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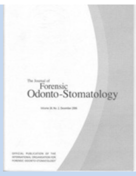

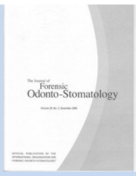

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SECTION IDENTIFICATION

Forensic Odontology in the Disaster Victim Identification Process

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ABSTRACT

Disaster victim identification (DVI) is an intensive and demanding task involving specialists from various disciplines. The forensic dentist is one of the key persons who plays an important role in the DVI human identification process. In recent years, many disaster incidents have occurred that challenged the DVI team with various kinds of difficulties related to disaster management and unique situations in each disaster. New technologies have been developed to make the working process faster and more effective and the different DVI protocols have been evaluated and improved.

The aim of this article is to collate all information regarding diagnostic tools and methodologies pertaining to forensic odontological DVI, both current and future.

It can be concluded that lessons learned from previous disaster incidents have helped to optimize working protocols and to develop new tools that can be applied in future DVI operation. The working procedures have been greatly improved by newly developed technologies.

KEYWORDS**:** forensic odontology, Disaster Victim Identification (DVI), human identification, mass casualty

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INTRODUCTION

Each natural or man-made disaster presents a different set of circumstances and, as a consequence, each event results in new challenges for identification teams. Although the exact number of deceased persons to define an event as a disaster varies by jurisdiction, it is widely agreed that mass fatality incidents always exert an onerous impact on local resources.

Dental DVI team leaders conduct training exercises to familiarize their team with standard operating procedures and to be better prepared for any kind of eventuality.[1] Attempts are made during training exercises to demonstrate the complex challenges using simulations and by studying previous responses and events. Interpol and other agencies have developed standardized forms to record dental traits at the time of PM examination. Similar forms are used to translate and transcribe the original data from collected AM dental records into a common nomenclature. These PM and AM data are entered into a computer database that will ultimately search for best possible matches. Examples of the most common computer applications are DVI System International from Plass Data® (approved and used by Interpol),[2] WinID® (North America) and DAVID® (Australia).

INTERPOL DISASTER VICTIM IDENTIFICATION PHILOSOPHY ON MASS CASUALTIES

The first manual on Disaster Victim Identification was issued in 1984 by the Interpol DVI Standing Committee to emphasize the multidisciplinary approach of victim identification. The Disaster Victim Identification Guide (Interpol) describes the basic principles of the Interpol philosophy in relation to DVI, and aims to stimulate DVI teams to apply 'the best practice' to obtain maximum results in DVI operations.[3,4]

The 2004 Asian Tsunami was a prime example of the application of Interpol's interdisciplinary disaster victim identification philosophy and how an international effort for DVI was set up and coordinated by the Interpol Secretariat General (IPSG) and DVI Standing Committee in Lyon. DVI teams from more than 20 countries took part in the identification process which, because of the complexity of the situation and different legal systems, had to be conducted in an internationally agreed upon way. After the incident, a thorough assessment and a review of all procedures and related issues were carried out on behalf of IPSG in Lyon to set new standards for the future.[5] Consequently new guidelines were implemented in the Guide.

DISASTER VICTIM IDENTIFICATION (DVI) PROCESS

Standard Operating protocols (SOP) for PM and AM procedures were established for fingerprinting, forensic pathology, forensic odontology and DNA profiling. Such protocols were found crucial in the quality of the entire DVI process, especially in case of rapidly decomposing bodies.

The overall identification process involves recovery-, AM-, PM- and identification teams. The mission and tasks of these various teams are outlined below, as well as the position of the forensic odontologist in each of these teams. It is clear that actions and operation of these teams should be interactive and well coordinated. A firm chain of command is thus essential.

Recovery team

The recovery team has the important task to collect evidence such as bodies and body parts, personal property from the disaster scene and to record the findings accurately. This requires accurate mapping

– photographic aerial overview or GPS mapping - of the disaster area, which allows the team to record in which part of the site the given evidence was recovered. Usually the incident site will be organized in a grid system.[3] Body numbering is done according to Interpol guidelines and has to be applied by all teams to avoid errors and creation of even more chaos. The given body numbering system – international country telephone code, site number and body number as applied in the Tsunami disaster (ex. 32-1-00596) – is the reference for future disasters. This unique body number has to stay with the body during the subsequent stages of the identification process and will be visible on all related documentation (forms, photographs).

It is recommended that a forensic odontologist is part of the recovery team, as the trained specialist has a better eye for dental evidence. In some cases, such as with charred bodies, it might be necessary for the odontologist on the recovery team to consolidate or describe the dental evidence on site before it is removed, to avoid destruction of the brittle dental substances during transportation to the mortuary.

AM team

The work of the AM teams starts with eliciting missing persons lists from each country and entering this information into a missing person database.

There is very limited information in this database; however, it can serve as a master list that should be compared with the names of those for which AM records exist. This AM information of the reported missing people is obtained through the missing persons' family members who will provide names of health care providers, where medical and/or dental AM information can be obtained.

After the missing persons' dentist has been contacted by the local police, a forensic dentist should allocate the dental AM data and materials. Other information sources such as specialists, hospitals, dental insurance companies should be contacted as well to obtain additional AM information. All available material (dental records, X-rays, CT scans, dental models, full face photographs, mouth guards, etc.) should be collected, with respect for the patient's rights to medical secrecy. The source and content of the original dental records will be carefully read, analysed and transcribed onto the AM F1/F2 Interpol forms before being transmitted to the identification centre. In case of any doubts, the forensic odontologist in the AM team should contact treating dentists to discuss the issue and clarify the problem.

The records (personal, medical, dental, DNA and fingerprints) forwarded by the specialists of countries with missing citizens will be entered into a central computer system: DVI System International (Plass Data®) or WinID® or DAVID® or other software available by trained and experienced forensic odontologists.

When AM fingerprint records are received with these records, they are scanned into a separate computer system called the Automated Fingerprint Identification System (AFIS).[2]

The quantity and quality of AM dental records is extremely variable across the world.[6] This is mainly due to differences in legislation in the way dental records are compiled and kept, their content and the legally required retention periods. Managing this AM information (searching, collecting, receiving, quality assurance, transcribing, tasking, analysing) from all countries is a crucial step in the quality system of a DVI process.[7] The importance of proper (complete and accurate) dental records should be emphasized to all dentists, dental and health organizations throughout the world.

PM or mortuary team

The temporary mortuary, where the PM information will be collected, needs to be constructed for body storage and examination, and established on premises affording the best possible facilities in the given circumstances (Fig. 1).[8] In the mortuary, the body will be thoroughly examined by a multidisciplinary team of specialists (fingerprint experts, policemen, pathologists, odontologists and DNA experts), who will register their findings on the pink PM Interpol forms.

Fig.1: Temporary mortuary in Thailand after the tsunami in 2004

Each body to be moved into the examination room for physical description should be placed under custody of a PM records officer, who follows the body through all the examination stages until it is returned for storage. The PM records officer should be in possession of all PM DVI forms for each body.

The first step is finger/palm print analysis by specialists from forensic police labs. The recovered fingerprints will be entered into the AFIS system for comparison with existing AM data. In the second phase, bodies will be photographed, followed by an extensive external description of the body, clothing and personal belongings. All these items are photographed preferably in colour after being cleaned and labelled, with the reference body number clearly visible. Personal effects such as documents, jewellery, watches, clothing and pocket contents may constitute valuable circumstantial evidence of identity, but never proof. They must be combined with other evidence to conclude to a positive identification.

During the next step, the pathologist starts the external and internal examination and description of the body. It should be standard practice to perform full autopsies on all disaster victims not only for identification and cause of death purposes, but also to assist in preventing or minimizing the effects of similar incidents in the future.

Dental examination in this phase is carried out by forensic odontologists. All dental related details will be registered on the PM F1/F2 Interpol forms. As a general rule, jaws should not be removed by dental experts unless a more specific examination is mandatory. To create more access to the dentition, a non-destructive method of mandibular dissecting technique is recommended.[9] This method allows an easy access to both maxilla and mandible and still enables a complete repositioning of the facial tissue after autopsy, so that the body can still be shown to relatives if required. All dental characteristics should be recorded by colour photography and radiography (Fig. 2). Dental age estimation is a major component of the identification process. Post- mortem dental age estimation allows forensic odontologists to focus on the search for a matching ante-mortem file on a specific age range among the possible candidates for identification from the missing persons list. Dental age estimation may be performed in different ways using morphological or radiological parameters that are all age-related. Obvious examples are tooth development in children and adolescents or morphological changes in adults (enamel wear, cementum incremental lines, root translucency or secondary dentine formation), which play an essential role in dental age assessments. In the context of identification, the most appropriate fitting age estimation method in relation to the presented evidence should be chosen out of all existing methods. This is an advantage compared with the aging of living individuals where for instance the methods of age estimation performed after tooth extraction have to be excluded. In essence, during identification, the dental age estimation protocols are divided in two groups based on the availability or absence of developing teeth. Therefore,

Fig. 2: Photographs of dental structures together with body number must be taken during the post mortem examination.

radiological dental investigations and preferably full body CT's are essential at the start of the identification process. They allow, if developing teeth are detected, to apply immediately the most appropriate age estimation method,[10-18] providing an instant age result. If all available teeth are fully developed, 2D radiographs can be used to apply the Kvaal technique[19] on the related monoradicular teeth. On 3D reconstructions of cone-beam computed tomography (CBCT) images, volumetric pulp tooth ratios can be calculated and implemented in an according age estimation technique.[20] After tooth development has been completed, methods on extracted and sectioned teeth will have to be considered. In this context, measurements of morphological changes related to age, need to be examined. The length of the apical translucency of the tooth roots[21] provides immediate age information on both intact and cut teeth. If possible the described methods on mature teeth are combined with methods taking into consideration attrition, periodontal attachment, cementum annulations, root resorption, and secondary dentine apposition.[22,23]

Genetic identification techniques provide a powerful tool in the identification of disaster victims. DNA analysis techniques currently in use complement other methods commonly used in disaster victim identification, especially when a body has been severely mutilated. As dental pulp material is a good source for DNA analysis, two vital teeth (canines/premolars) can be extracted and sent to the forensic DNA laboratories.[24]

Identification Centre

The Identification Centre handles and compares AM and PM documents forwarded from the AM and PM Units. In the different sections of the identification bureau - missing persons; ante-mortem; post-mortem; fingerprint; dental; DNA analysis and reconciliation - the quality controls and the transcription of the AM and PM documents take place. Results obtained from the specialised sections are fed back to the Identification Files Section to be combined into one master list of results (Fig.3).

Fig. 3: The identification team, working during Tsunami identification process, putting all AM and PM data into the Plass Data® software. The system matches two datasets (AM and PM) and the matches will be discussed again in the reconciliation board.

The identification software runs an automatic comparison and matching between the ante and post mortem data but the final judgment must be made by professional experts and be based on personal evaluation of the evidence.[2] The matches will then in the next stage be verified by the different experts on the

Reconciliation Board. The responsibility of this Identification Board is to check the results of comparison made by the various specialised sections. It is also responsible for scrutinising and eventual reconciliation of possible inconsistencies and will combine the results into one final list of identifications.

OTHER ASPECTS ASSISTING IN THE IDENTIFICATION PROCESS

During the recent years, many disasters occurred at different intensities. DVI teams must respond systematically, using all facilities and new technologies available in the process of victim identification. The fast growing technology and ever more powerful equipment are mostly in respect of the field of imaging processes.

Dental radiology

Many factors may affect PM-radiographic image collection: the presence of suitable dental X-ray equipment but also the body condition such as rigor mortis, positioning the victim's bodies and aiming the x-ray beam, electricity supply, working areas and equipment. Therefore, during mass disasters the recovery team needs to transport the remains to compartments - equipped with fixed dental x-ray unit(s) - suitable for performing dental autopsies. Even when electric power is supplied, the fixed x-ray devices can be damaged by constant line fluctuation as was reported during the Asian tsunami disaster.[25,26] Recently developed portable and handheld digital dental x-ray units can solve these practical forensic problems as shown during the Tsunami crisis in 2005 when Nomad® was introduced for the first time in mass disasters. These light weight and autonomic working devices can easily be brought next to the bodies, allowing an immediate forensic odontologic investigation in combination with digital imaging and management systems, enabling potentially an immediate AM-PM matching (Fig. 4).[27,28]

The dosimetry studies performed with the Nomad® portable X-ray machine, which is provided with circular lead-filled shields attached to the end of the exit tube, showed that exposure of the operator to leaking or backscatter radiation is below the maximum permissible for occupational dose.[29,30] A study reporting dose measurements carried out with Nomad® in normal conditions (i.e. patient seated in dental chair and operator in the "safe zone" provided by the circular lead-shield) as well as in "atypical" situations (i.e. forensic sites, field work, sedated patients) has demonstrated that the whole body exposure was equivalent to less than 1% of the occupational dose limit.[30] Another report[28] shows that exposure at the operator's hand was lowest when a protection shield was used or with the use of an exposure switch cable (distance > 1m).

