	95

Percentage Agreement	Kappa	Std. Err.	p-value
93.68%	0.9279	0.0383	<0.0001

Among the 384 panoramic radiographs:
Stage 0 is observed in ages 9 to 11 years.
Stage A is observed in 9 to 13 years of age.
Stage B is observed in 10 to 13 years of age.
Stage C is observed in 12 to 15 years of age.
Stage D is observed in 13 to 16 years of age.
Stage E is observed in age 13 to 21 years of age; however, it is noted that there is no developmental stage at ages 14, 19 and 20, possibly due to lack of samples assessed. Stage F is observed in ages 15 to 23 years. Stage G is observed in ages 16 to 23 years. Stage H is observed in ages 19 to 23 years. Table 5 shows the summary of age distribution per development stage of tooth #48.

Correlation was determined using Spearman Rank-Order Correlation Analysis to describe strength of association between two variables, Chronologic Age (CA) and Dental Age (DA). Table 6 shows results of the correlation. A strong positive correlation between Chronological Age (CA) and Dental Age (DA) was observed among the overall population (r=0.9518). The observed correlation was stronger among females (r=0.9595) compared to males (r=0.9445). All correlation coefficients were significant (p-value < 0.0001).

Mean Absolute Error of 1.05 and 1.06 and percentage error of 7.49 and 7.43 is observed within the male and female population, respectively, as shown in Table 7.

Table 5. Summary of Age Distribution per Developmental Stage of Tooth #48

	Developmental Stage									
Age	**o**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**Total No. of Teeth**
9	31 (83.8%)[a]	6 (16.2%)	0	0	0	0	0	0	0	37
10	24 (53.3%)	11 (24.4%)	10 (22.2%)	0	0	0	0	0	0	45
11	4 (11.1%)	17 (47.2%)	15 (41.7%)	0	0	0	0	0	0	36
12	0	10 (30.3%)	9 (27.3%)	14 (42.4%)	0	0	0	0	0	33
13	0	1 (4.2%)	8 (33.3%)	8 (33.3%)	5 (20.8%)	2 (8.3%)	0	0	0	24
14	0	0	0	14 (53.8%)	12 (46.2%)	0	0	0	0	26
15	0	0	0	1 (4.5%)	8 (36.4%)	10 (45.5%)	3 (13.6%)	0	0	22
16	0	0	0	0	10 (455%)	8 (36.4%)	3 (13.6%)	1 (4.5%)	0	22
17	0	0	0	0	0	15 (57.7%)	10 (38.5%)	1 (3.8%)	0	26
18	0	0	0	0	0	5 (25%)	14 (70%)	1 (5%)	0	20
19	0	0	0	0	0	0	10 (52.6%)	8 (42.1%)	1 (5.3%)	19
20	0	0	0	0	0	0	9 (60%)	5 (33.3%)	1 (7.7%)	15
21	0	0	0	0	0	2 (7.75%)	9 (34.6%)	7 (26.9%)	8 (30.8%)	26
22	0	0	0	0	0	0	2 (13.3%)	7 (46.75%	6 (40%)	15
23	0	0	0	0	0	0	1 (5.6%)	3 (16.7%)	14 (77.8%)	18
Total No. of Teeth	59	45	42	37	35	42	61	33	30	**384**

[a] Percentage of total number of teeth at that age

Table 6. Spearman Rank-Order Correlation for Males and Females

Correlation between DA and CA	**Males**	**Females**	**Overall**
Spearman correlation coefficient	0.9445	0.9595	0.9518
p-value (2-tailed)	<0.0001	<0.0001	<0.0001
N	215	169	384

Table 7. Mean Absolute Error and Percentage Error for Males and Females

	Mean Absolute Error	Percentage Error
Males (n=215)	1.05	7.49
Females (n=169)	1.06	7.43

DISCUSSION

The study showed a strong positive correlation between the Dental Age and Chronologic Age using the Modified Demirjian Scoring System among the study population. The association can be described that the chronologic age and dental age are directly proportional to each other. The observed correlation was strong in the population with females having r=0.9595 and males with r=0.9445 (p<0.0001). Studies conducted among South Indian,[20,21] Thai,[22] Israeli,[23] Spanish,[24] and Nigerian[25] populations revealed the same correlation results. The study of Mohammed[21] in 2014 found that third molar root development can be used to generate an age range for an individual of unknown chronologic age. Sarnat et al. concludes that indeed a high correlation is found between third molar development and chronological age.[23] Study results are consistent with other population based studies. [20-25]

Within the study population, initial formation begins at 9 years of age and root completion starts from 19 to 23 years of age.

The study of Mohammed, et al. among the South Indian population showed that initial formation occurred at mean age of 9 years and mean root completion is at 18.6 to 18.9 years of age.[21] Among the Israeli population, initial formation starts at 7 years of age and root completion begins to occur at 15 years of age.[23] The Nigerian population, on the other hand, showed initial third molar formation begins as early as 5 years of age to as late as 15 years of age.[25] Possible reasons for such differences can be attributed to racial variations as mentioned in the different studies of Harris, et al.[15] and Hashirm, et al.[16] Harris in 2001 stated that studies had suggested that African origin groups, with larger crowns and thicker enamel, spend less time in tooth formation than those of European descent.[15]

In this study, low percentage error among males and females indicate that CA and DA values of the two groups do not have significant difference within the population. This finding is consistent with other population groups such as the Israeli,[23] Nigerian,[25] Spanish,[24] South Indian,[20,21] and Thai[22] populations that reveal no significant difference between the CA and DA values.

Several scoring systems for third molar assessment are available. Though the study utilized the Modified Demirjian Scoring System, one of the most widely used, acceptable[21] and accurate scoring systems,[26] the goal is not to compare its accuracy with other scoring systems; it is suggested to also apply repetitive measurements and other techniques, and not adhere to a single age estimation technique. Since no scoring system can yet predict the actual chronologic age, it is important to apply other systems and techniques in order to make valuable conclusions in age estimation. Thus, exploring and testing other population based systems and techniques can be included in future research.

CONCLUSIONS

In this study, the use of Modified Demirjian Scoring System in age estimation of the population presented a strong positive correlation between the CA and DA values. The study also revealed no significant difference between the CA and DA of the male and female population. All results of the study are consistent with most population based studies already conducted.