In addition, new technologies provide new accessories for X-ray viewing in the field of disaster. Some companies have launched a portable X-ray machine with a viewing display attached to the machine itself. Others provide a small portable X-ray viewing gadget. When operated in a wireless environment, data and images

Fig. 4: Several portable dental x-ray devices are available on the market nowadays. (1) Nomad® (Aribex, Utah, USA) was first introduced in 2005 in the identification process after Tsunami disaster. (2) AnyRay® (VATECH Co., Ltd., Gyeonggi-do, Republic of Korea), (3) Rextar® (Sungwon Econet, Seoul, Republic of Korea) (4) ADX4000 (DEXCOWIN Co., Ltd. Seoul, Republic of Korea)

could thus be easily transmitted in both directions. This may help to facilitate the radiographic interpretation more rapidly than in the past.

Facial reconstruction

Today, thanks to the multidisciplinary approach towards an unidentified body, a tremendous amount of information concerning the victim can be obtained. Unfortunately even the biggest and most detailed post-mortem (PM) files are useless when no match with any AM file can be made. When confronted with a corpse that is unrecognisable due to its state of decomposition, skeletisation, mutilation or calcination, a cranio-facial reconstruction (CFR) should be considered. The goal of CFR is to recreate a likeness with the face of missing individuals immediately prior to their death. Presenting this reconstructed face to the public can get the identification-process out of the impasse by triggering recognition.[31-37]

Several 3D manual methods for CFR are currently being used. One of these reconstruction methods consists of physically modelling a face on a skull replica (the target skull) with clay or plasticine; however, this method requires a high degree of anatomical and sculptural expertise and, as a result, remains difficult and subjective. The progress in computer science and the improvement of medical imaging technologies during recent years have lead to the development of alternative computer-based CFR methods. A computer, compared to a human expert, is consistent and objective. Knowing all the modelling assumptions and given the same input data, a computer always generates the same output data. Furthermore, certain procedures can be automated so that the creation of multiple reconstructions from the same skull using different modelling assumptions (age, BMI, ancestry, gender, etc) becomes possible. As a result, the CFR process becomes accessible to a wide range of people without the need for extensive expertise.[38]

Virtual autopsy

The role of 3D imaging in the forensic field is growing quickly, not only in cranio-facial reconstruction (CFR) but also in the whole autopsy process.

Some institutions have already implemented CT in post-mortem forensic investigations, such as at the Armed Forces Medical Examiners autopsy room (Armed Forces Institute of Pathology, Washington, D.C., and Dover, Del., USA), where CT scans on military personnel killed in combat are used on a routine basis.[39]

During the Victorian Bushfire in 2009, CT scanning also proved to be very useful in the victim identification process.[40]

In Switzerland, the Virtopsy project implements a variety of imaging methods: 3D photogrammetry-based optical surface scanning, MSCT (Multi-slice CT) and MRI (Magnetic Resonance Imaging). Virtopsy is a non-invasive or minimally invasive approach that has several advantages to current forensic examination techniques, as it can help to provide precise, objective and clear documentation of forensic findings for testimony in court. This technique also helps improve quality assurance through digital data archiving and transfer. Because of its minimal invasiveness to the body, it can also improve judicature in cultures with low autopsy acceptance.[41]

In some cases, physical examination should still be performed in addition to the virtopsy to provide more physical and external information of the victims.

PAST AND FUTURE DVI

World globalisation has resulted in mass disasters these days often involving victims from many different nationalities, requiring the assistance of DVI teams from various countries often with different levels of expertise.[5,42]

After the Tsunami in 2004, all the DVI protocols were re-evaluated by an Interpol working group.[43-45] This Tsunami Evaluation report is a summary of what the DVI teams experienced during the 2004-2005 incident. In the current Interpol DVI guidelines, the methods of identification process are categorized into 2 groups: primary and secondary identification methods. [3,42,46] Primary identification means that the method by itself can lead to a 100 % scientific identification which is able to withstand global legal scrutiny. Forensic odontology, as one of the primary identification methods (DNA, forensic odontology, finger printing), has proved to be an effective identification method especially in large scale disasters with the overall ID rate of 83.3% in South-East Asia Tsunami.[25]

The Forensic Odontology Working Group of the Interpol DVI Standing Committee has been working to develop new guidelines and adapted forms (F1/F2). The content of the modified AM and PM forms will be simplified. Unnecessary parts will be removed. All captured data will be directly linked to the Plass Data® system.[2]

In terms of AM records, as internet access is growing rapidly, the AM records should be made available online when disaster occurs. This will help speed up the identification process and minimize possible risk of losing AM evidence during transportation. The AM team, the insurance companies and personnel who are dealing with the victims' data should follow the legal obligation of the medical confidentiality.[47, 48]

At this stage, Interpol DVI dental forms are being updated. Our suggestion is that as 3D data and virtopsy are more accessible, the DVI forms should be adapted to handle these 3D evidences. Civilians have better access to medical care. Images from multi-slice computed tomography (MSCT) and cone-beam computed tomography (CBCT) will become more widely available. It is possible that one of the potential victims of a future disaster may have these types of AM data, yet the old forms are not supporting this new type of evidence and thus, there is a need to revise the forms, considering new diagnostic material in more dimensions. More research should be performed on how to apply 3D information in the identification process more efficiently. If the PM records contain a 3D scan, the challenge will be to find a suitable match to the 2D AM datasets. To enable this, there is a need for new matching algorithms.

It is not only of crucial importance that rules and SOP's as written out in the Interpol DVI Guidelines are followed and applied by all DVI team members, but also that all specialists involved in DVI are suitably trained and qualified and will be deployed in appropriate roles. Different levels of experience with Interpol DVI guidelines, documents and standards, made clear the need for standardization**.**

Internationally agreed upon common minimum standards of training for the personnel of the different specialist sections would be beneficial to the international community. This of course requires specialists accredited to provide these training programs and also the creation of a framework for quality control within each speciality in the overall DVI process.

OVERALL CONCLUSIONS

Disaster victim identification (DVI) is a demanding task that can only be brought to a successful conclusion if properly planned, by selecting the appropriate forensic diagnostic tools and involving a team of well-trained key experts. Lessons learned since the Tsunami disaster in 2004 have changed the Interpol DVI vision and standards tremendously. The DVI community is moving forward and continuously improving the guidelines and protocols.

Forensic odontology as one of the primary identification methods is a dynamic field and has developed tremendously since the Tsunami in 2004. Recent developments in computer-aided 3D imaging have been applied for forensic odontology, forensic radiology, forensic craniofacial reconstruction and virtual autopsy. New research challenges include developing forensic diagnostic tools, with maximal use of remnants and information, increased efficacy for various forensic applications and optimized protocols for DVI operations.

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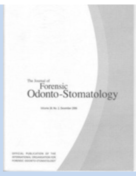
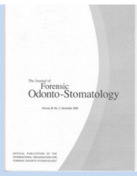

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SECTION DENTAL DAMAGE

Injuries to the Head and Face in Brazilian Adolescents and Teenagers Victims of Non-natural Deaths

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ABSTRACT

This study aimed to evaluate the occurrence of injuries to the head and face in adolescent and teenager victims of non-natural deaths. A retrospective study was undertaken by the analysis of medical forensic reports obtained from medical forensic examinations performed at the Department of Forensic Medicine of the city of Campina Grande, PB, Brazil, between January 2003 and December 2007. From a total of 607 reports issued during this time span, the study sample consisted of 423 reports (69.6%) referring to adolescents and teenagers of both genders, aged 12 to 18 years, who were confirmed to have died from external causes. The causes of death were encoded according to the Chapter XX of the International Statistical Classification of Diseases and Related Health Problems (ICD-10). The majority of victims were 17 year old males (25.8%). Firearms (33.3%) and transport accidents (32.2%) were the most common causes of death, with boys showing a 3.7 times greater likelihood of getting killed by firearms than girls. There was statistically significant relationship between the occurrence of transport accidents and gender. The majority of victims (71.6%) presented with multiple injuries throughout the body. There was statistically significant relationship between the occurrence of transport accidents and the presence of multiple injuries. A high percentage of the victims presented with injuries to the head and face. There was statistically significant relationship between the occurrence of transport accidents and the presence of injury to the head. Fatal gunshot wounds and transport accidents were the main causes of death of male adolescents and teenagers. The victims presented with multiple injuries, especially to the head and face, and the mandible was the most frequently injured facial bone.

***KEYWORDS*:** Maxillofacial Injuries; Wounds and Injuries; Accidents, Traffic

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INTRODUCTION

According to the World Health Organization (WHO), external causes of mortality are physical damage to the human body causing a fatal bodily lesion as a result of acute exposure to energy in amounts that exceed the threshold of physiological tolerance or impairment of function due to a lack of one or more vital elements (i.e. air, water, warmth)[1].

Unintentional (i.e. accidental) or intentional (i.e. deliberate) injuries due to external causes represent an important public health problem as they kill over 5 million people worldwide every year[2]. External causes are the leading cause of death among children and adolescents between 1 and 18 years of age around the world[3] and are responsible for the deaths of over 40,000 children in Europe[4]. In Brazil, they are the third most common cause of death, accounting for 15.2% of the deaths of individuals from 0 to 19 years[5]

Because it is the most exposed and poorly protected region of the body, the face is more frequently associated with a variety of traumatic injuries that may occur either alone or in combination with other organs or systems[6]. The main causes of facial injuries are transport accidents, physical assaults, falls and sports activities[7]. However, the etiology and incidence of injuries vary from one country to another, and even within the same country, according to social, cultural and environmental factors[7-9].

As external causes of morbidity and mortality are affecting ever younger age groups, several studies have focused on the importance of these events in the child and adolescent population[7,10-12]. The purpose of the present study was to characterize the injuries to the head and face in Brazilian 12-18-year-old adolescents and teenagers victims of non-natural deaths.

MATERIALS AND METHODS

A retrospective study was undertaken by the analysis of expert medical reports derived from medical forensic examinations performed at the Department of Forensic Medicine of the city of Campina Grande, PB, Brazil, between January 2003 and December 2007. From a total of 607 reports issued during this time span, the study sample included 423 reports (69.6%) referring to 12-18-year-old adolescents and teenagers of both genders who were confirmed to have died from external causes. The causes of death were encoded according to the Chapter XX (External causes of morbidity and mortality) in the International Statistical Classification of Diseases and Related Health Problems, 10th revision (ICD-10) (V01-Y98)[13].

According to the Brazilian legislation, all deaths from external causes and cases of sudden or suspicious death are autopsied at the departments of Forensic Medicine. A road traffic injury was defined as any injury (regardless of severity) that occurred while walking, bicycling, or riding in a vehicle due to a crash involving one or more vehicles (including bicycles) and originating or terminating on a roadway[14].

Data referring to the victims' gender, age, cause of death, number of injuries and anatomic location of injuries to the head, face and oral cavity and maxillofacial fractures (if present) were gathered from the forensic medical reports and transferred to specific registration forms, which were kept in folders classified according to the year and month of occurrence of the event.

This study was conducted in compliance with the ethical guidelines issued by the Brazilian Ministry of Health/National

Health Council Resolution 196/96 on research involving human subjects. The research project was approved by the Ethics Committee of the State University of Paraíba, Brazil.
All statistical analyses were performed using the Epi Info 2007 software (Centers for Disease Control and Prevention, Atlanta, GA, USA). The absolute and percent frequencies were obtained for data analysis (descriptive statistical techniques). The existence of statistically significant relationships between the variables was verified by means of bivariate analysis (Yates' chi-square test) using a value of □=.05

RESULTS

From the 423 forensic medical reports reviewed in this study, 79.9% (n=338) of the victims were male and 20.1% (n=85) were female. Figure 1 presents the distribution of victims according to the gender and year of study.
Most victims were among the older age groups, that is, 17-year-olds (25.8%) and 18-year-olds (24.1%) (Table 1).

Table 1. Distribution of deaths according to the victim's age.

	Sex						
	Male		Female		**Ratio**	**Total**	
Age (years)	n	%	n	%		n	%
12	20	66.7	10	33.3	2:1	30	7.1
13	17	62.9	10	37.1	1.7:1	27	6.4
14	19	67.8	9	32.2	2.1:1	28	6.6
15	41	74.5	14	25.5	2.9:1	55	13.0
16	56	77.7	16	22.3	3.5:1	72	17.0
17	93	85.3	16	14.7	5.8:1	109	25.8
18	92	90.1	10	9.9	9.2:1	102	24.1
Total	338	79.9	85	20.1	4:1	423	100.0

The mean male-to-female ratio was 4:1, increasing to 9.2:1 among the adolescents aged 18 years.
Wounding with firearms was the main cause of death (33.3%) followed closely by transport accidents (32.2%).(Table2). The analysis of mortality due to firearms revealed a statistically significant difference between genders, with boys showing a 3.7 times greater chance of getting killed by firearms than girls (P=0.000; OR=3.7 [1.96-7.18]).
Regarding the transport accidents, there was a predominance of motorcycle accidents (n=60; 44.1%), followed by accidents in which the children and adolescents were pedestrians (n=40; 29.4%) or were in a vehicle (n=23; 16.9%). This information was missing from 7 (5.1%) medical forensic reports. There was statistically significant relationship between the occurrence of transport accidents and gender (P=0.033; OR=0.57 [0.35-0.93]).