ACKNOWLEDGEMENTS

The Paediatric Dentistry Division of the Philippine Children's Medical Centre for all the help in the implementation of the study. The University of the Philippines Manila College of Dentistry and to my research supervisor, Dr. Cristina M. Laureta for the support in the completion of the study.

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APPENDIX

Modified Demirjian Scoring System

Stage o	– Crypt outline visible. No calcification
Stage A	– Calcification of single occlusal points without fusion of different calcifications.
Stage B	– Fusion of mineralization points; the contour of the occlusal surface is recognizable.
Stage C	– Enamel formation has been completed at the occlusal surface and dentine formation has commenced. The pulp chamber is curved and no pulp horns are visible.
Stage D	– Crown formation has been completed to the level of enamelocemental junction. Root formation has commenced. The pulp horns are beginning to differentiate but the walls of the pulp chamber remain curved.
Stage E	– The root length remains shorter than the crown height. The walls of the pulp chamber are straight and the pulp horns have become more differentiated than in previous stage. In molars, the radicular bifurcation has commenced to calcify.
Stage F	– The walls of the pulp chamber now form an isosceles triangle and the root length is equal to or greater than the crown height. In molars the bifurcation has developed sufficiently to give the roots a distinct form.
Stage G	– The walls of the root canal are now parallel, but the apical end is partially open. In molars, only the distal root is rated.
Stage H	– The root apex is completely closed (distal root in molars). The periodontal membrane surrounding the root and apex is uniform in width throughout.

Revisiting autopsies of death by mechanical asphyxia in the search for post-mortem pink teeth

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The authors declare that they have no conflict of interest.

KEYWORDS

Asphyxia,
Autopsy,
Forensic Dentistry,
Pink tooth

J Forensic Odontostomatol
2020. May;(38): 1-34:38
ISSN :2219-6749

ABSTRACT

Background: In contemporary forensic practice, Medicine and Dentistry combine forces to improve the search for the cause of death and human identification. The pink tooth phenomenon (PTP) is a cadaveric sign that allegedly manifests more often in victims of asphyxia. However, the scientific literature is scarce and controversial on this aspect. **Objective**: This study aimed at screening victims of mechanical asphyxia in order to search for post-mortem pink teeth. **Material and methods**: Retrospectively, autopsy reports from a local medico-legal institute in South Brazil were revisited in the search for victims of death by asphyxia. Autopsy reports of twenty one victims aged between 6 and 67 years (mean age: 40.6 years ±19.20; median: 37 years) were found and analyzed. **Results**: The different modalities of asphyxia included drowning (n=7; 33.33%), strangulation (n=1; 4.76%) and hanging (n=13; 61.91%). A single case of PTP was detected (4.76%) – a 26-year-old male victim of hanging. **Conclusion**: This study illustrates and highlights the unspecific aspect of the PTP. In practice, forensic experts must avoid interpretations of this phenomenon as conclusive evidence of the cause of death, such as asphyxia by drowning, strangulation or hanging.

INTRODUCTION

Violent deaths, such as homicides, suicides and accidents, figure amongst the reasons that more commonly justify medical or dental autopsies.[1] During the autopsy, the search for the cause of death plays an important role in forensic investigations.[2] From a scientific point of view, the study of death and its inherent signs is named thanatognosis – a field within thanatology.[3] Several death signs of medical interest may be retrieved from deceased bodies to support inferences about the cause of death.[4] Profuse froth from the nostrils, broken horns of the thyroid cartilage and sloped suspension peak marks are examples of cadaveric signs that may be found in victims of asphyxia by drowning, manual strangulation and suicidal hanging, respectively.[5]

While on one hand, several cadaveric signs may be detected by pathologists during the medical autopsy, a restricted number of signs are available and useful when it comes to the participation of Forensic Dentistry in the search for the cause of death.[6] The pink tooth phenomenon (PTP) represents a cadaveric sign eventually detectable in dental autopsies.[7-9] Throughout the years, the PTP was more commonly reported in cases of violent deaths by asphyxia, such as drowning.[10]

However, over time, the PTP was gradually denoted as a unspecific cadaveric sign that may manifest more evidently in deceased bodies retrieved from moist environments.[1]

The biomechanics behind the PTP also may involve the differences in pressure and their influence on pulp tissue. Under these circumstances, dentine might be stained by the haemoglobin from pulp red cells.[11] It is important to note that staining occurs in the dentinal tubules. These are distinctive structures formed during the dentinogenesis process.[12,13] From a biological perspective, the dentinal tubules enable hydration and sensory responses.[14] In relation to PTP, it is well known that the diameter of red cells prevents their entrance in the tubules, however, it must be noted that the size of tubules might be different between populations.[14] From a macroscopic view, stained teeth show the typical colour range from pink to brownish. In older victims, on the other hand, the colour might be discrete because the pulp tissue is less abundant as secondary dentine progressively grows, reducing the size of the pulp chamber.[15]

The controversial meaning and uncertainty behind the PTP justify more studies in the field. With the increase of scientific literature, stronger evidence may be used to support the interpretation of the PTP in the forensic routine. Based on the hypothesis that PTP is not an indicator of the cause of death (in this case, specifically for mechanical asphyxia), this study aimed to screen dental autopsies of violent deaths by mechanical asphyxia in order to search for the incidence of PTP.

MATERIAL AND METHODS

Ethical aspects and study design

This study was approved by a local medico-legal unit in South Brazil and received institution ethical clearance from the Committee of Ethics in Research (protocol: 63504916.6.0000.5419). A descriptive survey was designed and carried out.

Sampling and eligibility

The sample consisted of medical and dental autopsy reports retrospectively assessed from a local mortuary in South Brazil. Based on the inclusion criteria, only medical or dental autopsy reports of victims of violent deaths by mechanical asphyxia were considered. According to the exclusion criteria, reports with missing information of dental status were not eligible, as well as reports with missing information about the type of mechanical asphyxia.