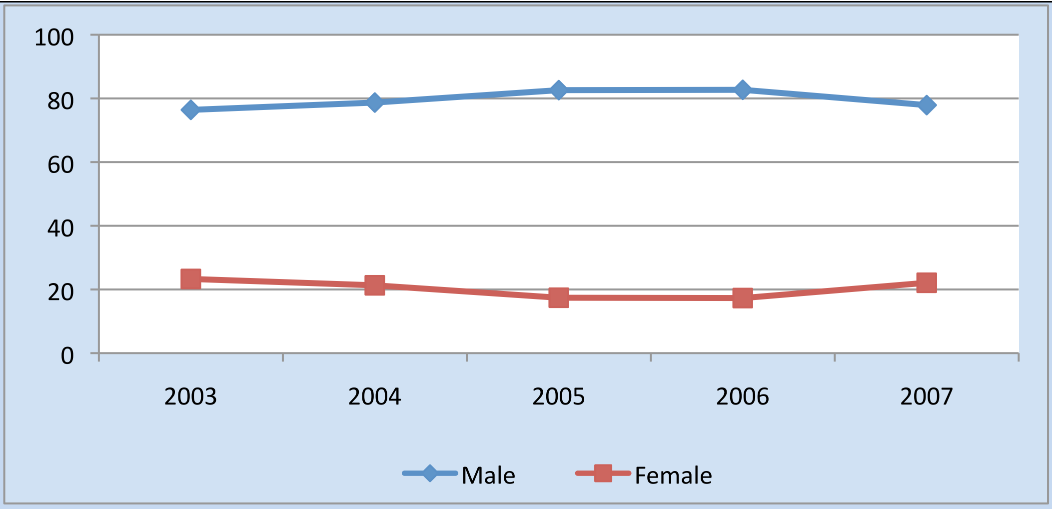

Figure1:Distribution of victims according to gender and year of study

Table 2. Distribution of deaths according to the cause of death and victim's gender

	Sex					
Cause of Death	Male		Female		Total	
	n	%	n	%	n	%
Motor vehicle accident	100	73.5	36	26.5	136	32.2
Drowning	49	75.4	16	24.6	65	15.4
Violence	10	83.3	2	16.7	12	2.8
Knife	18	90.0	2	10.0	20	4.7
Firearm	129	91.4	12	8.6	141	33.3
Electricity	3	75.0	1	25.0	4	0.9
Enforcamento	8	80.0	2	20.0	10	2.4
Poisoning	2	50.0	2	50.0	4	0.9
Undetermined	2	28.6	5	71.4	7	1.7
Intoxication	2	50.0	2	50.0	4	0.9
Other	3	60.0	2	40.0	5	1.2
Fall	9	75.0	3	25.0	12	2.8
Burn	1	100.0	0	0.0	1	0.2
NR*	2	100.0	0	0.0	2	0.5
Total	338	79.9	85	20.1	423	100.0

*Not reported

Overall most victims presented multiple injuries distributed all over the body (n=303; 71.6%), while 11.3% (n=48) of them presented with a single injury. A total of 72 victims (17.0%) did not show any injuries. There was statistically significant relationship between the occurrence of transport accidents and the presence of multiple injuries, namely that victims of motor accidents had a 46 times greater chance of being polytraumatized (P=0.000; OR=46,7 [11,3-192,7]).

The analysis of injury location revealed that 51.1% the victims had injuries to the head, while 49.9% presented only injuries to the face. Forty-one victims presented with maxillofacial fractures (9.7%) and 12.5% (n=53) with intraoral injuries, including 18 (34.0%) individuals with dental fractures affecting 52 teeth.

There was statistically significant association between the occurrence of transport accidents and the presence of injuries to the head (P=0.000), maxillofacial fractures (P=0.000) and intraoral injuries (P=0.000), as shown in

Table 3. Likewise, there was statistically significant association between the presence of multiple injuries and the occurrence of injuries to the head (P=0.000), maxillofacial fractures (P=0.000) and intraoral injuries (P=0.000) (Table4).

Most facial fractures occurred in the mandible (38.0%), followed by the nasal (22.5%), maxillary (16.9%), zygomatic (12.7%) and orbital (8.5%) bones, and the alveolar bone in a smaller proportion (1.4%) (Table 5).

Table 3. Association between transport accidents and presence of injury to the head, maxillofacial fracture and intraoral injuries

	Transport Accidents					
Variable	Yes		No		**P value**	**Odds Ratio**
	n	%	n	%		(IC=95%)
Injury to the head						1
Yes	108	50.0	108	50.0	P=0.000	6.39
No	28	13.5	179	86.5		(3.95-10.32)
Maxillofacial fracture						1
Yes	25	61.0	16	39.0	P=0.000	3.81
No	111	29.1	271	70.9		(1.96-7.41)
Intraoral injuries					P=0.000	1
Yes	30	56.6	23	43.4		3.24
No	106	28.6	264	71.4		(1.80-5.84)

Table 4. Association between presence of injury to the head, maxillofacial fracture and intraoral injuries and the existence of multiple injuries.

Variable	Multiple injuries Yes n	Yes %	No n	No %	P value	Odds Ratio (IC=95%)
Injury to the head						1
Yes	211	69.6	92	30.4	P=0.000	52.7
No	5	4.2	115	95.8		(20.84-133.46)
Maxillofacial fracture						
Yes	41	100.0	0	0.0	P=0.000	*
No	262	68.6	120	31.4		
Intraoral injuries					P=0.000	1
Yes	51	96.2	2	3.8		11.94
No	252	68.1	118	31.9		(2.85-49.87)

Table 5. Distribution of the facial fractures according to the localization

Localization	Frequency n	%
Mandible	27	38.0
Nasal	16	22.5
Maxillary	12	16.9
Zygomatic	9	12.7
Orbital	6	8.5
Alveolar	1	1.4
Total	71	100.0

DISCUSSION

Every year, injuries due to external causes account for 950,000 deaths of children and adolescents under the age of 18 worldwide[15]. It can be said that the life expectancy of thousands of young people is abruptly terminated by transport accidents, acts of violence or even injuries that occur at home or during moments of leisure[4].

In Brazil, from the total over all age groups of 131,471 deaths due to external causes registered in 2008, 1.8% and 10.3% occurred in the 10-14-year-old and 15-19-year-old age groups, respectively, with higher prevalence among males in both age ranges[5]. As reported by other authors[16-18] and confirmed in the present study, data analysis by gender and age shows that males and older adolescents are more frequent victims of fatal external causes.

Regarding the causes of death, this study showed that in 1.7% of cases it was not possible to establish the cause of death. In some cases, the corpses were found in an advanced stage of decomposition, which made it difficult for the forensic medical expert to establish the cause of death[19].

Unlike the results of studies performed in Switzerland[18], United States[10,20], Lithuania[21] and Manipal[22], in which transport accidents were mentioned as the main cause of deaths among adolescents, in the present study injuries caused by firearms were responsible for the majority of deaths, corroborating the results obtained in Fulton (Georgia)[17]. In the United States, fatal gunshot wounds rank second as the cause of death among youths from 1 to 19 years[23]. In addition to socio-cultural determinants, the violence in Brazil is primarily associated with abuse of alcohol and illicit drugs as well as with the easy access to firearms[24], which potentially increase the risk of death due to external causes[23].

The high morbity and mortality rates related to transport accidents in Brazil have been associated with the facts that private cars are usually the preferred mode of transport, and the road networks offers inadequate conditions in terms of safety[24]. Other additional factors include driving above the speed limit, driving under the influence of alcohol or drugs, inexperience of young drivers and above all lack of use of safety equipments (seal belts, airbags, age-appropriate restraint devices for children, and motorcycle helmets)[20,24].

Transport accidents, as the second major cause of deaths in the studied age group, were responsible for the majority of injuries to the head and face among the victims, which is consistent with previous findings[8,25-27]. In the United States, a study of children and adolescents revealed that transport accidents double the risk of facial fractures and that the association of a higher mortality rate with facial fractures results from the concomitant occurrence of severe injuries to the head[26].

The predominance of deaths involving motorcycles observed in this survey agrees only with the results of studies conducted in Estonia[28] and Iran[29], but the same result was not observed by other authors[10,25,30]. The fact that motorcycle riders have less physical protection compared to car drivers explains the higher risk during motorcycle crashes[31]. Motorcycle riders are at a higher risk for both collision traffic injuries as well as non-collision transport accidents that is not the case with other vehicles[29].

The transportation of children and adolescents on motorcycles is a common practice in small- and medium-sized Brazilian cities, such as Campina Grande, where the present study was conducted, because this is the main means of transportation among low socioeconomic groups [19]. The mortality of a teen driver is a complex phenomenon, which could partially be explained by the inherent characteristics of this group, such as immaturity, feelings of omnipotence, a tendency to overestimate their skills, little experience, limited ability to drive, and risky behaviors[32].

Maxillofacial injuries are common injuries that occur due to motor vehicle accidents. They can occur in isolation or in combination with concomitant injuries. In general, there are three broad divisions of maxillofacial injuries: facial bone fractures, soft tissue injuries, and dentoalveolar injuries[32].

The association between head, face and intraoral injuries and transport accidents and multiple injuries found in the present study shows that the head is severely injured in the majority of the cases. A study of Portuguese children and adolescents revealed that in all deaths due to transport accidents, all victims presented with multiple fractures, including injuries to the head[25].

Mandible and midfacial skeletal fractures are among the frequently reported facial bone fractures[33]. The present study found a large number of mandible fractures, which has also been observed in previous studies with children older than 10 years involved in motor vehicle accidents[8,25,26]. It is likely that, as the mandible is the only mobile bone of the face, it is more prone to fractures than the bones of the middle third of the face, which have greater bone support[34].

The relatively high incidence of injuries resulting from transport accidents indicates the necessity to reinforce legislation aimed at preventing road traffic accidents and thus to reducing maxillofacial injuries among children and adults[35].

CONCLUSION

Fatal gunshot wounds and transport accidents were the leading causes of death among male adolescents and teenagers. The victims presented with multiple injuries, especially to the head and face regions, and the mandible was the most frequently injured facial bone.

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SECTION TOOLS AND TECHNIQUES

The Tooth for Molecular Analysis and Identification : a Forensic Approach

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The authors declare that they have no conflict of interest.

ABSTRACT

The aim of this study is to optimize laboratory preparation of teeth for DNA identification. By sectioning the tooth topographically into two different radicular portions, it was analyzed whether these portions of mineralized tissue differ in the quantity and quality of DNA they contain.

25 teeth were subject to different experimental conditions and total DNA was quantified for each individual tooth's radicular portion: apical and remaining root, according to a 2003 study by Gaytemenn and Sweet.

We verified, with statistically significant figures, that the apical portion of the tooth is that which contains the greatest quantity of DNA. Different analytical procedures were studied for various polymorphic markers to evaluate the quality of the DNA.

We concluded that the tooth is topographically distinct in both DNA quantity and quality. The tooth's apical portion is the preferential choice in sample preparation of dental mineralized tissue for molecular analysis and identification.

KEYWORDS*: *tooth, apical root, identification, genetic profile

INTRODUCTION

The dentition is an organ, made up of different types of tissues which are structurally disposed for a specific function [1,2]. Enamel, the protective tissue is acellular, avascular and non-enervated and therefore unimportant for genetic analysis. Cement is responsible for tooth anchoring and suffers constant remodeling. Cement is composed of cement cells located in lacunae and can characteristically invaginate the interior of apical canaliculus and canaliculi[3]. Dentin, [2,3] characterized by its histological organization can be designated secondary or tertiary (which comprises sclerotic dentin).

The choice of a tooth as a sample for genetic analysis occurs only in situations of extreme degradation when other biological tissues are not considered suitable for sampling [4,5,6,7,8,9,10]. In practice, dental pulp, due to being highly vascularized connective tissue, was the first to be studied. However, for that same reason it is also the first part of the tooth to be degraded, therefore in this study mineralized dental tissues are being studied.

Through molecular analysis of the tooth, specifically mineralized tissues, we aimed to establish individual identification and genetic profile typing using DNA analysis.

Our specific objective was to study the apical radicular portion and the remaining radicular portion to analyze whether these portions differed in DNA quantity and quality.

MATERIALS AND METHODS

25 teeth belonging to individuals between the age of 18 and 87 were studied after informed consent was obtained. Equal proportions of intact teeth and those with caries or restorations were present. The teeth were exposed to different experimental conditions, some were buried in different mediums: pH=6 (n=7); pH=5 (n=3); pH=7 (n=5); buried in sand(n=7) for a period of 7 months while others subject to various atmospheric conditions (Iberian Peninsula) for two years (n=3). The material used for sampling was in different stages of genetic alteration after cell death [6,11,12].

Each tooth was chemically cleaned, with hypochlorite, and mechanically, with a rotary device, it was then pulpectomized (fig.1), and only the root portion kept. The root portion was then divided further into two portions, the apex and the remaining portion, in conformity to the topographic drawings of Gaytmenn and Sweet's 2003 study [13] (fig.2).

Figure 1: Sequence of the used tooth, cuts by Gaytmenn and Sweet

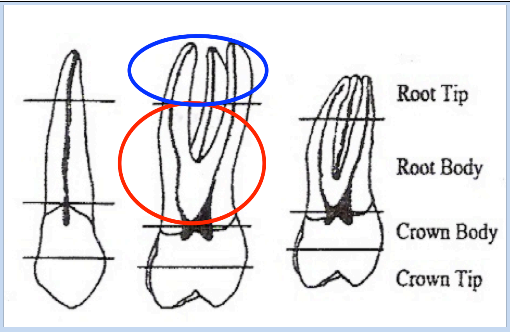

Figure 2: The Gaytmenn and Sweet guidelines

DNA was extracted using the ArchivePure commercial™ kit (5Prime®). Total DNA quantification was performed by real time PCR, by using the Human Quantifiler kit (Applied Biosystems®).

Total DNA quantification value was associated with the study viability of the polymorphisms which are most relevant for identification, namely autosomal STRs [14,15], with the aid of AmpFℓSTR® Identifiler™ and AmpFℓSTR® Minifiler™ commercial kits, both by Applied Biosystems.

Mitochondrial DNA analysis was performed on samples over which validation of the 7 markers or any autosomal marker was not viable [16,17,18, 19], and in which it was not possible to obtain an identifying autosomal profile.

The results of the quantitative study of DNA, performed on the apical and remaining tooth radicular portion, were analyzed from 25 paired samples. Quantification results were then statistically analyzed with SPSS (Statistical Package Social Science) software. Such analysis began by studying normality of the variable quantification through the Kolmogorov-Smirnov test. The non-parametric inferential median test was applied for the absence of normality within the variable quantification.

RESULTS

The results for the parameter total DNA quantification (ng/µL) obtained for each of the portions analyzed, the conservation medium and characterization of the analyzed polymorphisms are shown in Figure 3 and Table 1.

DISCUSSION AND CONCLUSIONS

Various types of teeth were sampled for this study: intact, carious, endodontically treated and restored. Teeth without caries were not included as referred to in the studies by Schwartz and colleagues (1991) [20] Chen, Sun e Wu (1994) [21] ou Alvarez-Garcia (1995) [7], López (1996) [22] and Utsuno e Minaguchi (2004) [23]. Over the years, the pulp has been considered the ideal source of DNA. Pulp degradation in extreme forensic situations, endodontic clinical procedures and difficulty anatomically in removing pulp tissue, makes, pulp tissue unsuitable for sampling. In this study the teeth were cleaned and pulpectomized and the mineralized tissues of two root portions were analysed.

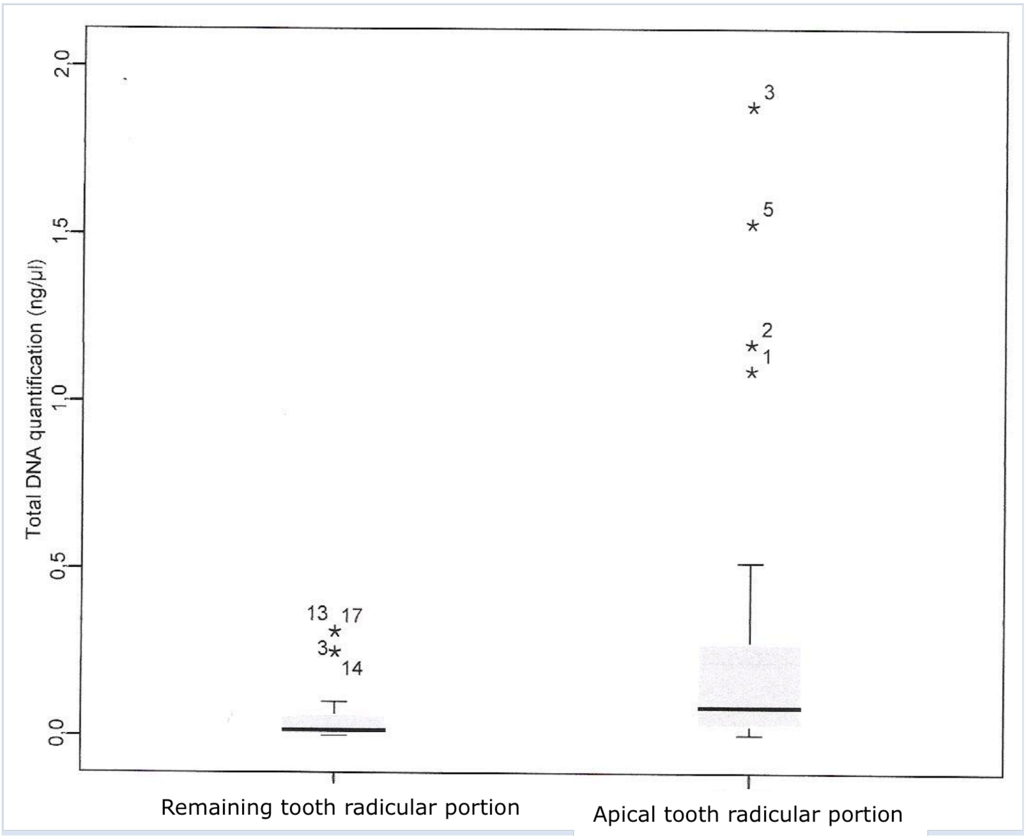

Figure 3: Quantification results of the apical radicular portion and the remaining radicular portion, statistically analyzed with SPSS.

There are several techniques for tooth preparation before DNA extraction [7, 20, 21, 22, 23], the most common is the crushing or grinding, which implies sample destruction. In this paper the tooth was preserved for morphological study, crown and restorations description. The main purpose of this research is to reduce the damage of archaeological specimens, maintain the crown morphology and validate access through the pulp, when this can yield potential samples [25,26].
The apical portion of the tooth was compared to the remaining radicular portion, and a topographic molecular analysis was made considering the two different portions of the root: the apical portion and remaining root.

The values obtained in the quantification of total DNA were higher for the apical portion than for the remaining root (table 1)the same applies to the number of genetic profiles that were obtained in each radicular portion. The type of the tooth did not affect the results obtained. Due to the tooth's anatomy, the pulp chamber communicates with the surrounding environment through the tooth's apical foramen located near the apex, and through which mineralized tissues of the erupted tooth maintain their activity. Elements resulting from cell death are disseminated through the apex: potential elements of DNA degradation and contamination.The pulp that fills the dentinal canalicules is degraded after death in a centripetal manner. Recent studies have focused on mineralized tissuesas an alternative source of DNA. One such study, published in 2003 by Gaytemenn and colleagues, shows that the

Table1: Characterization of samples according to conservation medium, total DNA identification and analyzed polymorphisms in 25 paired samples

SAMPLES	*MEDIUMS*	*RADICULAR TOOTH*	TOTAL DNA QUANTIFICATION (ng/□L)	*STR Profile*	*MITOCHONDRIAL*
1	2YEARS	REMAINING	0,006	*	ANALYZED
		APICAL	1,09	15 POLYMORPHISMS	**
2		REMAINING	0,1	*	ANALYZED
		APICAL	1,17	15 POLYMORPHISMS	**
3		REMAINING	0,311	15 POLYMORPHISMS	**
		APICAL	1,88	15 POLYMORPHISMS	**
4	pH6	REMAINING	0,019	*	ANALYZED
		APICAL	0,022	*	ANALYZED
5		REMAINING	0,003	*	ANALYZED
		APICAL	1,53	15 POLYMORPHISMS	**
6		REMAINING	0,0000	*	ANALYZED
		APICAL	0,03534	*	ANALYZED
7		REMAINING	0,024	*	ANALYZED
		APICAL	0,2396	14 POLYMORPHISMS	**
8		REMAINING	0,0605	*	ANALYZED
		APICAL	0,5172	15 POLYMORPHISMS	**
9		REMAINING	0,0061	*	ANALYZED
		APICAL	0,1027	*	ANALYZED
10		REMAINING	0,017	*	ANALISADO
		APICAL	0,113	*	ANALYZED
11	pH5	REMAINING	0,0158	*	ANALYZED
		APICAL	0,157	*	ANALYZED
12		REMAINING	0,033	*	ANALYZED
		APICAL	0,0846	*	ANALYZED
13		REMAINING	0,031	*	ANALYZED
		APICAL	0,033	*	ANALYZED
14	pH7	REMAINING	0,0250	*	ANALYZED
		APICAL	0,2955	12 POLYMORPHISMS	**
15		REMAINING	0,002	*	ANALYZED
		APICAL	0,0502	*	ANALYZED
16		REMAINING	0,0045	*	ANALYZED
		APICAL	0,047	*	ANALYZED
17		REMAINING	0,2491	15 POLYMORPHISMS	**
		APICAL	0,2756	15 POLYMORPHISMS	**
18		REMAINING	0,0012	*	ANALYZED
		APICAL	0,004	*	ANALYZED
19	SAND	REMAINING	0,0096	*	ANALYZED
		APICAL	0,0158	*	ANALYZED
20		REMAINING	0,0077	*	ANALYZED
		APICAL	0,031	*	ANALYZED
21		REMAINING	0,0605	*	ANALYZED
		APICAL	0,1084	*	ANALYZED
22		REMAINING	0,007	*	ANALYZED
		APICAL	0,004	*	ANALYZED
23		REMAINING	0,001	*	ANALYZED
		APICAL	0,039	*	ANALYZED
24		REMAINING	Indeterminated	*	ANALYZED
		APICAL	0,0069	*	ANALYZED
25		REMAINING	Indeterminated	*	ANALYZED
		APICAL	0,0020	*	ANALYZED

* Validated less than 7 markers, ** Not studied.

mid portion of the root is the ideal mineralized tissue for genetic analysis. Our results contradict their findings (Table 1 and Fig 1).

Our results can be explained based on the fact that apical canicular obliteration through the formation of tertiary dentin can preserve some of the pulpar contents in the topographic region, resembling a "mosquito in amber"; on the other hand cementogenesis in the apical portion can occur by invagination into the canaliculi in a rapid and disorganized fashion enabling the trapping of cementocites in its lacunae (fig. 4). In our study it was the apical portion that through its cellular content presented Ageing processes increase the quantity of collected DNA, which can be explained by the increased numbers of cell repairs and regenerative processes with increased sequestration of cells in the mineralized matrix (fig. 4).

Forensic laboratory practice shows that success in DNA typing depends on the conditions under which bodies are maintained and their capacity to degrade [6,11,12,24]. In order to promote extreme degradation of the pulp we have mimicked extreme forensic conditions as in prior studies by Pfeiffer and colleagues (1999), Burger and colleagues (1999) and

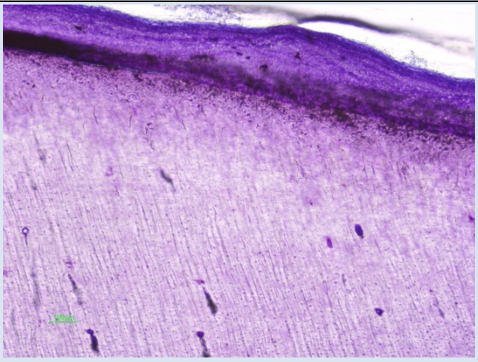

Figure 4: Microscopic photograph of cementogenesis in the apical root portion.

in studies by Alonso and colleagues (2001). To evaluate DNA quality, different polymorphisms were studied: 15 STRs and Amelogenin, the full profile for identification according to the European Standard .

We have verified that sand and pH=5 were the mediums that produced the poorest results in DNA analysis: less than seven nuclear markers were validated, only permitting the characterization of maternal lineage through the mitochondrial polymorphisms chosen for this study.

We verified that DNA quantity and quality are correlated [27]. For samples under 0,179ng/µL (tab. 1) mitochondrial DNA was used to obtain an ID. For the other samples we were capable of detecting more than seven autosomal markers, which are the preferred markers for DNA identification [16,17]. In samples 1,2,5,7,8 and 14, (Table 1) autosomal polymorphic validation for an ID (15 STRs) was obtained in the apical radicular portion while this was not possible in the remaining radicular portion. To increase the chances of compiling a complete profile from the samples (table1), we amplified with the more sensitive next generation kits, such as AmpFℓSTR® NGM™ PCR amplification kit (Applied Biosystems (D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01, FGA, D1S1656, D12S391, D10S1248, D2S441 and the gender determination *locus* amelogenin) and the PowerPlex® ESI 17 System (Promega) (D22S1045, D2S1338, D19S433, D3S1358, Amelogenin, D2S441, D10S1248, D1S1656, D18S51,D16S539, D12S391, D21S11, vWA, TH01, SE33, FGA AND D8S1179)[28,29].

We believe that these preliminary results warrant a further study with a larger sample size. .

This study emphasizes the value of the genetic analysis of mineralized tooth tissues as an alternative to pulp, especially in extreme forensic conditions.

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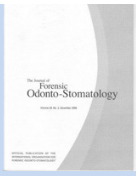

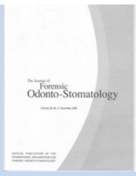

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SECTION TOOLS AND TECHNIQUES

Cementum Made More Visual

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The authors declare that they have no conflict of interest.

ABSTRACT

Dental cementum is a specialized calcified structure covering the root of a tooth. This study aims to investigate cementum using various stains which can be exceedingly useful in investigation, observation and diagnosis. 4µm sections of 25 extracted normal teeth, 25 cases of various cemental pathologies and 25 ground sections were stained using cresyl violet, H&E, toluidine blue and periodic acid Schiff and were observed under light and florescence microscopes. Cresyl violet showed best contrast amongst all stains in decalcified and ground sections under light and florescence microscopy. Under the fluorescence microscope, cementum floresced more distinctly than dentin and enamel. Among the cemental pathologies examined, osteoid and cementoid exhibited florescence but cementum and bone did not fluoresce. Incremental lines were prominently visualised with cresyl violet under fluorescent microscopy, which may aid in forensic determination of age. The present results demonstrate that cementum in normal decalcified teeth and cemento-osseous lesions, could be observed best using cresyl violet stain under florescence microscopy.