Variables and examiners

Within screened autopsy reports, the search variables were: I) the type of mechanical asphyxia, II) the sex and III) the age of the victim, IV) the aspect of the victim's face, V) the presence of pink teeth, VI) the type of material used for neck constriction, VII) the position of the knot, VIII) the position of the body and IX) the time between death and cadaveric examination. Variables VI, VII and VIII were applicable only in cases of strangulation or hanging. The search was performed by a main examiner – a forensic odontologist with two years of experience in the field, under the supervision of a second forensic odontologist with ten years of experience. The collected data were recorded in structured spreadsheets and descriptively analyzed.

RESULTS

A descriptive overview of the cadaveric findings is provided in Table 1. Twenty one cases of violent death by mechanical asphyxia were detected. The victims consisted of three females and 18 males aged between 6 and 67 years (mean age: 40.6 years ±19.20; median: 37 years). Within the modalities of mechanical asphyxia, drowning (n=3 females and 4 males) was found in 33.33% of the cases, strangulation (n=1 male) in 4.76% and hanging (n=13 males) in 61.91%.

In 13 cases (61.91%), the victim's face was cyanotic, while in seven cases (33.33%) the face was pale. In one case (4.76%) the analysis of the victim's face was not feasible because of advanced decomposition. The PTP was found only in a single case (4.76%) – a 26-year-old male victim of hanging.

The type of material used for neck constriction, the position of the knot and the position of the body were reported only for cases of hanging (n=13). Soft constriction material was used in 10 hanging cases (76.92%), while in two cases (15.38%) semi-rigid material was used. Hard constriction material was used in a single case (7.7%). The hanging knot was positioned on the lateral surface of the neck in seven cases (53.84%) and on the posterior surface in six cases (46.16%). In most of the hanging cases (53.84%), the position of the suspended body was not reported. Autopsy reports that provided this information

indicated complete and partial body suspension in 5 (38.46%) and 1 (7.7%) hanging cases, respectively.

The time between death and autopsy was reported only in cases of drowning (n=7). Four (57.14%) of these cases reported a time since death longer than 24 hours, while 3 cases (42.86%) reported an interval less than 24 hours.

Table 1. Case-specific characteristics of the victims of mechanical asphyxia

Asphyxia	#	Age	Face	PTP	Material	Knot	Position	Time
Drowning	1	37	Cyanotic	No	-	-	-	<24h
	2	29	Cyanotic	No	-	-	-	>24h
	3	11	Pale	No	-	-	-	>24h
	4	26	-	No	-	-	-	>24h
	5	34	Cyanotic	No	-	-	-	<24h
	6	34	Pale	No	-	-	-	<24h
	7	19	Cyanotic	No	-	-	-	>24h
Strangulation	8	6	Cyanotic	No	-	-	-	-
Hanging	9	52	Cyanotic	No	Rigid	Posterior	-	-
	10	57	Pale	No	Semi-rigid	Lateral	-	-
	11	60	Cyanotic	No	Soft	Posterior	Complete	-
	12	54	Cyanotic	No	Soft	Lateral	Complete	-
	13	56	Cyanotic	No	Soft	Posterior	Complete	-
	14	67	Pale	No	Soft	Posterior	Partial	-
	15	19	Pale	No	Semi-rigid	Posterior	-	-
	16	53	Pale	No	Soft	Lateral	-	-
	17	26	Cyanotic	Yes	Soft	Lateral	Complete	-
	18	73	Cyanotic	No	Soft	Lateral	-	-
	19	64	Pale	No	Soft	Lateral	-	-
	20	44	Cyanotic	No	Soft	Lateral	Complete	-
	21	33	Cyanotic	No	Soft	Posterior	-	-

#: number given to each cadaver for didactic purposes; PTP: Pink tooth phenomenon; -: information not applicable or not available; times correspond to the time from death to autopsy; position indicates if the body was completely or partially suspended after hanging.

DISCUSSION

Over the last 190 years, the PTP was reported and studied in the routine of Forensic Medicine and Dentistry.[16-25] Originally, in 1829, Bell[7] described the PTP in victims of mechanical asphyxia – more specifically in cases of drowning and strangulation. Sequentially, other studies also reported the PTP within victims of the same cause of death.[10,22,23] Because of the recurrent

manifestation of the PTP in cases of mechanical asphyxia, less experienced forensic experts began to (mis)interpret the former as a pathognomonic sign of the latter. Consequently, the recent scientific literature strives to clarify this scenario by pointing out the PTP as a cadaveric finding unspecific for the cause of death.[1] This study aims to revisit dental autopsy reports of victims of mechanical asphyxia in order to demonstrate that the PTP is not necessarily a cadaveric marker of the cause of death.

Out of the autopsy reports of the twenty one victims sampled in this study, initial information about sex and age were retrieved. The ratio between males and females was 1:6, respectively. The higher number of males is justified by the fact that, globally, men are more involved in violent deaths than women [26] and violent deaths explain the need for autopsies.[1] In relation to age, the scientific literature shows that the PTP may be more common in young victims because the dentinal tubules reduces in size in adults[27] and because the volume of the pulp chamber decreases with the continuous deposition of secondary dentine throughout life. As a result, less blood is available in the pulp to promote the reddish colouration.

The rationale behind the PTP is explained in different ways by the scientific literature. While on one hand, the opinion that the colouration that characterizes the PTP results from the haemoglobin that penetrates the dentinal tubules is indisputable,[20] on the other hand, the mechanism that induces the extravasation of blood cell components to the dentine is uncertain. Increase in blood pressure, rupture of blood vessels and consequent haemorrhage in the pulp chamber figure among the explanations.[26] Explanations for the increased blood pressure relies on the peri- mortem blood congestion in the head and eventually in the pulp chamber.[27] However, due to the scarce experimental studies in the field and the ethical limitations of working with animal models,[1] certainty of haemorrhage in the pulp chamber after asphyxia may not be guaranteed.[25]

In particular, the controversial contribution of blood congestion in the head to trigger the PTP becomes even more uncertain when it comes to the fact that several cases of strangulation and hanging do not lead to post-mortem tooth colouration.[22] The autopsy reports revisited in the present study corroborate this scenario by showing a single victim with PTP (victim of hanging) compared to the other thirteen victims of mechanical asphyxia by strangulation or hanging that did not present with PTP. The same counts for drowning, especially because blood congestion in the head is not necessarily a thanatological sign of this modality of mechanical asphyxia.[22] In this study, all the victims of death by drowning did not present post-mortem pink teeth. According to the scientific literature, cases of drowning that show the PTP may be justified by the position of the head of the drowned cadaver (downwards), which allows blood flow towards the head.[22]