KEYWORDS**:** dental cementum; microscopy; pathology

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INTRODUCTION

Cementum is an avascular, inorganic, dense, inert, extremely narrow layer of tissue whose sole purpose is that of affording attachment for a tooth to its articulation. Various authors have suggested the possibility of age estimation from acellular cementum incremental lines.[1-6] Human cementum has been shown in studies to exhibit a strong propensity for fluorescence.[7]

The dentino-enamel junction is well defined in light microscopy. In contrast, the distinction between root dentine and cementum is exceedingly ill defined; so much so that, in decalcified haematoxylin and eosin [H&E] stained sections, it is difficult to distinguish between them. This may be because both are mesodermic in origin with similar histologic character and physiological function. Various stains have been used for cementum but not many are easily available and economical. An inexpensive and distinctive stain for cementum can be extremely useful in investigation, observation, diagnosis & teaching. A search of the published literature has shown that, at present, there are only few techniques[8] which will differentially stain the cementum of human teeth.

Various controversies exist about the cemento-osseous lesions affecting oral cavity. This group of lesions are characterized microscopically by fibrous stroma containing various combinations of bone and cementum- like material. Histologically, they may be indistinguishable from other fibro-osseous lesions, except by the clinical and radiographic findings.[9] Thus differential staining of cementum can be of great importance in highlighting the nature and biological behaviour of such lesions.

The present study concerns a successful differential staining technique for human cementum and aims to investigate cementum of decalcified and ground sections using various stains like Cresyl Violet, Hematoxylin and Eosin, Toluidine Blue and PAS under conventional light & fluorescent microscopy. We have also studied the differential staining of cementum in various pathological cemento-osseous lesions.

MATERIALS AND METHODS

25 normal human teeth which were extracted for orthodontic reasons from KLE VK Institute of Dental Sciences, Belgaum were used for the study. Institutional Review Board and Ethical Committee approval was obtained prior to the start of the study**.** None of the teeth were extracted because of periodontal disease, which destroys the periodontal fibres and stops the formation of cementum in the affected areas. These normal human teeth were decalcified, formalin fixed & paraffin embedded. 25 cases of cemental pathologies which included histopathologically diagnosed cases of peripheral cemento-ossifying fibroma, central cemento-ossifying fibroma and cementoma were retrieved from the archives of the Department of Oral and Maxillofacial Pathology. 4µm sections taken from these tissues and 25 longitudinally cut ground sections were stained using Hematoxylin and eosin,[10] PAS,[11] toluidine blue[12] and cresyl violet.[8] The stained sections were examined using conventional light microscopy (Magnus MLX-Bi) and fluorescent microscopy (Leica™ DM2500) independe ntly by five experienced oral pathologists. The sections were graded from 1 to 4 according to the contrast of cementum in various stains (Table 1).

RESULTS

When sections were viewed under light microscope and approximate average of the grades by five oral pathologists were calculated, cementum was best distinguished in cresyl violet stained decalcified and ground sections (Table 2). Toluidine blue also enabled better differentiation than PAS and hematoxylin and eosin under light microscope (Table 2; Fig.1). In ground sections differentiation between cementum and dentin was more appreciable in cresyl violet than the other three stains (Table 2; Fig. 1).

Table 1: Grading for distinguishing cementum from dentin and periodontal ligament in decalcified and ground sections; in cemento-osseous lesions differentiation of cementum from cementoid.

GRADE 1	Cementum distinguishable with difficulty
GRADE 2	Moderately distinguishable
GRADE 3	Differentiation of cementum easy
GRADE 4	Cementum brightly contrasted

Table 2: Differentiation of cementum from dentin in decalcified and ground sections under various stains as observed under light and florescence microscope (approximate average of the grades of the intensity of staining by five oral pathologists for 20 tooth sections)

Grade	**Cresyl violet stain**		**Periodic acid Schiff (PAS) stain**		**Toluidine blue**		**Hematoxylin and eosin (H&E) stain**	
Light microscopy								
	Decal.	**Gr.**	**Decal.**	**Gr.**	**Decal.**	**Gr.**	**Decal.**	**Gr.**
1	0	0	4(20)	1(5)	1(5)	3(15)	**19(95)**	**17 (85)**
2	0	5(25)	**15(75)**	**16(80)**	2(10)	**15(75)**	1(5)	2(10)
3	1(5)	**15(75)**	1(5)	3(15)	**17(85)**	2(10)	0	1(5)
4	**19 (95)**	0	0		0	0	0	0
Florescence microscopy (green light)								
1	0	0	0	0	0	0	1(5)	2(10)
2	0	0	4(20)	**17(85)**	0	0	3(15)	**12(60)**
3	2(10)	**12(60)**	**14(70)**	3(15)	0	0	**13(65)**	4(20)
4	**18(90)**	8(35)	2(10)	0	0	0	3(15)	2(10)

Decal= % Decalcified sections, Gr=% Ground sections

Under florescence microscope maximum contrast was seen in all cresyl violet stained sections using green light excitation of wavelength 590 nm. Cementum showed red fluorescence as compared to enamel, dentin and PDL (Fig.2). This florescence was brighter than that seen in PAS and hematoxylin and eosin stained sections. Incremental lines were prominently seen as they did not show florescence under fluorescence microscopy (Fig. 2). These incremental lines were more noticeable at approximately apical third of the root and furcation area where cementum is more prominent.

Under light microscope maximum contrast between cementum and cementoid, and bone and osteoid was seen in cresyl violet and toluidine blue. PAS showed better contrast of cementum than H&E stained sections (Table 3). Hematoxylin and eosin stained sections of cemental pathologies showed cementum as basophilic spheroidal lobules and cementoid as eosinophilic deposit around it. Bone was seen as trabeculae with eosinophilic osteoid deposit around it. In PAS stained sections cementum was stained magenta in color.

Cementum was stained blue surrounded by purple cementoid in toluidine blue and in cresyl violet cementum was stained purple. The most appreciable contrast was observed in cemental pathologies stained with cresyl violet, where osteoid and cementoid showed brilliant red florescence under florescence microscope with green light excitation but cementum and bone did not fluoresce (Fig.3; Table 3).

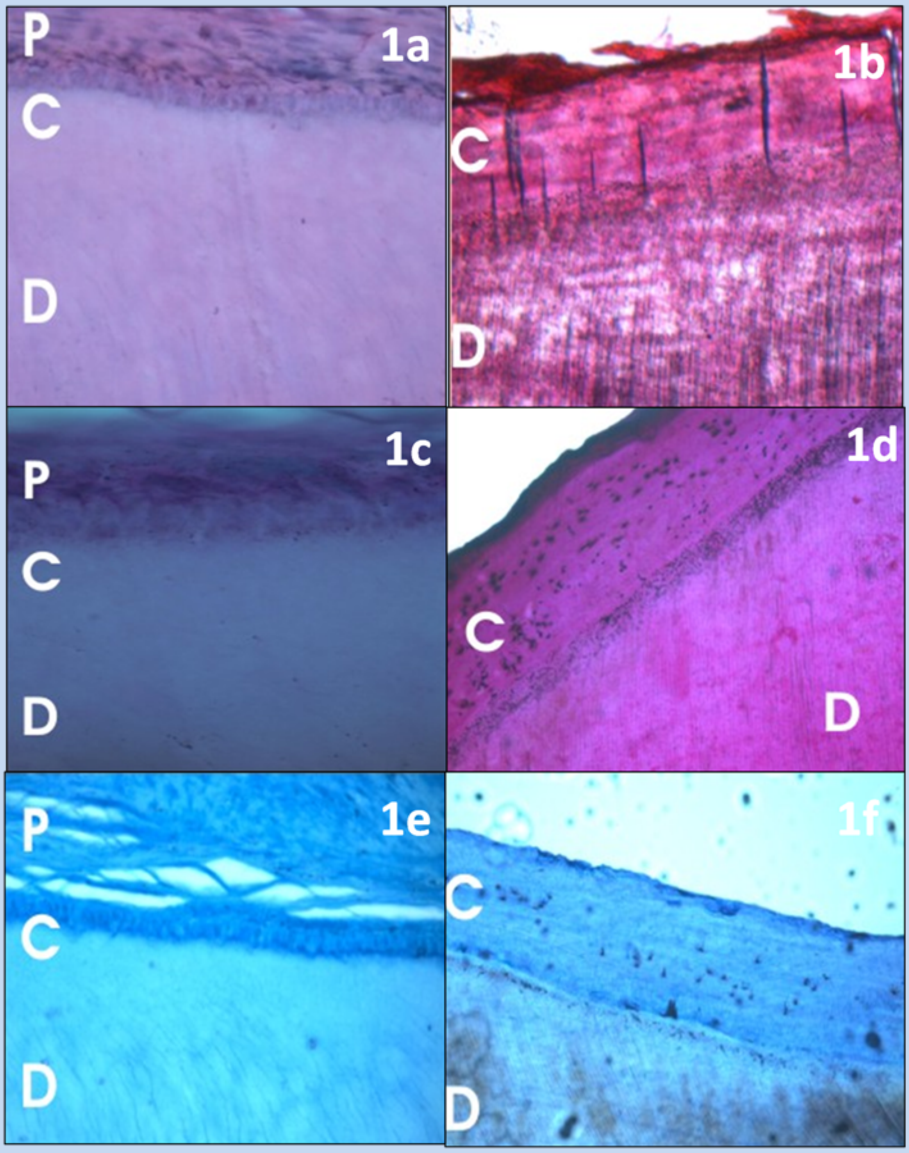

Figure 1: Normal cementum in various stains as observed under light microscope. (1a) Hematoxylin and eosin stained (H&E) decalcified section showing periodontal ligament (P), cementum (C) and dentin (D) (magnification 40 X). (1b) H & E stained (magnification 40 X) ground section showing cementum (C) and dentin (D). (1c) Periodic acid Schiff's stained (PAS; magnification 40 X) decalcified section showing better contrast of cementum (C) from dentin (D) and periodontal ligament (P) than H&E. (1d) PAS (magnification 40 X) of a ground section showing better contrast of cementum (C) from dentin (D) than H&E. (1e) Toluidine blue stain (magnification 40 X) of a decalcified section showing better contrast of cementum (C) from dentin (D) and periodontal ligament (P) than PAS and H&E. (1f) Toluidine blue stain (magnification 40 X) of a ground section showing better contrast of cementum (C) from dentin (D) than PAS and H& E.

DISCUSSION

In the present study cresyl violet stain exhibits better contrast of cementum in decalcified and ground sections than other stains under light microscopy with a clear and even background. Toluidine blue stain

showed better contrast than PAS and H &E (Fig. 1). The contrast was more appreciable in PAS stained sections than H & E. This suggests that cresyl violet staining affords the best appreciation of cementum in decalcified and ground sections.

Further, contrast was better using fluorescence microscopy than light microscopy since the stained cemental bands, but not the incremental lines, fluoresced after staining with cresyl violet, PAS and hematoxylin and eosin. Incremental lines in cementum are most prominently seen in sections stained with cresyl violet excited by green light observed under fluorescent microscopy, a property which may be exploited in forensic studies (Fig. 2d). Since incremental lines are not destroyed by acids and stain differently than the remaining cementum, it is likely that they possess an organic structure which differs from the cementum[8]. It was observed that the width of incremental lines varied significantly from one region to another. The lines seemed to spread and were better distinguished in apical third of root. Incremental lines are considered to represent periods of varying activity in matrix formation and mineralization during cementogenesis. [13] Apposition of cementum occurs in phases resulting in two types of layers with different optical and staining properties. Narrow, dark staining incremental lines are separated by wider bands of pale staining cementum. The distance from one line to the next represents a yearly increment deposit of cementum in many mammals, and counting these lines has been used routinely to estimate the age of the human beings. [8]

In the present study very distinct demarcation between dentin and cementum can be made as the cementum floresced more than dentin and enamel using cresyl violet seen under florescence microscopy (Fig. 2). Cresyl violet shows red fluorescence with green light of wavelength 590 nm in contrast to dentin and PDL. This staining is easy to perform with good shelf life. Cresyl violet was resistant to bleaching and fluorescence persisted for several days with the background fluorescence being minimal. Another interesting finding was the florescence of cementum seen in H & E stained sections. This could be probably due to staining of collagen fibres with eosin Y as occurs in other types of connective tissue. This study demonstrates the successful differential staining of the human cementum, in decalcified sections of teeth, showing the deeply stained layer of cementum (C), contrasting markedly with the dentin (D) and the periodontal membrane (P) (Fig. 2). This was consistent with Sigrid I. Kvaal et al. who concluded that human cementum could be best observed using decalcified sections stained with cresyl violet. [8] The possible explanation for the findings in the present study could be due to the fact that human cementum contains much more organic material & hence fluoresced strongly than dentin & enamel. It is probable that the fluorescing substances in cementum are associated with the organic fraction.