Other contributing factors to be considered in the occurrence of the PTP are the decomposition process and the moist and wet environments. The former plays an important role breaking erythrocytes to produce the colouration of teeth.[17] This process is evidently relevant considering that the average diameter of the erythrocyte (7.5µm) itself is larger than the average diameter of the dentinal tubules (3µm).[16] After the microscopic pathway through haemolysis, blood cell components, especially haemoglobin, are available to penetrate the dentine and culminate in the macroscopic coloured aspect of the PTP. Moist and wet environments not only accelerate the process of decomposition, but also maintain the solubility of tooth tissues enabling the diffusion of blood products from the pulp chamber to the dentine.[17]

It is important to note that the PTP is detected during the cadaveric examination of the oral cavity. Hence, the present study highlights the importance of dental autopsies as an adjuvant to medical autopsies. Trained professionals, namely forensic odontologists, must be consulted for specialized forensic expertise in the oral cavity. Using knowledge from clinical and Forensic Dentistry, these experts may not only detect post-mortem pink coloured teeth, but also distinguish it from ante-mortem coloured teeth (e.g. from dental trauma).

Future studies in the field should focus on establishing experimental set ups with animal or human models following proper ethical standards. Body farms emerge as alternatives to be explored in the context of the PTP. Among the advantages of these alternatives is the possibility of testing the exact influence of decomposition and environment over the PTP in a controlled scenario.[28]

CONCLUSIONS

The dental autopsy cases revisited and analyzed in this study confirmed the PTP as an unspecific thanatological sign in the cause of death. In particular, the PTP was detected in a single case out of the twenty one victims of violent death by mechanical asphyxia, namely drowning, strangulation and hanging.

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Comparison of accuracy of age estimation in Indian children by measurement of open apices in teeth with the London Atlas of tooth development

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The authors declare that they have no conflict of interest.

KEYWORDS

Age estimation,
Indian children,
Cameriere's method,
London Atlas,
Forensic odontology

J Forensic Odontostomatol
2020. May;(38): 1-39:47
ISSN :2219-6749

ABSTRACT

Aim: The aim of this study was to evaluate and compare the accuracy of two age estimation methods in Indian children by using the open apex method proposed by Cameriere et al and the London Atlas of Tooth Development.

Materials and method: Three hundred and thirty five archived digitised panoramic radiographs of healthy children (165 males and 170 females) in the age group of 5 to 15.99 years were retrieved and analysed. The observations were entered in the SPSS software (Version 19.0). The paired t-test and independent samples t-test were applied to assess the differences between chronological and estimated age in both genders.

Results and conclusion: Inter-observer reliability was found to be excellent with Cronbach Alpha to be 1.000 and 0.997 for Cameriere's and London Atlas age estimation methods respectively. The difference of 0.59 years (SD ±1.32 years) was highly significant and indicated a consistent underestimation of age using Cameriere's method. While, applying the London Atlas, the difference of -0.03 years (SD± 0.69 years) was not significant and indicated a little overestimation of age. No significant difference was observed for both genders with the methods. Our results revealed that the methods are reliable for age estimation in Indian children, however, the London Atlas is simpler to use and is more accurate than Cameriere's method.

INTRODUCTION

Children are the future of any nation, however, in recent times, increasing trend of juvenile crimes, escalating cases of immigrants and child abuse are being reported globally, making the estimation of age in children more significant medico-legally. Furthermore, exact age assessment in the paediatric population becomes mandatory in various fields like forensic medicine, endocrinology and orthodontic treatment planning.[1,2] Though there are different methods of age estimation like the secondary sexual characteristics, the biomarkers, bone and dental development, most of the techniques are expensive and are not very accurate.[3]

Dental age, however, is, considered to be a reliable, easy and quick method of age assessment in children as there is minimal variability observed due to the calcification rate that is not much affected by environmental factors.[4-6] Furthermore, teeth are the most indestructible mineralised structures which survive for several years, hence, examination of teeth is considered the most reliable method of age estimation.[3]

Amongst all the imaging methods, the method which is cost effective, uncomplicated and gives an excellent overview of the dental maturity is orthopantomography.[7] Some recently introduced methods of age estimation which are more precise, reliable and use panoramic radiographs for age assessment, include Cameriere's open apices method in children[8] and the London Atlas of Human Tooth Development and Eruption.[9]

Cameriere et al, 2006[8] developed a linear regression formula to assess the chronological age in a European population. Popularly recognised as Cameriere's equation, it estimates age by measuring the open apices of seven permanent mandibular teeth on the left side of the jaw. While, an innovative and simple approach to age estimation was introduced by AlQahtani et al in 2010,[9] who developed a comprehensive atlas of human tooth development to determine age between 28 weeks intrauterine and 23 years. This method utilised the Moorrees et al's[4] and Bengston's[10] tooth developmental stages. There is no study done in an Indian paediatric population which compares the accuracy of Cameriere's with the London Atlas method of age estimation. Thus, the present study was carried out to estimate the accuracy of Cameriere's formula and the London Atlas of Tooth Development in assessing the actual age of Indian children. The second objective was to analyse if there was any difference in the accuracy of these two methods. Additionally, the study aimed to determine if there was a difference between the dental age of male and female subjects using Cameriere's equation and the London Atlas of Tooth Development.

MATERIALS AND METHODS

This was a cross-sectional retrospective study which examined the digitised orthopantomographs (OPGs) retrieved from the archives of Department of Oral Medicine and Radiology. Only best quality radiographs were selected of 335 healthy Indian children aged between 5.00 and 15.99 years. All the subjects were categorised into 11 age groups with equal distribution of 15 males and 15 females in each age group, except Group 5 which had 20 females (Table 1).