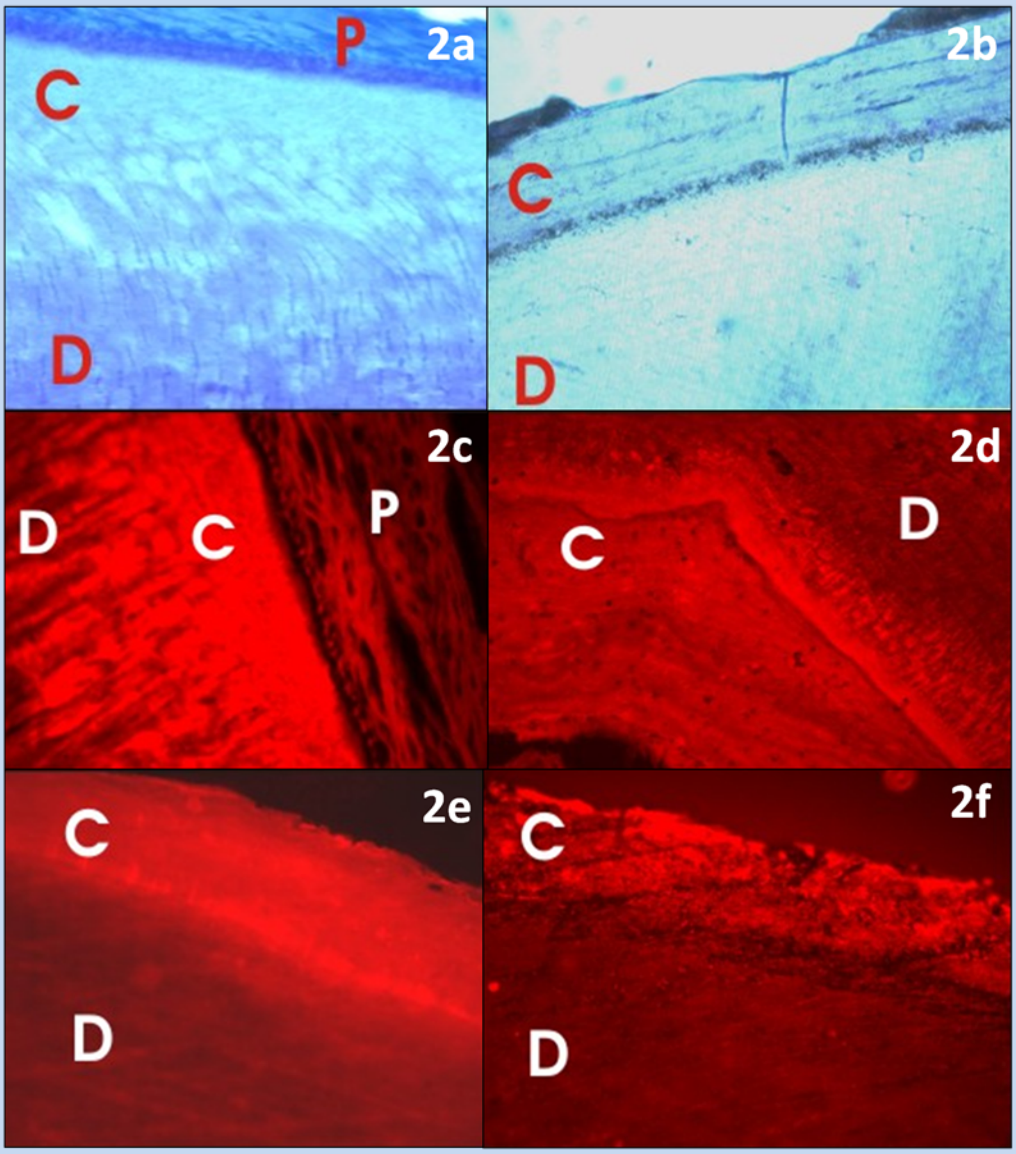

Figure 2: Normal cementum in cresyl violet stain as observed under light and florescence microscope. (2a) Cresyl violet stained decalcified section (magnification 40 X) showing best contrast of cementum (C) from dentin (D) and periodontal ligament (P) than other stains under light microscopy. (2b) Cresyl violet stained ground section showing best contrast of cementum (C) than other stains under light microscopy (magnification 40X). (2c) Cresyl violet stained cementum shows red fluorescence with green light of wavelength 590 nm in contrast to dentin and PDL as seen under florescence microscopy (magnification 40X). (2d) At the bifurcation of a mandibular molar incremental lines in cementum are prominently seen as they do not show florescence with cresyl violet under fluorescent microscopy (magnification 40X). (2e & 2f) Cresyl violet stained ground sections showing distinctly demarcated cementum (C) from dentin (D) as cementum show red florescence under fluorescence microscopy (magnification 40X).

In the present study the sections from cemental pathologies showed calcified material which is more appropriately considered to be cementum. This belief is based upon a number of criteria. A globular accretion pattern with basophilic tendency is evident in these lesions under plain light. In a trabecular form the trabeculae are usually “molded” and do not have the rather sharp angles that are seen in bone. With plain light microsocopy, solid masses of secondary cementum are virtually indistinguishable from bone. In cementum typical osteoblasts and rimming of cells is not seen. In comparison with bone, the lacunae in cementum contain fewer recognizable cellular elements. The use of these criteria for the distinction between bone and cementum was valid in a high percentage of cases observed in the present study. Cementum, while is undoubtedly a phylogenetic derivation of bone, is at the same time different in a number of respects. Cementum appears to be destined to form dense, sclerotic masses without organization that are incapable of remodelling and appears to be resistant to osteoclasis. [14]

Another interesting finding in the present study was that under florescence microscopy, sections of cemental pathologies with osteoid and cementoid showed florescence but cementum and bone did not fluoresce at all (Fig. 3), though in decalcified and ground sections cementum showed red florescence when excited by green light. This could possibly be due to alteration in structure and biochemical composition of cementum which is affected by several diseases. [5] This could make cementum lose its property to fluoresce in cemental pathologies, thus suggesting that when cementum is uncalcified (cementoid) it shows florescence probably due to increased organic proportion, whereas when calcified it loses its florescent properties. We hypothesise that the inorganic portion is abundant in pathologic cementum which replaces the organic portion making the cementum non florescent. Alternatively, the fluorescing substances may be lost in the cementum formed in these pathologies. Further studies are required to investigate the biochemical composition of cementum in cemental pathologies.

CONCLUSION

Cresyl violet showed better contrast of cementum than toluidine blue, PAS and H&E in decalcified and ground sections under light and florescence microscopy. Cementum fluoresced appreciably more than dentin and enamel under florescence microscope when stained with cresyl violet and excited with green light. In cemental pathologies, osteoid and cementoid showed florescence but cementum and bone did not show fluorescence. Incremental lines are clearly seen with cresyl violet under fluorescence microscopy, which could play an important role in forensic sciences

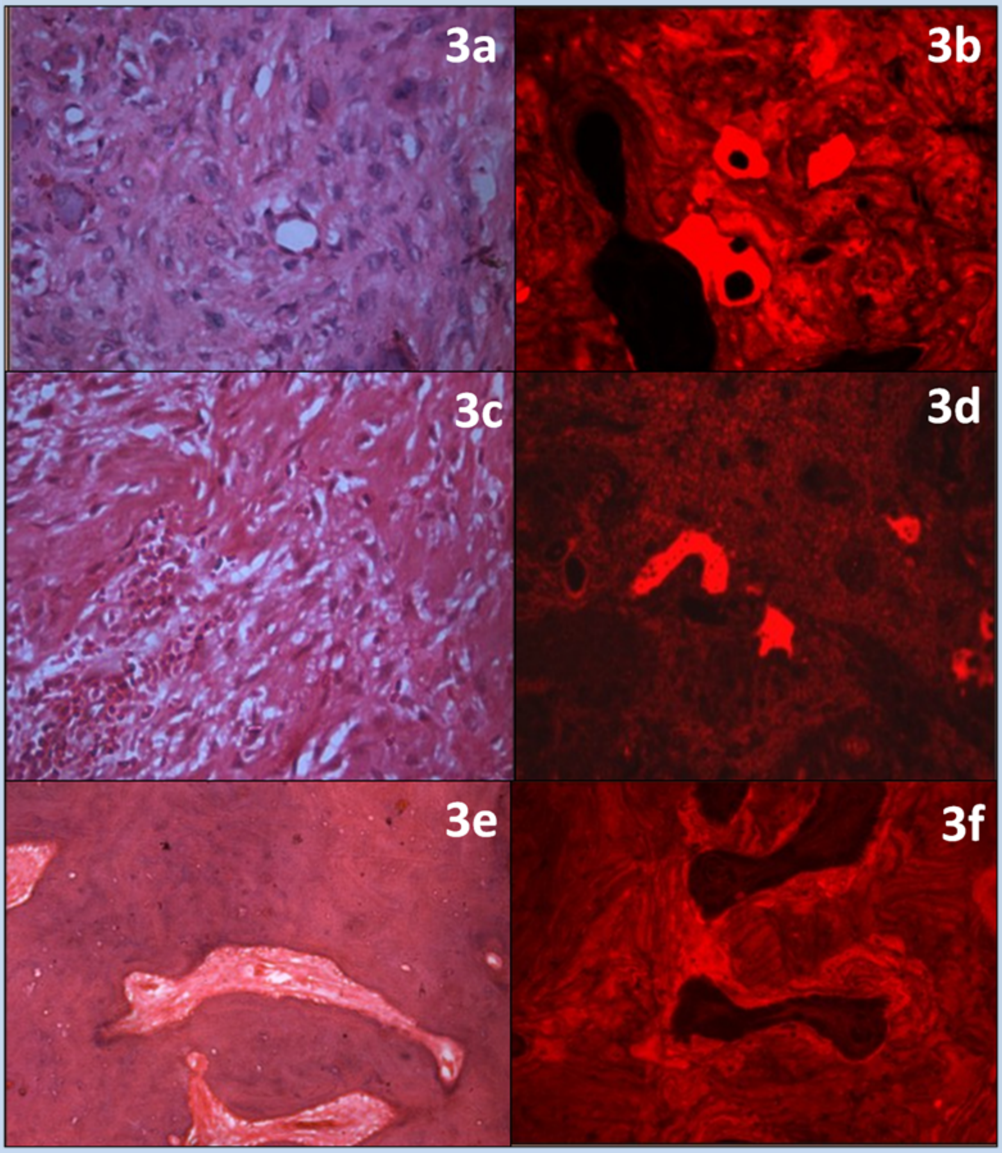

Figure 3: Various cemento-osseous lesions stained with H &E and cresyl violet observed under light and florescence microscopy. (3a) Phomicrograph showing H&E stained peripheral cemento ossifying fibroma with small round basophilic globular masses having calcific foci in the centre resembling cementum surrounded by eosinophilic cementoid in fibrocellular stroma (magnification 40 X). (3b) Cresyl violet stained section of peripheral cemento ossifying fibroma showing red fluorescing cementoid with non fluorescing calcified centre resembling cementum. Bony trabeculae seen on left side shows no florescence (magnification 40X). (3c) H&E stained central cement-osseous fibroma showing irregular bony trabeculae with interlacing collagen fibers interspersed by active, proliferating fibroblasts (magnification 40X). (3d) Cresyl violet stained section of central cemento-ossifying fibroma showing fluorescing osteoid in the centre. Few oval to round fluorescing cementoid like globules with non fluorescing calcified centre are seen on right side (magnification 40X). (3e) H&E stained section of cementoma showing sheets of cementum like tissue with scattered reversal lines. (3f) Cresyl violet stained section of cementoma showing calcified cemental trabeculae with cementoblasts at their borders, surrounded by fluorescing cementoid (magnification 40X).

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SECTION ANTHROPOLOGY ARCHEOLOGY

Sex determination by linear measurements of palatal bones and skull base

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ABSTRACT

Genetically determined sexual dimorphism is not restricted to reproductive organs. All body structures show sexual differences which emerge during puberty and persist lifelong. The aim of this study is to obtain a reliable method for sex determination through the analysis of linear measurements of palate bones and skull base. One hundred skulls of both sexes, 50 from males and 50 from females, aged between 22 and 55 years, from the São Gonçalo Cemetery of Cuiabá, capital of Mato Grosso state, Brazil, were analyzed. Distances between the incisive foramen, right and left greater palatine foramens and the basion were measured with a digital caliper. Finally, data were tabulated and statistically analyzed. Measurements showed significant sexual dimorphism, except the distance between the right and the left greater palatine foramens. The superior expression of sex dimorphism corresponded to the distance from the basion to the incisive foramen. The authors obtained two mathematical models for sex determination, with a reliability rate of 63% and 65% respectively.

KEYWORDS**:** Forensic anthropology, anthropometry, forensic dentistry, sex, skull, palate

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INTRODUCTION

Anatomic differences between men and women go much beyond body physiognomy or the presence of primary and secondary sexual characters. Male and female human skeletons, despite having the same bones, exhibit a number of differences. However, they are not always apparent and are sometimes difficult to identify in the sex differentiation process.[1,2]

Before puberty it is virtually impossible to diagnose sex by visual examination of the human skull. After this period, as a result of hormone action, distinctive sexual characteristics become more apparent, male muscles gain mass and power, and bones begin to exhibit significant differences between sexes.[3,4] However, there is no single male skull trait that identifies a skull as being male or female. Rather, it is a set of traits that determines one sex or the other. [4, 5, 6]

Male skulls are significantly larger, heavier, and thicker in addition to having greater cranial capacity, whereas in women, protuberances, crests and processes tend to be smaller and smoother. [6, 7, 8]

It is well known that the credibility and reliability of human identification processes is directly related to the amount of data available for the individual. Thus, assessment of all truly dimorphic bones in the human skeleton, would be an ideal condition for determining sex. However, there are numerous cases in which a complete skeleton is unavailable, hindering this type of identification.