Table 1. Age and gender distribution in the sample studied

Groups (of Sample studied)	Age (years)	Boys (n)	Girls (n)	Total (n)
1	5.5.99	15	15	30
2	6.6.99	15	15	30
3	7.7.99	15	15	30
4	8.8.99	15	15	30
5	9.9.99	15	20	35
6	10.10.99	15	15	30
7	11.11.99	15	15	30
8	12.12.99	15	15	30
9	13.13.99	15	15	30
10	14.14.99	15	15	30
11	15.15.99	15	15	30
	Total	165	170	335

The uniformity in age and gender distribution was maintained purposely to achieve accuracy across the age range and gender. All the radiographs were taken for routine diagnostic and treatment purposes; no radiographs were taken particularly for this study. Poor quality unclear radiographs, as well as those showing pathology, dental anomalies, previous orthodontic treatment, severe dental caries, periapical cysts, grossly destructed teeth and crowns, were excluded from the study. The chronological age of participants was calculated by subtracting the date of the birth from the date on which radiographs were taken. Coding was done for all participants, and the observer was blinded to their actual age. Only the subject's gender was revealed to the examiner.

While using Cameriere's method, all seven left permanent mandibular teeth were examined. In single rooted teeth, the distance between the inner side of the pulp canal at the apex was measured (Ai, i= 1,..5). In multirooted teeth, the sum of the distances between the inner sides of

the pulp of the two apices was calculated (Ai, i=6,7). Normalised measurements were obtained by dividing the measurement of the apices by the tooth length from the highest cusp tip to the lowest root tip (Li, i=1,....,7) (Figure 1).
Finally, dental maturity was calculated using the normalised measurements of the open apices of the seven left permanent mandibular teeth, xi, i=1,.....,7, their sum, s, and the number of teeth (No), with root development complete. Dental age in Indian children was calculated by putting all the values in Cameriere's equation formulated according to linear regression model:

Dental age = 8.971+0.375g +1.631X5 +0.674 No-1.034s-0.176s. No,
where g stands for gender, X5 = A5/L5, s = sum of normalised open apices, and No = number of teeth showing complete root development.

When the London Atlas of Tooth Development was used for age estimation, OPGs were examined to assess the development stages for all primary and permanent teeth on the right side of both upper and lower jaws. Subsequently, the dental age of the individual was calculated by using available software on the website: http://www.atlas.dentistry.qmul.ac.uk. The tables were filled by observing specific figures of the development stage and level of alveolar eruption of the tooth and matching these with the panoramic radiographs of each participant; the dental age calculator feature automatically displayed the dental age.
To test inter-observer reproducibility, a random sample of 35 panoramic radiographs were examined by two observers at fifteen days interval. Reliability analysis showed inter-observer Cronbach Alpha to be 1.000 and 0.997 for Cameriere's and London Atlas age estimations respectively, suggesting excellent agreement. All the calculated values obtained from both age estimation methods, were entered in an excel file and subjected to SPSS (Statistical Package for the Social Sciences) software, (version 19.0) analysis.
Dental age (Estimated age) was subtracted from the chronological age (Actual age): a positive result indicated underestimation and a negative one indicated overestimation of age. A paired t-test was applied for each of the two methods with a significance level of P< 0.001, to calculate the bias which is the mean difference between the predicted and chronological ages.

Figure 1. Cameriere's method of measuring open apices and tooth length (on Adobe Photoshop)

RESULTS

Cameriere's Method

The mean chronological age of the entire sample was 10.238 ± 3.160 years. The mean estimated age of the whole sample using Cameriere's method was 9.639 ± 2.486 years. The difference of 0.59 years (SD ±1.32 years was highly significant and indicated a consistent underestimation of age. The bias was highly significant in all age groups (Table 2).

Independent samples t-test was applied to measure the differences between male and female participants. No statistically significant difference (p = 0.154) was observed between males and females. The mean difference between the actual and predicted ages was less in male subjects as compared to female subjects (Table 3).

Figure 2 depicts the paired t-test results for the entire sample as well as for each year interval. Age was significantly overestimated in the children between the age of 5 to 9 years, on the other hand, in children of age range from 11 to 15 years, it was significantly underestimated.

The paired t-test results for male and female participants for each year interval are observed in Figure 3. Non-significant overestimation of age was seen in age groups 1,2,3 and 5 in both genders. While underestimation of age was seen in age groups (4,6,7,8,9,10,11) for both males and females. However, in the age group 3 (7-7.99 years), males showed underestimation of age.

Table 2. Paired t-test for Cameriere's method showing the bias for the sample population

Measure of accuracy	N	Mean	± SD	SE mean	Significance	95% CI Lower	95% CI Upper
Bias	335	0.59773	1.32365	0.07232	0.000**	0.4555	0.7399
Absolute Difference	335	1.11048	0.93464	0.05106	0.000**	1.1010	1.2109

**p<0.001; Highly significant SD = Standard deviation, SE = Standard error, CI = Confidence interval

Table 3. Independent samples t-test for Cameriere's method to express bias for gender

Measure of	Sex	N	Mean	± SD	SE mean	Significance
Bias	M	165	0.4931	1.2961	0.1009	0.154
	F	170	0.6994	1.3459	0.1032	
Absolute Difference	M	165	1.0755	0.8723	0.0679	0.500
	F	170	1.1445	0.9929	0.0761	

F = Female, M =male, SD = Standard Deviation, SE = Standard error

Figure 2. Paired t-test for Cameriere's method showing the bias for the sample population

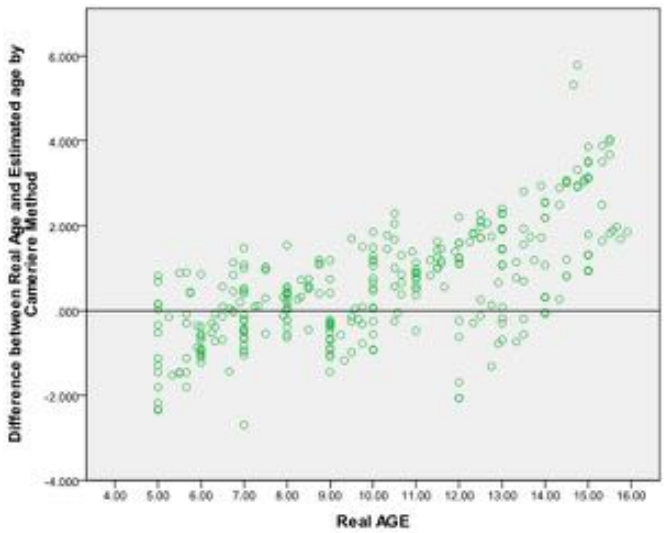
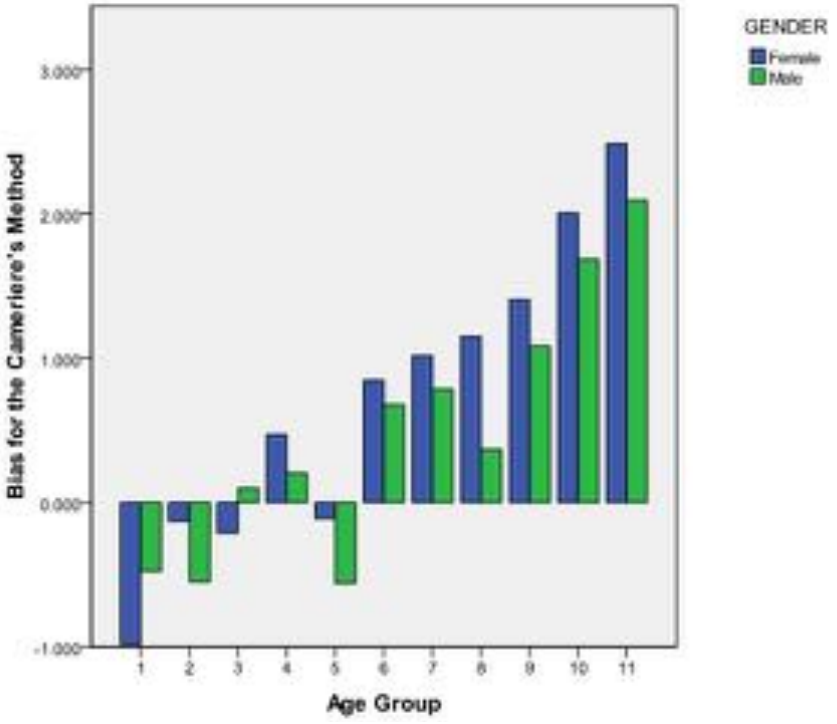


Figure 3. Independent samples t-test for Cameriere's method to express bias for gender



London Atlas of Tooth Development

The mean chronological age of the entire sample was 10.238 ± 3.160 years, while the mean estimated age of the whole sample was 10.267 ± 3.033 years when the London Atlas was applied to determine the age of the subjects. The difference of -0.03 years (SD± 0.69 years) was not significant and indicated a little overestimation. The bias was statistically significant in all the age groups (Table 4). To analyse and compare the differences between both genders, independent samples t-test was used. No statistically significant difference (p = 0.321) was observed between male and female participants (Table 5).The mean difference between the chronological and estimated ages was less in female subjects than in male subjects.

The paired t-test was applied to estimate the accuracy of age intervals of the entire sample. Differences between the actual and estimated age for the entire sample at each year interval are illustrated in Figure 4. Applying the London Atlas method of age assessment to the study sample, there was non-significant underestimation and overestimation in all age groups, hence it was found to be more accurate.

Paired t-test applied was used to test the accuracy of different age intervals in both male and female subjects as depicted in Figure 5. While non-significant, overestimation of age was noticed for both genders in age groups 1 to 6 and underestimation was observed in age groups 7 to 11. However, overestimation of age was seen in males in the age group 11 (15-15.99 years), while, underestimation of age was observed in males in the age group 5 (9-9.99 years).

Table 4. Paired t-test for the London Atlas method showing the bias for the sample population

Measure of accuracy	N	Mean	± SD	SE mean	Significance	95% CI Lower	95% CI Upper
Bias	335	-0.02955	0.69944	0.03821	0.440	-0.10472	0.04562
Absolute Difference	335	0.54048	0.44397	0.02426	0.000**	0.49276	0.58819

**p<0.001; Highly significant SD = Standard deviation, SE= Standard error, CI= Confidence Interval

Table 5. Independent samples t-test for the London Atlas method to express bias for gender

Measure of accuracy	Sex	N	Mean	± SD	SE mean	Significance
Bias	M	165	-0.06812	0.616293	0.047978	0.321
	F	170	0.00788	0.771636	0.059182	
Absolute Difference	M	165	0.49794	0.367464	0.028607	0.084
	F	170	0.58176	0.505007	0.038732	

F = Female, M= Male, SD= Standard deviation, SE = Standard error

Figure 4. The London Atlas method showing bias for age estimation in different age groups

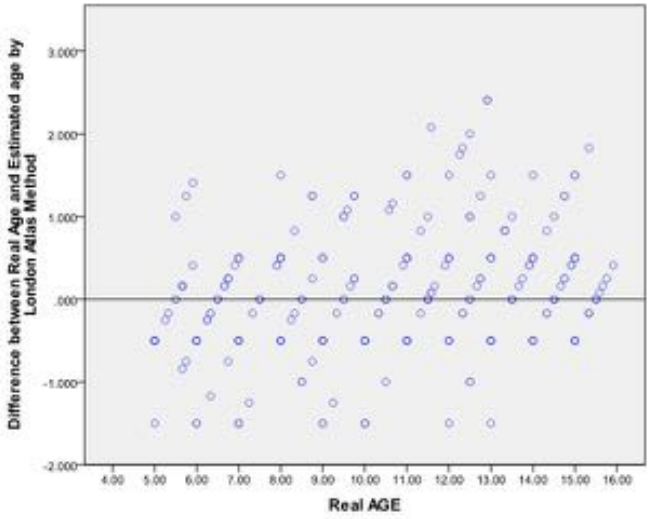
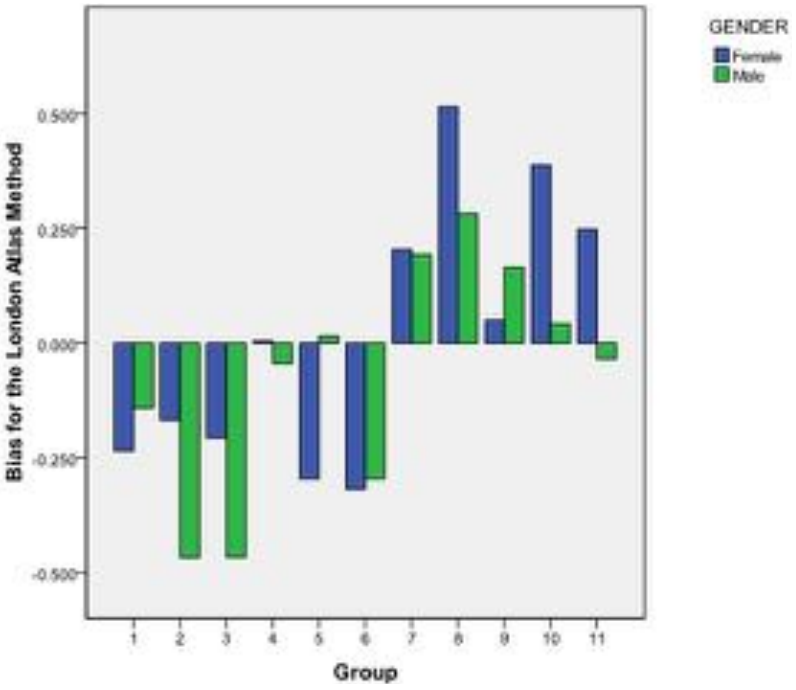