The verification of dissimilar features and dimensions involving the sexes, in addition to facilitating the human identification process, makes it more reliable since correct sex determination reduces the pool of evaluated persons by one half. [2,9]

Given that male proportions are larger than their female counterparts, we sought to establish a sex differentiation parameter,

based on relevant anatomic points present when the skull base is visualized, since the male palate is larger than the female's[7, 10, 11] and the skull base is very important in determining sex.[12]

The aim of this paper is to develop an accurate sex determination method using linear measurements of the palatal bones and skull base.

MATERIALS AND METHODS

Sampling

The sample consisted of 100 skulls (50 males and 50 females), aged between 22 and 55 years, with no malformations, apparent abnormalities, severe pathologies or traumatic sequelae, from persons who died in the second half of the 20th century. The skulls were obtained from the São Gonçalo Cemetery of Cuiabá, capital of Mato Grosso state, Brazil.

Data collection

The following linear measurements of the palatal bones and skull base, expressed in millimeters, were taken by a single investigator using a digital caliper (Digimess®, São Paulo, Brazil).(Figure 1):

- incisive foramen - right greater palatine foramen (IF-RGPF);
- incisive foramen - left greater palatine foramen (IF-LGPF);
- right greater palatine foramen – left greater palatine foramen (RGPF-LGPF);
- basion - incisive foramen (Ba-IF); and
- incisive foramen - point located at the center of an imaginary line joining the right and the left greater palatine foramens (IF-RGPF/LGPF).

To evaluate the intra-examiner reliability, 25 skulls were re-measured after seven days.

Statistical analysis

Data were entered into an Excel file and analyzed using Statistical Package for the

Social Sciences (SPSS) software, version 13.0. Intra-examiner reliability was checked by the paired t-test (systematic error) and Dahlberg's index (casual error), and the remaining data by the Kolmogorov-Smirnov test (normal distribution of the measurements), t-Student test (statistically significant difference between the sexes), and stepwise discriminant analysis model

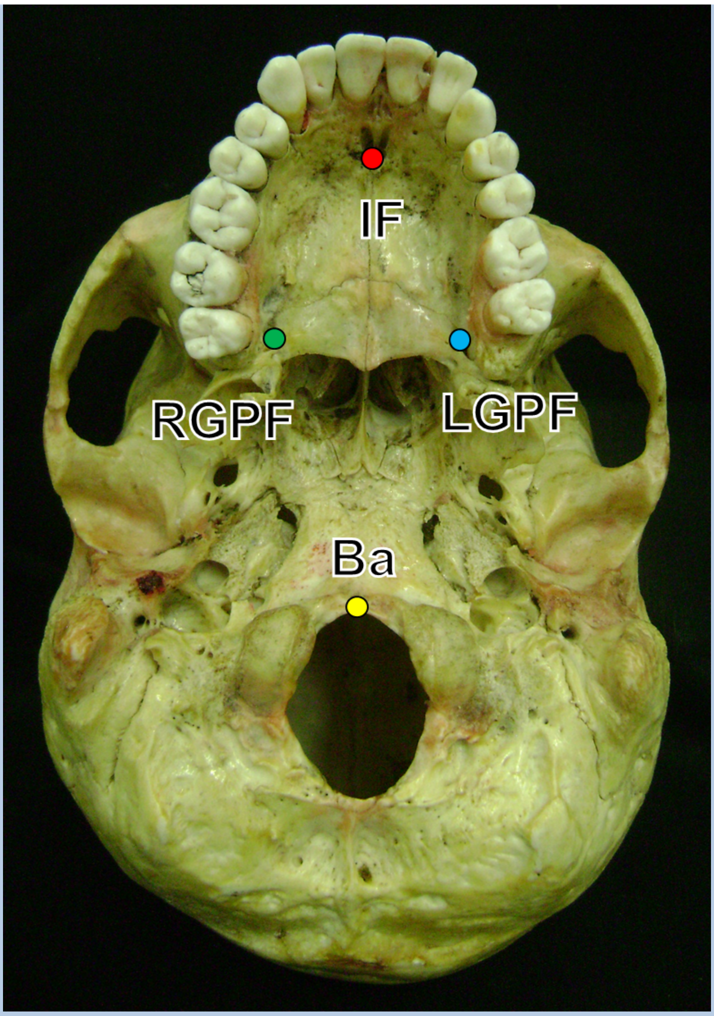


Fig.1: Illustration of anthropometric points used in this research: Incisive foramen (IF, red), right greater palatine foramen (RGPF, green), left greater palatine foramen (LGPF, blue) and basion (Ba, yellow)

RESULTS

With regard to differences between the averages obtained for each sex (Table 1), IF-RGPF, IF-LGPF, Ba-IF and IF-RGPF/LGPF showed statistically significant differences between males and females ($p<0.05$), while RGPF-LGPF did not.

The cut-off value for the most significant measurements is shown in Table 2; numbers above this value represent males and below it, females. Although IF-LGPF showed a higher overall percentage of sex differentiation than Ba-IF(65%), the latter best discriminated between sexes, due to the mean difference observed (3.66mm) (Table 2), demonstrating greater difference between males and females (Table 1).

The highest expression of sexual dimorphism corresponded to the Ba-IF variable ($p=0.004$); values less than 87.0 mm indicate females while those above this value represent males.

The analysis of all possible measurement combinations displayed a similar discriminatory power to Ba-IF, considered separately. Thus, two logistic regression models for sex determination, with an accuracy rate of 63% and 65% respectively, were obtained and expressed by the formulas below:

Sex=-0.194xIF-RGPF+0.268xIF-LGPF+0.141xBa-IF–0.034xIF-RGPF/LGPF– 14.264

Sex = Ba-IF – 87. 0

In both functions, results less than zero indicate females and those above zero suggest males.

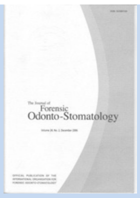
Table 1: **Mean values of variable measurements**

Variable	Female		Male		P-value[1]
	mean	*SD*	*mean*	*SD*	
IF-RGPF	41.78	3.40	43.32	3.10	0.020 *
IF-LGPF	42.19	3.53	43.96	2.96	0.008 *
RGPF-LGPF	34.69	2.46	34.95	2.26	0.593 ns
Ba-IF	85.10	6.45	88.76	5.85	0.004 *
IF-RGPF/LGPF	37.13	3.70	38.99	3.83	0.015 *

(1): t *Student*testsignificance level
(*): statistically significant difference (p<0.05)
(ns): not statistically significant difference (p>0.05)
Measurements in mm

Table 2: Cut-off value that maximizes the accuracy and percentage of correct classification among the sexes.

Measurement	Cut-off value	% of correct classification		
		General	*Female*	*Male*
IF-RGPF	43.27	65%	76%	54%
IF-LGPF	43.03	67%	70%	64%
Ba-IF	86.92	65%	64%	66%
IF-RGPF/LGPF	38.32	64%	66%	62%



DISCUSSION

Human identification consists of a series of steps to individualize individuals and establish their identity.[8] In this respect, forensic anthropology plays an important role in reconstructing the biological profile, taking into consideration its four main components: ancestry, age, stature and sex.[13,14] These factors can be determinants in a unique subject or a large number of unidentified corpses and skeletal remains. Such is the case of mass disasters, where correct sex identification reduces the pool of possible missing persons to just 50% of the population. [2,9]

Over the years, several scientific methods have been used to diagnose sex: visual inspection, anthropometric measurements, time and sequence of dental eruption, X-rays, microscopic observation of internal bone structures, physical and chemical analysis of calcified dental and bone tissues, sex-chromatin and DNA examinations. The choice of one or more of these will depend primarily on the quantity and state of preservation of human remains available for study. The degree of simplicity, practicality and accuracy, as well as costs, must also be considered. [15]

Adult human skulls are composed of a set of bones that are extremely rich in information concerning sexual dimorphism, which can be assessed both qualitatively and quantitatively. The robustness, size and cranial capacity in men are larger than in women. Methods based on visual morphological examination and anthropometric measurements are practical, easy to apply, inexpensive and reliable, as demonstrated by numerous and prestigious international studies.[6, 16] With the measures proposed, we sought to quantify this difference in order to establish parameters that can reliably determine the sex of an unidentified skull.

The sample analyzed was composed of skulls from the São Gonçalo Cemetery of Cuiabá, capital of Mato Grosso state, in Midwest Brazil. As to ancestry, the country contains one of the largest mixed populations in the world.[17] After more than five centuries of interethnic admixture between Amerindians, Europeans, Africans and Asians, Brazilians should be considered authentic members of an ancestral group, whose genetic traits have been reported by Pena et al.[18]and Santos et al.[19] It is important to remember that there is an enormous variability among peoples, making it essential to use sex determination methods grounded in the parameters of each population. [20, 21,22]

The present study focused on palatal bones and skull base measurements of 50 males and 50 females in order to verify the existence of sexual dimorphism. The Ba-IF linear distance was the most statistically significant measurement ($p=0.004$), corroborating the findings obtained by Francesquini Júnior et al. [23]

With respect to incisive foramen to left and right palatine foramen measurements, the results observed here agree with those obtained by Moreira et al.[10] who found sex differentiation for both the left and right greater palatine foramen. This was not observed by Teixeira,[7] who obtained a statistical difference only for the distance between the incisive foramen and the right greater palatine foramen.

In contrast to the findings of the present study, Saliba[24] found sex differentiation when measuring the distance from the right greater palatine foramen to the left greater palatine foramen. Methathrathip et al.[11]and Teixeira[7] also found statistically significant intersex differences in the distance between the palatine foramen and the interpalatine suture, which represents half the distance proposed here.

Finally, it is important to draw attention to the fact that sex determination and human identification are fundamental individual rights and that, according to Brazilian law, dentists are ethically and legally qualified to perform such a task.[25]

CONCLUSION

Sex is a helpful tool in the human identification process and its determination is a complex task. The measurements analyzed in this study exhibited sexual dimorphism, excepting for the distance between the right and left greater palatine foramens. The most statistically significant was the distance from the basion to the incisive foramen.

Two logistic regression models for sex determination were developed, with a reliability rate of 63% and 65% respectively.

The method proved to be valid, simple, reproducible and inexpensive, but not foolproof. For this reason, it should only be used exclusively when more accurate techniques cannot be applied, in accordance with the anthropological premise of using the largest possible number of identification methods.

It should also be noted that any human identification method must be tested and validated on local samples due to the increasing level of human variation and interethnic admixture.

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SECTION AGE ESTIMATION

The variability of lower third molar development in Northeast Malaysian population with application to age estimation

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ABSTRACT

This study aimed to assess the variability of the lower third molar (tooth 38 and 48) development in Northeast Malaysian population with respect to the side of dentition, to generate age prediction models and to compare the outcome with other studies. A total of 1080 orthopantomograms of Northeast Malaysian population aged between 14 and 25 years (540 males and 540 females) from the Hospital Universiti Sains Malaysia's archive which met the inclusion and exclusion criteria were selected and the maturity stages of toothth 38 and 48 were scored using Demirjian's stages (A-H). The findings showed a wide variation of the development of lower third molars in the Northeast Malaysian population. The roots developed earlier in males than in females. The development of the dentition on opposite sides of the mandible was synchronously in females and males. A multiple regression analysis shows that 71.1% of variance in age was explained by sex and developmental stage of tooth 48. An age prediction model was generated from the regression analysis: [Age = 7.117 + 1.907*(stage of tooth 48) – 0.432*(sex)] with mean prediction errors between -0.17 to 3.14 years. The obtained data in the current study are useful for references and determining age of unidentified human remains for identification investigation.

***KEYWORDS*:** Third molar development, Age estimation, Multiple linear regression, Northeast Malaysian, Demirjian's stages

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INTRODUCTION

Age estimation is useful for human identification[1] and in determining legal age for criminal responsibility.[2] Both applications are related to local legal requirements and can be applied to ageing both human remains and living people.[3]

Dental evidence is of great importance for forensic age estimation procedures as teeth are the most durable structure of the human body[4] and they give results of acceptable accuracy for assigning the age.[5] Moreover, dental post-mortem data can predict the age of a person from approximately 18-20 weeks 'in utero' until the last tooth is lost.[6]

Dental age can be evaluated in young children with higher accuracy because many teeth are undergoing development and mineralization simultaneously.[7] However, most of teeth have completed their development by approximately 14 years old,[8] leaving only the third molars to continue maturing until a later age.

The third molars are variable teeth in terms of position, size, shape, timing of formation and eruption and agenesis,[9] nevertheless, many studies found that the third molar development was applicable for age estimation.[2, 10-14]

There is no specific trend of sexual differences in the third molar development was reported across several populations and geographical areas. Studies on American Whites,[10] Texas Hispanic,[15] Europeans such as Belgian,[16] Spanish,[11] Turkish[17] and Austrian[18] and several Eastern Asians[2, 14, 19] reported that the third molar development is more advanced in males than in females. Few reverse findings are reported among North Indian[12] and American Black.[10]

Since there is no information regarding to age estimation in Northeast Malaysian population, the present study aimed to assess the variability of the lower third molar (tooth 38 and 48) development in Northeast Malaysian population aged between 14 to 25 years in both sexes, with respect to the side of dentition, and to generate an age prediction model. The accuracy and the regression analyses were also compared with other publications.