Figure 5. The London Atlas method showing bias in different age groups in male and female subjects



DISCUSSION

In view of the UN convention on the Rights of the Child,[11] age estimation in children and adolescents becomes the prime concern for the forensic fraternity and is one of the most relevant issues today in forensic medicine. According to its preamble, a child has the right to be registered and granted a nationality and according to article 12 of its constitution, the child has the right to express their opinions in accordance with their age and maturity.[11] Juvenile delinquency is a serious concern for India as recent years have witnessed a rapid rise in criminal cases involving minors. Furthermore, violation of the child's basic rights like child labour, other forms of exploitation, non-registration of births are prevalent in India. Thus, it becomes more important to estimate accurate age in juveniles in the Court of Law. Thus, we chose children between 5 and 15.99 years of age as our target study population. Different age estimation methods have been tested in Indian children, for instance, a study conducted on Indian children concluded that Willem's method was the most accurate followed by Demirjian and Chaillet's methods.[12]

Various studies on different populations have assessed the accuracy of Cameriere's method using the measurement of open apices in teeth. This method proved to be reasonably accurate in all the populations including Italian, European, Indian and Saudi Arabian children.[7,13-15] While the London Atlas has also been found to be fairly precise in estimating age in different populations, Cameriere's linear regression equation has been tested for accuracy in an Indian population earlier, where the results revealed that an open apex method in children was highly accurate with the morphological variables explaining 88.5% of the variations in predicted age.[13] However, to the best of our knowledge, no research has been undertaken so far in Indian subjects assessing the difference in the accuracy of age estimation applying these two non-invasive radiographic techniques. Thus, we compared the accuracy of both these methods in an Indian population (age 5-15.99 years) visiting the OPD of Oral Medicine and Radiology Department.

Cameriere et al, 2006[8] tested the stepwise multiple regression formula in Italian white children and observed that age can be predicted more accurately and efficiently by using this method. Moreover, when tested and compared with Willems and Demirjian method in White Italian, Spanish and Croatian children, it was again found to be a more reliable and precise method for age assessment in young children.[13] The study emphasised the suitability of the sum of normalised open apices (s) and number (No) of teeth with complete root development as accurate morphological parameters for determining age in juveniles. On the other hand, in a sample of Italian children between 11 and 16 years of age, the authors compared four age estimation methods.i.e. Demirjian, Willems, Cameriere and Haavikko and observed that Cameriere's method underestimated the age by one year for both genders, while other methods were found to be more accurate.[16] The results of our study are more congruent with the latter study as, in the current study, a statistically significant underestimation of age was observed for all age intervals. Our results are also more similar to other age estimation studies carried out in different populations like Saudi, Iranian, Turkish and American children, where Cameriere's formula invariably underestimated the age.[15,17-19]

However, no significant difference was found between male and female subjects and the underestimation of age was uniform between the genders in several studies using Cameriere's formula.[15,17,18] The present study was in agreement with the results of these studies and showed no statistically significant difference between the genders. Contrast results were observed in Mexican and Bosnian Herzegovinian populations, where overestimation of age was reported in females.[20,21]

The London Atlas of Human Tooth Development determines age based on tooth development stage and the level of alveolar eruption. The use of software (http://www.atlas.dentistry.qmul.ac.uk) makes the technique convenient and practicable.[9] When the London Atlas was compared with the Schour and Massler estimation chart and Ubelaker estimation chart, it was found to be reasonably accurate and no significant difference was found for most age groups, except some. The study sample included white and Bangladeshi populations and the authors observed that tooth formation showed minimum variation during infancy but revealed most variability after the age of 16 years.[9]

Alshihri et al, 2015[22,23] assessed the suitability of the London Atlas of Human Tooth Development for age estimation in Saudi Arabian children and adolescents and found a significant difference between mean estimated and actual age. Further, there was a significant difference in the accuracy of age estimation between males and females. In females, the frequency of overestimation of age was higher as compared to males, emphasising the requirement of development of designate charts for each gender. This signified that hormonal changes during growth or puberty affect the tooth formation stages.[24] On the other hand, the results of a cross-sectional study carried out in Iranian children indicated high accuracy with no significant differences between the mean chronological age and mean dental ages using Smith's method and the London Atlas but suggested the latter to be simpler to use.[25] Our findings were in congruence with the results of the latter study.

When the London Atlas of Human Tooth Development was used in Indian children, the results of the present study observed non-significant underestimation and overestimation in all age groups. The difference of -0.03 years was not significant and indicated a little overestimation, implying that it was more accurate than Cameriere's stepwise linear regression equation. The London Atlas was also applied to estimate age in a Portuguese population and no significant difference was observed between male and female subjects either.[26] The observations of our study are in agreement and indicated no statistically significant difference between male and female participants. The mean difference between the chronological and estimated ages was less in female subjects than in male subjects.

In Saudi children, there was a significant difference between the dental and actual age of the subjects when the London Atlas was used for age estimation.[22] While, underestimation of age was a common finding in Saudi[22] as well as American populations,[19] overestimation of age was noticed in two different studies conducted in Portugal.[26,27] In contrast to above studies, using the London Atlas, the results of the present study observed non-significant underestimation and overestimation in all age groups.

With the London Atlas, age is shown as an average.e.g.10.5 represents the mean of an age range from 10.00 to 10.99 years. While, when using Cameriere's formula, 10.5 implies 10 years and 6 months. Thus, there is more likelihood of error of six months with the London Atlas and the bias between predicted and actual age may be overstated.[15]

In the present study, results from both the methods did not show any significant difference between male and female participants. Though overestimation and underestimation of age was observed in all age groups with both the methods, a non-significant difference was found when the London Atlas method was applied, implying it to be a more accurate technique. It has certain advantages, using both the upper and lower jaws, inclusion of both deciduous and permanent dentition developmental stages and observing the level of alveolar bone eruption of the teeth, implying that it is more accurate.

CONCLUSIONS

Considering the results of the present study, both Cameriere's method of open apices and the London Atlas of Human Tooth Development are reliable for accurate dental age estimation in Indian children. While Cameriere's method requires precise calculations and relies more on the expertise of the observer, the London Atlas method is relatively convenient and simple to use. The latter method uses software programme (available in different languages) to make accurate calculations.

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A contribution for the forensic odontologist's safety in the autopsy room

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The authors declare that they have no conflict of interest.

KEYWORDS

Covid-19;
autopsy;
forensic odontology

J Forensic Odontostomatol
2020. May;(38): 1-48:50
ISSN :2219-6749

ABSTRACT

The Covid 19 pandemic we are currently facing all over the world is undoubtly an emergency condition which exposes all workers to the risks of a serious infection. The personnel working in the autoptic room is at high risk for the possible contact with infected material from corpses, splashes and aerosols diffusion, and must be always prepared to stay safe and healthy. We offer to the forensic odontologists' community a short summary of some old and new hints on the treatment of dead bodies in the autopsy room during this pandemic emergency time.

We are undoubtly living a weird and dangerous time and any forensic operator must be aware of the risks in the treatment of living and dead bodies.

A vast amount of Literature has been recently written on Covid 19 treatment and Covid 19-related behavioural guidelines [1-4].

We would like to just summarize for our IOFOS Colleagues some old and new hints on the treatment of dead bodies in the autopsy room during this pandemic emergency time.

It is largely known already that the COVID-19 (Coronavirus Disease-19) is nowadays the most urgent health emergency worldwide and all professionals – included pathologists and forensic odontologists – are often called to offer support in the diagnosis and treatment of patients affected by this disease.

There are actually four hazard groups (HG) of infectious biological agents, classified according to the following considerations: the likelihood that it will cause disease by infection or toxicity in humans; how likely it is that the infection would spread to the community; the availability of any prophylaxis or treatment. At the moment, as far as any effective therapy of the Covid 19 serious illness is known and no primary prophylaxis is available due to the lack of a vaccine, we can say that the Sars-Cov 2 is provisionally categorised at the third of four HG levels of infectious biological agents, causing a serious risk of infection for professionals [1,4].

All that said, despite the high infectivity of the virus, the risks for personnel operating in the morgue facilities could be considered relatively scarce if some supplementary precautions are carefully applied by the personnel to the standard universal precautions for infection prevention; the personnel in the autoptic room, at risk for the possible contact with infected

material from corpses, sharp injuries, splashes and aerosols diffusion, must be always prepared for the possible presence of the Sars-Cov 2 infection in the dead body. [5,6] The infection can be caused by dermal inoculation, inhalation, ingestion, contamination of intact skin or oral, nasal, eye mucous membranes.

The scientific community has not found yet how long this kind of coronavirus resists - vital and infectious - in a dead body. The tests, moreover, often offer false negative results[5].

We think that it is important to share information that our research group at the University of Firenze (Italy) found that the swabs resulted positive in some dead bodies kept in the mortuary refrigerator at between 0°C and 4°C and tested after several days (7-14 days) post-mortem. The results of the aforementioned research, in disagreement with some other previously published articles, will be released soon. All bodies, except those whose swabs resulted certainly negative, during the Covid-19 pandemic time should be considered and treated in the autoptic room as potentially infected by Sars- Cov 2.

In the clear intent of offering a technical support to all the professionals (forensic pathologist, odontologist, other personnel) involved in the autoptic activities, we summarize some technical and practical hints as follows:

- The forensic personnel (forensic pathologist, odontologist, other personnel involved, FP) must be previously and properly educated about risks and prevention; the autoptic procedure must be properly programmed in advance. The room door must always be closed and the openings kept to the minimum[4].
- The number of the FP working in the autopsy room should be limited; we all know, however, that this issue can be balanced by the need of support from collaborators who can write or take photographs during the autopsy. The use of a video-registration of the procedures should therefore be usefully implemented.
- Antiseptic hand hygiene procedures must always be carefully followed in compliance with the WHO sequence[7,8]: antiseptic hand washing with alcoholic solution or antiseptic soap and water after each contact with the body.
- Special attention must be dedicated to avoid accidentally touching the face.
- Personal protection equipment (PPE) should be worn as follows: disposable headgear; double pair of cut-resistant protective disposable gloves which must extend to cover wrists: the second pair can be changed frequently, if needed; respiratory filter FFP2/N95 or FFP3/N99 in case of aereosol generating procedures, for which surgical masks do not provide adequate protection[9]; face protection gear (goggles or protective visor); disposable whole-body suit or long-sleeved waterproof or fluid-resistant gown and surgical cap; disposable rubber boots and waterproof shoe protectors.
- Strong attention must be paid to the correctness of the donning and doffing procedures.
- All the necessary equipment must be on hand range to avoid leaving the autopsy area
- Oral and nasal secretions should be previously cleared by suction.
- TC and CBCT x-rays shoud be preferred: they are carried out more quickly than the time consuming oral x-rays and allow to obtain a OPG-like image of the teeth (panorex)
- According to IOFOS recommendations[10], even more valid nowadays, we recommend to avoid any maxillary split or resection, since bone sectioning can produce potentially infected aerosol.

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