MATERIALS AND METHODS

Materials

Orthopantomograms (OPG) from the Hospital Universiti Sains Malaysia's archive were screened. Poor quality OPGs and those subjects with obvious dental pathology, known history of chronic medical illness and hormonal deficiency were all excluded. Non locals, based on the information from their new registration identification card number in the dental records, were also excluded.

All OPGs had been taken using Orthoralix 9200 (Finland) with different xray doses depended on the patient's body size, 70kV (small), 74kV (medium) and 78kV (large). The distance between subject and X-ray source was set at 0-14mm (default=7mm) and the exposure was 12 seconds. The study was granted ethical clearance to access dental records by the Universiti Sains Malaysia Human Ethics Committee. The handling of dental records complied with the highest standard of ethics.

Based on sample size calculation, a total of 1080 orthopantomograms of Northeast Malaysian population aged between 14 and 25 years (540 males and 540 females) were required. The calculation of the sample size was based on these parameters: the mean difference of 1.5 years with 80% power and alpha 0.05 which consists of 45 subjects in each study group (45 x 12 age groups x sex). Standard deviation was estimated as 2.5 years.[18]

Methods

The mineralization of both mandibular third molars was assessed according to the method described in Demirjian *et al*[5] using the eight grade scheme where stages A to D described the crown formation, while stages E to H describe root development.[5] Intra and inter-observer reliability was tested by re-examining 30 OPGs after a week interval.

Statistical analysis

Intra and inter-observer reliability were analyzed using Kappa Agreement.[20] Mean ages with standard deviation at each developmental stage were calculated and analyzed. Sex differences in age of attainment were tested using independent t-test. Side differences of age of attainment were assessed by Spearman '*rho*' correlation coefficient. A prediction model and its accuracy were tested using stepwise regression method in a multiple linear regression analysis. The stepwise method criteria used probability of F (entry of 0.05 and removal of 1.0). All statistical analyses were performed using PASW 18.0[21] and significance level was set at 0.05.

Results

Intra-observer reliability was excellent with a high value of agreement of 0.96 ($p<0.001$) for both sides of third molars. Inter-examiner reliability was also as high as 0.84 ($p<0.001$) for tooth 38 and 0.88 ($p<0.001$) for tooth 48.

Tables 1 and 2 show the distribution and the frequency of Demirjian's stages of lower third molars (tooth 38 and 48) according to chronological age group and for both sexes. In this sample the root apex for tooth 38 begins to close (Stage H) at age 18 years for both sexes. However, for tooth 48 one female case reached stage H at 17 years old.

DISCUSSION

The intra- and inter-observer reliability were high so it is unlikely that they biased the results and interpretation of our study.[22] This supports the popularity of Demirjian's atlas approach which is not dependent on the length of root or any metric measurement. The present study focused on the lower third molars but not the maxillary third molars due to the difficulty in assessing the latter teeth reliably as adjacent structures on the orthopantomograms (e.g. floor or posterior wall of maxillary sinus, zygomatic arch) superimpose the maxillary teeth.[2] One female subject reached complete root development before the age of 18 years old. Thus, this may limit the forensic use of the third molar development for estimating legal age. However, further study with larger sample size at stages development of G and H would be required before this conclusion can be made.

The results of this study showed that the development of third molars in Northeast Malaysians was more advanced in males than in females. The root development in males was ahead of females by more than 6 months; while the sexual dimorphism was less obvious in crown development, where the difference was less than 5 months. These findings are consistent with other Eastern Asians (0.2 - 1.5 years)[2, 14] and Europeans (0.2 – 1.49 years).[23, 24]

Multiple regression analysis demonstrated the level of association between chronological age, the tooth development stages and sexes. As development stage of tooth 38 and 48 were strongly correlated, a

Table 1: Frequency of Demirjian's stage of left third molar

Chronological age groups (years)	**Demirjian's stages**													
	Females							**Males**						
	C	**D**	**E**		**G**	**H**	**Total**	**C**	**D**	**E**	**F**	**G**	**H**	**Total**
14	11	27	7				45	12	23	9	1			45
15	7	18	14	5	1		45	4	13	19	8	1		45
16	2	15	19	8	1		45	3	8	14	14	6		45
17		5	12	19	9		45		3	8	15	19		45
18		3	6	13	16	7	45			4	11	21	9	45
19		1	1	18	14	11	45			4	6	21	14	45
20				4	23	18	45				4	15	26	45
21				2	22	21	45					9	36	45
22				1	12	31	45					5	40	45
23					9	36	45					5	40	45
24					5	40	45					2	43	45
25					1	44	45					1	44	45
Total	20	69	59	70	114	208	540	19	47	58	59	105	252	540

multicollinearity problem was detected (*VIF*>10). Thus the analysis was completed using tooth 48 and sex only. Previous publications[12, 16, 17] generate separate/unique sex prediction models, however, our study included sex into the model since the interaction between sex and tooth development did not contributet significantly to the model (p>0.05). This allows the unique contribution of each tooth's development and sex to age being quantified. Thus, one prediction model can be used to predict age for either males or females among Northeast Malaysians.

Every scientific evidence should have a sound methodology, known error rate and acceptabilityby the scientific community to be admissible in court.[25] Thus, our study provided the error rates and SD. The average prediction error is less than a year except for few age groups, in which the predicted error was up to 3.14 years. The SD values were also comparable with other publications for Belgian Caucasian and Thail population.[16, 26] So far, there is no study reporting the mean error and the SD values of each age group for comparisons with our study.

The regression model should be used cautiously when the stage of tooth has reached stage H. The prediction model is limited to the predicted ages of 22.37 years for females and 21.94 years for males, as the highest value of "8" (stage H) was

Table 2: Frequency of Demirjian's stage of right third molar

Chronological age groups (years)	Demirjian's stages													
	Females							Males						
	C	D	E	F	G	H	Total	C	D	E	F	G	H	Total
14	13	22	10				45	12	25	7	1			45
15	8	15	18	3	1		45	4	17	16	6	2		45
16	3	12	21	6	3		45	3	5	18	14	5		45
17		5	13	20	6	1	45		3	6	18	18		45
18		2	7	12	19	5	45			4	13	23	5	45
19			2	18	17	8	45			2	8	21	14	45
20		1		4	25	15	45				3	17	25	45
21				2	24	19	45				1	13	31	45
22				1	11	33	45					7	38	45
23					7	38	45					5	40	45
24					6	39	45					2	43	45
25					2	43	45					1	44	45
Total	24	57	71	66	121	201	540	19	50	53	64	114	240	540

considered for the developmental stage. This explains why the error (between observed and predicted age) was increased in older age group and always under-predicted. Other methods of dental age estimation should be considered for cases with stage H such as pulp size changes[27] and estimation of aspartic acid racemization in dentine.[28]

The trend of R^2 values of our study was comparable with other Asian populations but higher than reported R^2 values for European ancestry. This conclusion can be made since the scoring method was also Demirjian *et al*.[5] method. The value of R^2 0.711 is considered high and perhaps better than the skeletal age.[29]

CONCLUSION

A wide variability exists in the development of third molar teeth. Correlation between sides generally strong, and the third molar root developed earlier in males than in females. The age

Table 3: Descriptive statistics and sex differences in chronological age of left third molars development for males and females

Demirjian's stage	Females			Males			Mean diff	95% CI	t-statistics	df	p value
	n	Mean (years)	SD	n	Mean (years)	SD					
Stage C	20	14.55	0.686	19	14.53	0.772	0.024	-0.45,0.50	0.10	35.0	0.920
Stage D	69	15.16	1.232	47	14.81	0.947	0.351	-0.05,0.75	1.73	112.3	0.086
Stage E	69	15.98	1.225	58	15.84	1.142	0.138	-0.35,0.62	0.57	112.2	0.573
Stage F	70	17.80	1.528	59	17.03	1.474	0.766	0.24,1.29	2.89	124.7	***0.005***
Stage G	114	20.15	2.011	105	19.03	2.031	1.129	0.59,1.67	4.13	215.2	***<0.001***
Stage H	208	22.63	1.984	252	22.37	1.980	0.260	-0.11,0.62	1.40	441.3	0.162

SD, standard deviation; n, sample size; mean diff, mean difference; CI, confidence interval; df, degree of freedom

Table 4: Descriptive statistics and sex differences in chronological age of right third molars development for males and females

Demirjian's stage	Females			Males			Mean diff	95% CI	t-statistics	df	p value
	n	Mean (years)	SD	n	Mean (years)	SD					
Stage C	24	14.58	0.717	19	14.53	0.772	0.057	-0.41,0.52	0.25	37.3	0.805
Stage D	57	15.19	1.302	50	14.72	0.882	0.473	0.05,0.90	2.22	99.0	***0.028***
Stage E	71	15.93	1.291	53	15.81	1.257	0.118	-0.34,0.58	0.51	113.8	0.609
Stage F	66	17.92	1.460	64	17.20	1.449	0.721	0.22,1.27	2.83	128.0	***0.005***
Stage G	121	20.09	2.033	114	19.16	2.033	0.933	0.41,1.46	3.52	232.2	***0.001***
Stage H	201	22.73	1.910	240	22.48	1.930	0.252	-0.11,0.61	1.37	427.0	0.170

SD, standard deviation; n, sample size; mean diff, mean difference; CI, confidence interval; df, degree of freedom

prediction model for the Northeast Malaysians *i.e.* 7.117 + 1.907(stage of development) – 0.432(sex) is applicable for age prediction with the averages SD 1.82 years and 1.90 years for females and males respectively. However, this prediction model should be cautiously applied when the stages of the tooth development has reached stage H. The applicability of this model is also limited to stages C to H due to the design of the study and statistical analyses. Further study on younger age group (stages A and B) and focusing on legal age would be beneficial.

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Table 5: Relationship of tooth developmental stages between sides in both sexes

Sex	Correlation coefficient	p
Females	0.936	<0.01
Males	0.915	<0.01

Table 6: Un-standardized coefficients of multiple regression analysis[a]

Predictors	Unstandardized Coefficients		t-statistics	p value	95% Confidence Interval for *B*	
	B	Std. Error			Lower Bound	Upper Bound
(Constant)	7.117	0.253	28.098	<0.001	6.620	7.613
Stage of development at right third molar	1.907	0.037	51.537	<0.001	1.834	1.980
Sex	-0.432	0.114	-3.802	<0.001	-.655	-0.209

[a] Lower left third molar (tooth 38) was omitted due to multicollinearity *VIF*>10

Table 7: Mean of prediction error (observed – predicted) and standard deviation for sex and chronological age

Chronological age groups (years)	Females		Males	
	Mean prediction error (years)	Standard deviation	Mean prediction error (years)	Standard deviation
14	-0.58	1.36	-0.17	1.28
15	-0.53	1.76	-0.68	1.84
16	-0.42	1.84	-0.80	1.93
17	-0.87	1.80	-1.41	1.75
18	-1.38	1.94	-1.35	1.59
19	-0.89	1.64	-1.06	1.59
20	-0.74	1.47	-1.03	1.18
21	-0.22	1.03	-0.31	0.98
22	0.13	1.00	0.32	0.79
23	0.91	0.73	1.25	0.58
24	1.89	0.70	2.10	0.45
25	2.67	0.63	3.14	0.47
average		1.82		1.90

Negative sign indicates over-prediction (observed – predicted); Codes used for prediction model: Stage of development 1 to 8 equation to A to H; sexes 0=female, 1=male; example of model prediction for stage H, female: Age of prediction = 7.117 + 1.907(8) -0.432(0)=22.37 years. If males (code=1), the predicted age is equivalent to 21.94 years.

Table 8: Summation of multiple linear regression analysis results on third molar development from different studies.

Population/study	*R^2*	Age prediction model/regression formula	SD(years)
Northeast malaysian	**0.711**	**age = 7.117 + 1.907(stage of tooth 48) – 0.432(sex)** **whole sample and sex variable was included**	**refer to table 7**
Belgian caucasian[16]	**0.37 (f)** **0.48 (m)**	**separate male and female**	**1.56 (f)** **1.52 (m)**
Turkish[17]	**0.61 (f)** **0.57 (m)**	**separate male, female and whole sample**	**n/a**
Thai[26]	**n/a**	**separate male and female, and specific for each jaw**	**1.71-1.90**
North indian[12]	**0.63 (f)** **0.62 (m)**	**separate male, female and whole sample**	**n/a**
Spanish[23]	**0.45 (f)** **0.54 (m)**	**n/a**	**n/a**
Turkish[24]	**0.56 (f)** **0.57 (m)**	**n/a**	**n/a**
Korean[2]	**0.81 (f)** **0.84 (m)**	**n/a**	**n/a**
Romanian[30]	**0.94 (f)** **0.95 (m)**	**n/a**	**n/a**

(F) Females; (M) Males; SD Standard deviations; all predictions models and coefficients of determination are within 95% CI. N/A data were not available

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